SEQUENCE SPECIFICITY IN TRANSCRIPTION AND TRANSLATION

Richard Calendar and Larry Gold, Organizers

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Sequence Specificity in Transcription and Control

Prokaryotic Promoters

0784 ACTIVATION OF LAC TRANSCRIPTION, Jay D. Gralla, Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, CA 90024

Transcription of the *lac* promoter can be activated by both negative DNA supercoiling and by catabolite activator protein. We have studied the magnitude, mechanism, and DNA sequence requirements for this transcriptional activation. A series of circular templates which differ only in the extent of negative supercoiling were prepared and assayed for transcription rate *in vitro*. This basic experiment was repeated using a family of *lac* promoter variants which differed by point mutations in each of the three prokaryotic promoter elements, -10 region sequence, -35 region sequence, and spacer length. In general, the introduction of negative superhelicity into promoter-bearing DNA leads to an initial exponential rise in transcription rate followed eventually by a modest decline. The maximum stimulation achieved varies very significantly among promoters. It is a slight two-fold for *lac* UV5 containing the consensus -10 element and is forty-fold for *lac* p^s which contained a single substitution in the region. Possible mechanisms for these effects will be discussed.

CAP protein also stimulates transcription. CAP was found to act as a promoter-clearing protein *in vitro*. That is, it can catalyze the removal of RNA polymerase bound improperly to the *lac* promoter region. Experiments will be presented which bear on the issue of how RNA polymerase is re-directed to the proper promoter site.

Lastly, a novel methodology for footprinting DNA-protein complexes will be described. This method gives high resolution data and is ultra-rapid since neither restriction cleavage nor blotting is required. Several applications, including footprints of regulatory proteins on supercoiled DNA, will be presented.

0785 DEVELOPMENTALLY REGULATED PROMOTERS IN A SPORE FORMING BACTERIUM, Richard Losick, Peter Zuber, Michele Igo, Cellular and Developmental Biology, Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, MA 02138; and Charles Moran, Department of Microbiology and Immunology, Emory University School of Medicine, 502 Woodruff Memorial Building, Atlanta, GA 30322

The transcription initiation regions of the *Bacillus subtilis* genes *spoVG* and *ctc* are composed of overlapping promoters that are separately utilized by the minor RNA polymerase holoenzyme forms $E\sigma^{37}$ and $E\sigma^{32}$. We have constructed specialized transducing phages bearing fusions of *spoVG* and *ctc* to the *E. coli lacZ* gene in order to identify nucleotide sequences required for expression and regulation of these genes *in vivo*. *spoVG* transcription is induced at the onset of sporulation, and this RNA synthesis is dependent upon the products of a class of developmental regulatory genes known as the *spo0* loci. We have been able to establish by means of deletion analysis that *spo0*-dependent regulation of *spoVG* is exerted at or near the sites of $E\sigma^{37}$ and $E\sigma^{32}$ interaction with the overlapping *spoVG* promoters. An upstream AT-rich box (positions -50 to -76) is shown to enhance strongly the *level* of *spoVG* transcription but is not required for the normal *pattern* of *spoVG* expression. *spoVG* can be contrasted with *ctc* whose expression is *spo0*-independent and whose induction takes place under nutritional conditions distinctly different than those that induce spore formation. We show that the *level* of *ctc* transcription and its *regulation* is entirely determined by DNA sequences that extend no further upstream than position -48 (that is, without dependence upon upstream AT-rich sequences). Nucleotide substitution mutations at "-35" and "-10" positions, which impair *ctc* recognition by $E\sigma^{37}$ and $E\sigma^{32}$ *in vitro*, are shown to prevent its expression and induction *in vivo*. Our results suggest that the activity of *spoVG* and *ctc* is controlled by regulatory proteins (e.g. *spo0* gene products in the case of *spoVG* and other unidentified regulatory gene products in the case of *ctc*) that act directly or indirectly *in conjunction* with minor RNA polymerase forms at sites close to or overlapping with promoter sequences.

Losick and Youngman, *Microbial Development*, eds. R. Losick and L. Shapiro, Cold Spring Harbor, 1984, p. 63.

Zuber and Losick, Cell *35*: 275-283, 1983.

Tatti and Moran, J. Mol. Biol. *175*: 285-297.

0786 MECHANISMS OF ACTIVATION OF *ESCHERICHIA COLI* RNA POLYMERASE.

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Experiments performed *in vitro* have revealed many similarities and also considerable diversity in the mechanisms of transcription activation. In all cases the activator protein binds to a site close to the promoter. In all cases activator binding results in an increase in transcription initiation frequency. The diversity displayed by several activation mechanisms is summarized in the accompanying table. The biochemical studies have shown that the binding step (K_B) or isomerization step (k_2) or both steps can be directly enhanced by different activators. Activation can also occur indirectly as a result of the activator (a.k.a. repressor) excluding RNA polymerase binding at an overlapping promoter. The activated promoters can also be separated into groups based on the effects of promoter mutations and DNA supercoiling. Activation of λP_{RM} and *lac*P$^+$ is superimposed in a simple way upon the effects of mutation in these promoters (both up and down mutations have been examined). Activation of λP_E and λP_I by *c*II protein is more complex because up (P_I) and down (P_E) mutations affect the mechanism and the extent of activation. DNA supercoiling does not always result in increased promoter activity (λP_{RM} ± *c*I). DNA supercoiling has been shown to contribute significantly to the activation of *lac*P$^+$ and of λP_E and λP_I. In the latter three cases supercoiling might enhance activator binding, polymerase interaction, or both. The effects of activator concentration, promoter mutations, and DNA supercoiling found *in vitro* are in good agreement with experiments performed *in vivo*.

Promoter	Activator	Activation *in vitro* K_B	k_2	Indirect	Activation *in vivo*	References
1. λP_{RM}	*c*I	-	11x	-	8 - 10x	1) 2)
2. *lac*P$^+$	CAP-cAMP	>20x	-	3x	25 - 50x	3) 4)
3. λP_E & λP_I	*c*II	100x	100x	-	∿ 100x	5) 6)

1) Hawley, D.K. and McClure, W.R. (1983) *Cell* *32*, 327-333.
2) Shih, M.-C. and Gussin, G.N. (1983) *PNAS* *80*, 496-500.
3) Malan, T.P., Kolb, A., Buc, H. and McClure, W.R. (1984) JMB, in press.
4) Malan, T.P., and McClure, W.R. (1984) *Cell*, in press.
5) Shih, M.-C. and Gussin, G.N. (1983) *Cell* *34*, 941-949.
6) Hoopes, B.C. and McClure, W.R., unpublished.

0787 PROMOTERS FOR BACTERIOPHAGE N4-VIRION RNA POLYMERASE,

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Coliphage N4 early RNA synthesis requires the activity of a phage-coded, virion-encapsulated RNA polymerase composed of one 320,000 MW polypeptide. *In vitro*, the enzyme is inactive on all native DNAs tested including the linear, double-stranded 72 Kb N4 genome. However, denatured N4 DNA is transcribed with *in vivo* specificity. The sequences of three sites of transcription initiation on the N4 genome have been determined. Extensive sequence homology is present from position -18 to position +1, with a conserved G + C rich heptamer centered at -12. Another conserved feature is the presence of short inverted repeats, centered around the heptamer and encompassing conserved and non-conserved sequences. In order to test the relevance of the sequences and inverted repeats to transcription initiation, we have cloned the promoter in M13 vectors. We have introduced changes in the non-conserved bases of the inverted repeats by oligo-directed site-specific mutagenesis. Preliminary evidence suggests that the inverted repeats are necessary for activity when single-stranded DNAs are used as templates.

Although at a low level, denatured non-N4-DNA templates support transcription by the N4 virion RNA polymerase. Initiation is specific, and the sequence of several of the sites on ϕX174, SV40, T7, BPV, pBR322 and M13 DNAs is being determined. Available data suggests that three sets can be considered wild type N4 promoters where substitutions, insertions and deletions have occurred.

Although N4 early transcription is independent of the host RNA polymerase, two *E coli* functions are required *in vivo*: single-stranded DNA binding protein (ssb) and DNA gyrase. We have found that N4 virion RNA polymerase can transcribe double-stranded DNA *in vitro* if the DNA carries a promoter, is supercoiled and *ssb* is present. No other single-stranded DNA binding proteins (fd, T7, T4, N4, etc.) can substitute for *E. coli ssb*. The topology of the initiation complex as well as the role of *ssb* are under investigation.

Promoters A

0788 A TRANSCRIPTIONAL ACTIVATOR ELEMENT IS LOCATED UPSTREAM OF THE HUMAN U2 SMALL NUCLEAR RNA GENE

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We are studying the human U2 snRNA promoter, a powerful polymerase II promoter without a TATA-box. A set of deletions, insertions, and rearrangements of sequences upstream of the U2 cap site have been constructed and tested for their ability to direct human U2 RNA synthesis after microinjection into Xenopus oocytes. Deletion of sequences between positions -291 and -218 reduces the activity of the template 5 to 10 fold, and destroys the ability of the template to compete with a coinjected U1 gene. When a fragment containing activator sequences is placed in its natural orientation closer to the U2 cap site, transcription is still activated, suggesting that function of the element is at least partially independent of position. Activation of initiation at the normal U2 cap site is lost if the element is inverted, either in its natural position at -198 or closer to the gene at -61. Aberrant initiation sites upstream of the U2 cap site, however, are activated by the element independent of orientation. The 68 bp fragment containing the upsteam activator element also contains a highly conserved sequence homology found in the human U1 gene, as well as in U2 genes from rat and Xenopus. Deletion of just 4 bp in the center of this homology destroys the activity of the element. The U2 transcriptional activator may be different from other enhancer elements because its ability to stimulate correct U2 initiation is extremely sensitive to orientation, although it can activate aberrant transcription independent of orientation.

0789 C. elegans RNA POLYMERASE II GENES

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Sanford, Golomb and Riddle (J. Biol. Chem., 258: 12804-12809, 1983) have identified a gene, ama-1 IV, which appears to code for a subunit of RNA polymerase II. This gene was initially defined by the dominant mutation m118, positioned near dpy-13 on linkage group IV. Strains carrying ama-1(m118) are resistant to α-amanitin and produce an altered RNA polymerase II enzyme. To continue the analysis of RNA polymerase in C. elegans, we have: (1) isolated 7 lethal alleles of ama-1, including one temperature-sensitive allele and one gamma-ray-induced chromosomal rearrangement, (2) to date identified six essential genes near ama-1 (defined by 15 lethal mutations) one or more of which, by analogy with yeast (Ingles et. al., Proc. Natl. Acad. Sci. USA., 81: 2157-2161, 1984), may encode other polymerase subunits, (3) constructed a detailed genetic map of the region surrounding ama-1, and (4) used molecular clones encoding regions of the Drosophila RNA polymerase II large subunit gene (Greenleaf, A.L., J. Biol. Chem., 258: 13403-13406, 1983) to isolate recombinants from a C. elegans gene bank.

We are currently characterizing these recombinants, particularly the structure of the promoter of this house-keeping gene. Also, we are selecting genetic revertants of certain lethal ama-1 alleles in an attempt to identify suppressor mutations defining genes encoding other RNA polymerase II subunits, or other genes affecting transcriptional efficiency.

0790 A NOVEL ARRANGEMENT OF SHORT SEQUENCE REPEATS IN THE PROMOTER REGIONS OF THE C. ELEGANS YOLK PROTEIN GENES

Tom Blumenthal, Karen Denison, Sarah Kirtland, Jerome Cane, and John Spieth, Indiana University, Bloomington, IN 47405.

The C. elegans yolk proteins are encoded by a six member gene family. The transcripts from these genes accumulate to very high levels in the intestine of the adult hermaphrodite, but are not found in juveniles, males, or other hermaphrodite tissues. In order to identify possible cis-acting elements involved in this developmental regulation, we have determined the nucleotide sequences surrounding the 5' ends of five of the yolk protein genes. Whereas, the protein coding sequences are highly conserved, the 5' flanking sequences are largely diverged. However, we have identified three different presumptive regulatory sequences embedded in the first 250 bp upstream of each of the five genes. One 7 bp element is present between four and six times per gene, in either orientation. This sequence is always present in one orientation at about position -180 and, also, usually in the opposite orientation just 5' of the TATA box. The other two sequence elements are each present between positions -85 and -150 of all five genes. They are usually immediately adjacent to each other but in differing orders and orientations. We suggest these three sequences may signal the high level stage-specific, tissue-specific, and sex-specific expression of the yolk protein genes.

0791 ANALYSIS OF THE PROMOTERS OF THE E.COLI rrnB GENE, Imre Boros, Jörn Belter, Tamás Lukacsovics, Pal Venetianer, Institute of Biochem. Biological Research Center, Hungarian Academy of Sciences, Szeged, H.
The two strong tandem promoters of the E.coli rrnB gene were cloned in a stable multicopy plasmid joining the natural terminator region of the gene immediately to the promoters. Measuring the transcription from the individual promoters both in vitro and in vivo we found that the higher structure of the template DNA strongly affects the rrnB promoter activity and mutations preceeding the rrnB P_1 -35 region cause dramatic alterations in the promoter properties. A short part of the E.coli lac operon was inserted after the rrnB promoter/s/ and by creating in vitro deletions between the rrnB and lac promoter regions plasmids ensuring the highest lac expression were selected. Some of these plasmids carry a hybrid promoter region: rrnB P_2 -35 region with lac or lac-rrnB fused -10 region. These promoters combine the advantages of the two original promoters: they are regulatable through the lac repressor--operator system and although none of them contains the believed ideal 17 bp spacer between the two consensus regions they preserved the very strong character of ribosomal promoters.

0792 SUPERCOILING RESPONSE OF MUTANT *LAC* PROMOTERS, James A. Borowiec and Jay D. Gralla, UCLA, Los Angeles, CA 90024

We have examined the effects of negative DNA supercoiling on the rate of open complex formation between *E. coli* RNA polymerase and mutant *lac* promoters *in vitro*. In particular, extensive rate data was collected on the *lac* p^S promoter over a wide range of negative superhelical densities. The presence of low to moderate levels of DNA supercoiling increases the transcription rate at the p^S promoter in an exponential manner. Further additions in the amount of supercoiling past these levels halt the exponential increase and result in a minor reduction of the rate. The maximum rate achieved is over 40-fold greater than the transcription rate from the fully relaxed promoter and occurs at or near the superhelical density of form I plasmids isolated from the bacterial cell. The rate enhancement was found empirically to be a linear function of the free energy of supercoiling of the plasmid, that is, the increase in rate is a linear function of the square of the number of superhelical turns contained in the plasmid.

Comparison of the supercoiling response between mutant *lac* promoters suggests that slight modifications of the promoter structure can significantly affect the response of a promoter to DNA supercoiling. A method for comparing the supercoiling response among promoters is presented.

0793 ORGANIZATION AND FUNCTION OF THE PROMOTERS FOR THE TWO LINKED δ-CRYSTALLIN GENES. T. Borras, G. C. Das, J. M. Nickerson, J. W. Hawkins, A. B. Chepelinsky and J. Piatigorsky. Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20205.

δ-Crystallin, the major protein of the developing chicken lens, consists of at least two polypeptides with molecular weights of 48K and 50K (present in a ratio of 3:1, respectively). There are two extremely similar δ-crystallin genes, 4.2 Kb apart, each containing 17 introns. Only δ1 (the 5' gene) is known to be active in the lens; no cDNA has been isolated yet for δ2. The 5' flanking region of both genes are rich in GC and have TATA boxes, however, only δ1 has a CAAT box and an upstream core-enhancer like sequence. Interestingly, the 5' flanking region of δ1 shows several-fold greater promoter activity *in vitro* in a Hela cell extract and *in vivo* using the pSVO-CAT expression vector in transfected lens epithelia, than the corresponding flanking region of δ2. The *in vitro* transcription experiments were performed with about 600 bp of flanking sequence from each gene, while the *in vivo* experiments were conducted with 365 bp of flanking sequence. The δ1 promoter region was shown by S1 mapping to initiate RNA synthesis *in vitro* at the same major sites as the authentic gene in the intact lens. Thus, the 5' flanking sequences of the δ1 gene appear to have inherently stronger promoter activity than those of the δ2 gene. Detailed analyses can now be undertaken to understand the bases for the differential regulation of the two members of this small family of specialized, lens genes.

Sequence Specificity in Transcription and Control

0794 DISCONTINUOUS TRANSCRIPTION OF mRNAs IN TRYPANOSOMES.

Borst, P., Kooter, J.M., De Lange, T. and Cornelissen, A.W.C.A.

Many, if not all, mRNAs in *Trypanosoma brucei* start with the same sequence of 35 nucleotides, separately encoded in mini-exon repeats (1,2). The mini-exon repeats are transcribed into a 141-nt precursor RNA with the 35-nt sequence at its 5'end (3). Three lines of evidence show that (part of) this RNA is linked in a second step to pre-mRNA. (i) The 141-nt RNA is the only transcript of the mini-exon repeat synthesized at high rate (3). (ii) We have obtained evidence that a gene for a variant surface protein on a chromosome that contains no mini-exon repeats nevertheless yields a mRNA with the 35-nt sequence (4). (iii) Whereas transcription of mini-exon genes is more than 90% inhibited by 200 μg α-amanitin per ml, variant surface protein (VSG) gene transcription is insensitive to 1000 μg α-amanitin (unpublished results). VSG genes are presumably transcribed by a separate RNA polymerase.

1. De Lange, T. et al. Nucl. Acids Res. (1984), 12, 3777-3790.
2. De Lange, T. et al. Nucl. Acids Res. (1984), 12, 4431-4443.
3. Kooter, J.M. et al. EMBO J. (1984), 3, 2387-2392.
4. Van der Ploeg, L.H.T. et al. Cell (1984), in press.

0795 AUTOGENOUS REGULATION OF A DNA REPLICATION GENE, *dnaA*, IN *E.COLI*,

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dnaA is an essential gene whose product is required for the initiation of DNA replication in *E. coli* K-12. Results of various *in vivo* experiments have indicated that regulation of the expression of *dnaA* may play a central role in regulating the frequency of initiation of DNA replication. Using transcriptional and translational fusions of the *dnaA* gene to the *lacZ* gene, we have obtained *in vivo* evidence which indicates that the *dnaA* gene product regulates its own synthesis at the level of transcriptional initiation. Results from a deletion analysis of the *dnaA* promoter/regulatory region suggest that both *dnaA* promoters are regulated by the *dnaA* gene product and that a site between the two promoters is responsible for the regulation. DNase protection experiments showed that purified *dnaA* protein binds to a site between the two *dnaA* promoters. We have also shown that one of the the two *dnaA* promoters is regulated by DNA methylation. We are currently investigating the dual role of the *dnaA* protein in its own regulation and in the initiation of DNA replication. To this end we have genetically defined two domains in the *dnaA* protein: one involved in autogenous regulation (DNA binding), and the other involved in the initiation of DNA replication.

0796 GENBANK AS A RESEARCH TOOL.

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GenBank, the national nucleotide sequence database, is a computer-based data bank of all published DNA and RNA sequences. The database is available on-line, on tape, and in hardcopy book form (Andersen et al. (1984) Nucleotide Sequences 1984, IRL Press, Oxford). As of September, 1984, the database contains close to 3.5 million nucleotides in over 4000 entries. In addition to being a convenient reference for researchers interested in individual sequences, the database has been and is being designed to anticipate strategies that scan over many entries in search of similar features. We have also developed algorithms and software to accomplish these searches, and are especially interested in sequence comparison algorithms (used for identifying local homology, consensus sequences and hairpin structures), prediction of protein coding regions, and correlation of primary sequence data with both secondary and tertiary structure and functional roles in the cell.

Sequence Specificity in Transcription and Control

0797 IN VITRO ASSEMBLY OF TRANSCRIPTION COMPLEXES ON GENES TRANSCRIBED BY RNA POLYMERASE III, Michael Carey, Stephen P. Gerrard and Nicholas R. Cozzarelli, University of California, Berkeley, CA 94720

We studied the assembly of RNA polymerase III transcription complexes on the 5S RNA gene of *Xenopus* and the VAI gene of adenovirus. Complete transcription complexes and subassemblies were formed on these genes *in vitro* using transcription factor A and RNA polymerase III from *Xenopus* and factors B and C from HeLa. To demonstrate these complexes, the DNA and factors were passed through a Sepharose 4B column. The excluded material containing DNA and bound factors was assayed for transcription after addition of polymerase. For the 5S gene, factor A bound by itself and allowed factor C to bind, which in turn allowed factor B to bind. Only factor C alone bound to the VA gene but it allowed factor B to bind. The specific subassemblies formed imply that the order of addition to a 5S gene is factors A,C and B, and to the VA gene it is factors C and B. To study the stability of these assemblies we determined the effect of high ionic strength. Factors B and C were preincubated with the VA gene in the presence of high salt or allowed to prebind under standard conditions and then raised to high salt. Factor binding was analyzed after purification by Sepharose 4B chromatography. Surprisingly 50% of the prebound complexes remain associated with the DNA even after 2.5 min washes with 0.8 M KCl. Kinetic data indicate a half life of dissociation of 5 min in 0.45 M KCl. Only 10% of the complexes are able to form in 0.8 M KCl but 50% of the normal amount of factor C binds.

0798 AMANITIN-INSENSITIVE TRANSCRIPTION OF MOUSE β^{MAJ}-GLOBIN 5'-FLANKING SEQUENCES CORRELATES WITH mRNA EXPRESSION, David P. Carlson and Jeffrey Ross, University of Wisconsin, Madison, Wisconsin 53706

RNAs initiate upstream of the canonical cap site of the mouse β^{maj}-globin gene. Like the human β-globin gene, the mouse β^{maj}-globin gene has associated RNAs with heterogeneous 5' termini within 235 nucleotides upstream of the mRNA cap site. Synthesis of most of them in vitro is insensitive to low levels of α-amanitin, indicating that they are transcribed by a polymerase other than RNA polymerase II. The upstream RNAs extend into the structural gene and bind to oligo dT cellulose under standard conditions. The stable, mature RNAs contain approximately 800 nucleotides in mouse reticulocytes. Induction of mouse erythroleukemia cell differentiation by dimethylsulfoxide stimulates accumulation of the upstream RNAs. Both upstream RNAs and mRNA begin to accumulate between 24 and 48 hours after addition of inducer. Therefore, expression of the upstream RNAs closely correlates with the expression of the β-globin in MEL cells.

0799 TWO NON-CONTIGUOUS ELEMENTS ACT IN A POSITION AND ORIENTATION DEPENDENT MANNER TO INDUCE SV40 LATE GENE EXPRESSION WITH LARGE T ANTIGEN. S. Chandrasekharappa, S. Hartsell & K. Subramanian, Univ of IL Health Sciences Cntr, Chicago, IL 60617.

The late promoter of SV40 has been studied using a transient expression vector containing the chloramphenicol acetyl transferase(CAT) gene placed downstream from the late control region and under conditions similar to the late phase of the lytic cycle of SV40(i.e., in the presence of T antigen and autonomous replication of the template). In monkey cells producing T antigen the CAT activity under the late control region is induced by approximately 1,000-fold above the basal level. By deletion and point mutagenesis two domains of the late control region required for efficient induction with T antigen have been identified. Domain I(ORI) is the minimal replication origin region containing T antigen binding sites I and II. Domain II(ENH) consists of the 72-bp repeat and a 19-bp downstream sequence up to nucleotide 270. Domains I and II should act synergistically since the absence of either one or the other decreases induction efficiency by two orders or magnitude. For maximum induction by T antigen both these domains should be in the same order with respect to the CAT gene as in the wild type late promoter. Switching the order of the ORI and ENH domains results in a considerable decrease in induction efficiency. Keeping the order of the ORI and ENH domains the same as in the wild type late promotor, the reversal of orientation of the ENH domain does not affect induction efficiency. Both the ORI and ENH domains should be present together for efficient induction since separation of the domains by placing one upstream and the other downstream, and *vice versa*, from the CAT gene results in the loss of induction. Direct interaction between the ORI and ENH domains must be important for efficient late promoter function.

0800 AN AUTOREGULATORY PROTEIN IS REQUIRED FOR P1 PLASMID REPLICATION, Dhruba K. Chattoraj and Ann L. Abeles, NCI-Frederick Cancer Research Facility, Frederick, MD 21701

The unit-copy P1 replicon is ca. 1.5-kb long and contains three adjacent, fuctionally discete segments. These are i) a 245-bp region that acts as an origin, ii) a region (*repA*) that encodes an essential replication protein, and iii) a region that exerts a negative control on replication. Both the origin and the negative control regions contain a set of 19-bp imperfect repeated sequences. In vivo and in vitro experiments indicate that the two sets of repeats interact with and apparantely compete for the limited amount of RepA protein. Gene fusion and primer extension assays show that the promoter for the *repA* gene maps within the origin repeats and that the RepA protein negatively regulates its own promoter (autoregulation). The autoregulatory circuit could adjust for any reduction of RepA concentration caused by binding of RepA by the control repeats (titration). However, we postulate that when titration is complete, a delay in the autoregulatory response allows the protein concentration to rise transiently to a level sufficient for the origin function.

Research sponsored by the National Cancer Institute, DHHS, under contract NO. N01-CO-23909 23909 with Litton Bionetics.

0801 LENS-SPECIFIC EXPRESSION OF THE CAT GENE PROMOTED BY 5' FLANKING SEQUENCES OF THE MURINE αA-CRYSTALLIN GENE IN EXPLANTED CHICKEN LENS EPITHELIA.

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We have developed a system using explanted embryonic chicken lens epithelia to express foreign recombinant genes containing crystallin DNA regulatory sequences introduced by calcium phosphate transfection. Optimal results were obtained using lens epithelia from 14 day-old embryos transfected 1 day after explantation and assayed 3 days later. When DNA sequences (-364 to +45) of the murine αA-crystallin gene were inserted in the pSVO-CAT expression vector of Gorman et al (Mol. Cell. Biol. *2*, 1044-1051, 1982) in the same orientation as in the crystallin gene, they promoted chloramphenicol acetyl transferase (CAT) activity in the transfected epithelia. Sequences 87 to 364 bp upstream from the murine gene cap site were required for CAT gene expression. These crystallin gene regulatory sequences did not promote CAT expression in primary cultures of embryonic chicken fibroblasts, NIH-3T3 cells, L-cells or BSC-1 cells. Our experiments thus demonstrate that the explanted embryonic chicken lens epithelium is an advantageous recipient for identifying lens cell-specific regulatory sequences of crystallin genes and implicate a DNA region upstream of the TATA box for regulation of the murine αA-crystallin gene. These experiments also suggest that explanted epithelia of other tissues may be useful for studying the expression of foreign genes.

0802 CELL SPECIFIC EXPRESSION OF A CLONED HUMAN α_1-ANTITRYPSIN GENE, G. Ciliberto, L. Dente, R. Cortese, EMBL, 69 Heidelberg, FRG

We have cloned and sequenced a full length cDNA from a human liver library, coding for α_1-antitrypsin. The corresponding genomic segment, from a EMBL3 lambda human library has also been cloned and characterized. By sequence analysis, S_1 mapping and primer elongation we have identified the promoter and the transcription initiation point. A human α_1-antitrypsin minigene has been constructed, lacking the 2nd and 3rd exon codifying for α_1-antitrypsin. The α_1-antitrypsin minigene is efficiently and correctly transcribed and spliced following transfection only in the human hepatoma cell line Hep3B but not in HeLa cells. A 1176 base pair DNA segment, deriving from the 5' flanking region of the α_1-antitrypsin gene, was cloned in the unique BglII site of plasmid pA10cat2 (Cell, *30*, 403, 1984), in both orientations. The construct carrying the α_1-antitrypsin DNA segment in the "physiological" orientation is expressed, following transfection only in the human hepatoma cell line Hep3B but not in HeLa cells.

Sequence Specificity in Transcription and Control

0803 TRANSCRIPTIONAL REGULATION OF THE 3-HYDROXY-3-METHYLGLUTARYL COENZYME A REDUCTASE GENE IN RAT LIVER,

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The rate of *de novo* synthesis of cholesterol is regulated by the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase. The activity of this enzyme in rat liver can be induced over 1,000-fold by feeding cholestyramine (a resin which sequesters bile acids) and mevinolin (a potent inhibitor of reductase activity). The 1,000-fold increase in reductase activity is due in part to a 100-fold increase in the level of reductase mRNA (Clarke et al; J. Biol. Chem., 259,10439, 1984). Reynolds et. al; (Cell, 38, 275, 1984) have shown that the promoter region of the reductase gene in a CHO cell line shares homology with the GC rich, 21 bp repeats of the SV40 early promoter. We have used isolated rat liver nuclei in a "run off" transcription system to investigate the role played by transcription in the regulation of reductase mRNA levels. Levels of reductase RNA were quantitated by hybridization to filters containing a reductase cDNA. Rats induced by a diet supplemented with cholestyramine and mevinolin to have high levels of reductase mRNA exhibited levels of reductase transcription which were 15-20 fold higher than in rats fed an unsupplemented diet. Over 90% of the transcription of the reductase gene was inhibited by concentrations of α-amanitin which selectively inhibit Pol II. To determine whether the increased transcription corresponded to sense or to anti-sense RNA, the relative amount of transcription directed off of the reductase coding and noncoding strands was determined using filters containing the appropriate cloned single stranded reductase cDNA. Transcription off the noncoding strand of the reductase gene was approximately 15-20% that of the coding strand. We conclude from these studies that reductase transcription can be induced under conditions which stimulate synthesis of cholesterol, and that the increased transcription rate accounts for much, but not all of the elevated level of reductase mRNA.

0804 PURIFICATION OF rRNA STABLE TRANSCRIPTION COMPLEXES AND IDENTIFICATION OF THE REQUIRED TEMPLATE SEQUENCES,

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Mouse rRNA synthesis occurs on template molecules that are activated for transcription by stably associating with transcription factors (Nuc. Acids Res. 11: 3795; 7043). To identify the DNA sequences required for formation of this stable complex, 5'and 3' rDNA deletion mutants were tested for their ability to stably bind transcription factors and thereby preclude synthesis from subsequently added wild type rRNA genes. A large rDNA region is involved: a proximal domain (residues ~-30 to +5) is essential to allow any detectable binding and the stability of this complex is sequentially increased by the presence of several additional rDNA domains that extend in the 5' direction out to residue ~-140. These regions exactly coincide with the various domains of the rDNA promoter, indicating that the active promoter contributes to the stability of the transcription complex.

We have also found that the rDNA stable transcription complex can be quantitatively and selectively sedimented from in vitro transcription reactions. Complete transcriptional activity can be recovered on the pelleted active template under conditions where 90% of the input rDNA molecules and 98% of the extract proteins remain soluble; the factors also do not sediment in the absence of template. Thus, sedimentation of the stable complex effects a major and rapid purification of polymerase I transcription factors. Moreover, polymerase II and III active templates can be similarly isolated by centrifugation of in vitro reactions.

0805 EXPRESSION OF THE SATELLITE BACTERIOPHAGE P4 LATE GENE CLUSTER,

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P4 has 3 late genes, *sid*, δ, and *psu*. These genes are clustered at the right end of the genetic map and we have shown that they are all transcribed in the same direction from a promotor near the start of the *sid* gene. P4 δ^- mutants are deficient in the expression of the P4 late promoter. P4 late transcription is delayed until 45 minutes after infection in the absence of a P2 helper. When P2 is present, late transcription begins 15 minutes after coinfection, at the same time P2 late transcription begins. In the presence of a P2 helper, late transcription is not affected by a mutation in the P4 δ gene. The -35 region of the *sid* promoter shares no homology with the late gene promoters of its helper phage P2 or with the known classes of *E. coli* promoters.

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             -35                        -10         +1
E. coli      TTGACA                     TATAAT
P2 late      ATAGC                      G-AA-CT     ↓              FMet
P4 late  AATGCGTCGTGTTGTTGTCCGGTGTACGTCACAATTTTCTTAACCTGAAGTGACGAGGAGCCGGAAAATG
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0806 THE CHARACTERIZATION OF PROMOTERS AND TERMINATORS OF RNA TRANSCRIPTION CARRIED BY IS30, Brian P. Dalrymple and Werner Arber, Biozentrum, University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland
Using plasmids carrying various fragments from IS30 (a resident mobile genetic element of *E. coli* K12) inserted in front of a promoterless *galK* gene several promoters of transcription have been identified. In a similar system insertion of fragments of IS30 between the *lacUV5* promoter and the *galK* gene has allowed us to identify sequences with terminator activity. The long open reading frame of IS30 is transcribed from a weak promoter (P_A), the proposed -35 region of which is in the left hand terminal inverted repeat. Transcription from this promoter is not detectable immediately outside the right hand end. A second promoter (P_B) is present in the right hand end of IS30, but transcription from this promoter is generally repressed. On the reverse strand a third promoter (P_C) has been identified preceding a short open reading frame. A sequence with weak termination activity follows this open reading frame. A much stronger terminator of transcription maps in the first two hundred bases of IS30 (but on the opposite strand to the large open reading frame). So far it is not clear whether this terminator is preceded by an additional active promoter.

0807 OVERLAPPING Tn*10* *tet* PROMOTERS COMPETE FOR RNA POLYMERASE, David W. Daniels and Kevin P. Bertrand, University of California, Irvine, CA 92717.

The tetracycline resistance determinant in transposon Tn*10* consists of two genes, the *tetA* resistance gene and the *tetR* repressor gene, that are transcribed from divergent overlapping promoters. There are two *tetR* promoters (P_{R1} and P_{R2}) with transcription startpoints 20 bp apart. The *tetA* promoter (P_A) overlaps both P_{R1} and P_{R2}, such that the P_A-P_{R1} startpoints are 17 bp apart and the P_A-P_{R2} startpoints are 37 bp apart. We isolated nine point mutations in P_A, all but one of which lie outside the -35 and -10 regions of P_{R1} and P_{R2}. To study *tet* transcription from supercoiled DNA templates, we constructed a plasmid in which the divergent *tet* promoters are flanked on both sides by the efficient *rrnB* t_1 transcription terminator. Transcription of this template by purified *E. coli* RNA polymerase yields short P_A, P_{R1} and P_{R2} RNAs. Comparison of supercoiled and linear templates indicates that supercoiling stimulates P_A, inhibits P_{R2}, and has little effect on P_{R1}. With a supercoiled P_A^+ template, P_A is substantially more active than either P_{R1} or P_{R2}. With supercoiled templates carrying P_A-down mutations, P_A activity is reduced as expected, however P_{R1} activity, and to a lesser extent P_{R2} activity, are substantially increased. These results suggest that P_A competes with both P_{R1} and P_{R2} for RNA polymerase.

0808 STRUCTURE AND EXPRESSION OF THE LENS FIBER CELL-SPECIFIC β35-CRYSTALLIN GENE OF THE CHICKEN EYE LENS. G. C. Das, M. A. Thompson, J. F. Hejtmancik and J. Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20205.

Development of the chicken lens is characterized by the sequential synthesis of three major classes of crystallins (α, β and δ). Each crystallin mRNA displays a characteristic temporal and spatial pattern in the developing lens. δ-Crystallin is the first crystallin to appear and accumulates during lens induction whereas the β-crystallin mRNAs accumulate rapidly near the end of the embryogenesis. The β-crystallins consist of several polypeptides. β35 (35K polypeptide) is particularly interesting because, unlike the other β-crystallin polypeptides, it appears only in the lens fiber cells. We have isolated the gene for β35 and are characterizing its promoter region by functional analysis *in vitro* and *in vivo*. The β35 cDNA and most of the gene have been sequenced. Each exon encodes a structural motif of the protein. A 600 bp fragment flanking the 5' region of the gene shows characteristic features of eukaryotic promoters, including a high GC-rich structure. This putative crystallin promoter is recognized *in vitro* in a Hela cell extract. S1 nuclease mapping of the *in vitro* synthesized RNA shows several transcriptional initiation sites, clustered in a narrow region. Detailed characterizations of the fiber-cell specific promoter *in vitro* and *in vivo* and its comparison with other crystallin promoters are underway.

0809 ANALYSIS OF β-CASEIN GENE EXPRESSION BY GENE TRANSFER, Yvonne David-Inouye, Craig C. Couch, William K. Jones, Li-yuan Yu-Lee, Jeffrey M. Rosen, Baylor College of Medicine, Houston, TX 77030

Caseins are milk proteins whose synthesis in the mammary gland is regulated by a complex interaction of peptide and steroid hormones. In particular, the level of β-casein gene expression in lactating mammary tissue is increased 260-fold over the level in the virgin gland. Lambda clones containing varying regions of the β-casein gene have been isolated from a rat DNA library. The gene is composed of 9 exons, 8 introns, and spans 7.2 kb of DNA. Nearly the entire β-casein gene has been sequenced. To identify those sequences important for the hormonal regulation of this gene by prolactin and glucocorticoids, the β-casein gene is being used in gene transfer studies. Two constructions have thus far been made. The entire β-casein gene with either 2.3 or 1.3 kb of 5' flanking DNA have been cloned into the BamHI site of the expression vector pBMTH1, constructed by G. Pavlakis. The vector contains the entire BPV genome, the mouse metallothionein gene, and a pBR322 segment, pML2. The recombinant expression vector has been transfected into mammary epithelial cells; the human T-47D breast cancer cell line, an epithelial mouse mammary cell line, COMMA-1D cells, and DMBA-induced rat mammary cells. These epithelial cells have been demonstrated to respond to prolactin and glucocorticoids. Transfection of T-47D cells with the β-casein-BMTH1 vector followed by selection with cadmium and zinc has resulted in 25 clones which are now being analyzed for rat β-casein gene expression.

0810 A CAT BOX BINDING PROTEIN FROM HeLa CELL EXTRACTS, Alex Elbrecht, Sophia Y. Tsai, Ming-Jer Tsai and Bert W. O'Malley, Baylor College of Medicine, Houston, TX 77030

Most eukaryotic genes require sequences upstream from the TATA box for proper and efficient transcription in *in vitro* and *in vivo* systems. One of these sequences is called the CAT box and is usually located between nucleotides -70 and -80 of the cap site. Although no function has been attributed to this conserved sequence it is clear that deletion results in a marked decrease in transcription for both the ovalbumin and globin genes. We have identified a protein component of HeLa cell extracts that binds to the CAT box region. The protein is present in a fraction termed P1000 obtained from a phosphocellulose column eluted with 1M KCl. Through reconstitution experiments and using a runoff transcription assay, we have shown that this fraction is required for correct initiation of transcription of the ovalbumin gene. To map the CAT box binding protein it was necessary to use various exonuclease footprinting methods - conventional DNase 1 footprinting not being sensitive enough. For the ovalbumin gene 5'-flanking region, the binding site spans 20-25 base pairs (depending on which DNA strand is used) from approximately nucleotides -68 to -90. A similar binding site is protected on the globin gene promoter. Through further fractionation and perhaps isolation of the binder we hope to determine the effect of this binding activity on transcription of the ovalbumin and globin genes.

0811 TRANSCRIPTIONAL REGULATION OF DIHYDROFOLATE REDUCTASE, Peggy J. Farnham and Robert T. Schimke, Stanford University, Stanford, Ca 94305

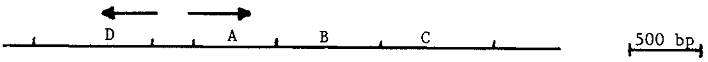
We have studied the transcription of the dihydrofolate reductase gene as cells progress through the cell cycle. We used the techniques of DNA-excess filter hybridization and *in vivo* pulse-labeling of a synchronous population of cells to measure the transcription rate of the mouse dihydrofolate gene in the different phases of the cell cycle. Our results indicate that the dihydrofolate reductase gene is cell cycle regulated at the transcriptional level. The transcription rate is low in G1, increases six fold at the beginning of S phase, decreases almost immediately thereafter and remains low throughout the remainder of S and into G2. This cell cycle regulation seen in the G1 to S transition is achieved by increasing the rate of transcription from a single promoter region. The major site of transcription initiation is in the most 3'-ward of three contiguous 48 base pair repeats. A secondary start site is located in the same relative position in the middle repeat. These repeats do not contain the usual CAAT or TATA boxes that many eukaryotic genes utilize, but instead contain the sequence CACAAATA, which may be a combination of the two signals, and the sequence GGGCGG, a hexanucleotide present in the SV40 early promoter region. The fact that the promoter of DHFR is similar to promoter regions of other housekeeping genes suggests that this type of regulation may not be unique to dihydrofolate reductase.

0812 QUANTITATIVE MEASUREMENTS OF PROMOTER STRENGTH, Nancy A. Federspiel and Vernon T. Oi, Becton Dickinson Monoclonal Center, Mountain View, CA 94043

We are in the process of constructing vectors containing various eukaryotic promoters driving the β-galactosidase gene. Following transfection of these vectors into mouse tissue culture cell lines, it is possible to measure the transient level of expression of the β-galactosidase gene by a colorimetric assay utilizing o-nitrophenyl- -D-galactoside. The relative strengths of various promoters, such as the SV40 early promoter, Class I and II promoters, the β_2 microglobulin promoter, and immunoglobulin light and heavy chain promoters can thus be compared in cell lines where the parental gene is normally active or inactive. In addition, the interactions of eukaryotic enhancer elements, such as from SV40 and the immunoglobulin genes, with homologous or nonhomologous promoters can be measured, again in cell lines which normally express or do not express these genes.

0813 EARLY NODULATION GENES OF *RHIZOBIUM MELILOTI*, Robert F. Fisher, Thomas Jacobs, John T. Mulligan, Thomas T. Egelhoff, Janice K. Tu, and Sharon R. Long, Stanford University, Stanford, CA 94305

The soil bacterium *Rhizobium meliloti* is able to establish symbiotic nitrogen-fixing root nodules with its host plant, alfalfa. Early events in the infection process include bacterial attachment to plant cells, characteristic deformation (marked curling) of host root hairs, and invasion of host cells by way of an infection thread. We have cloned the genes involved in the initiation of nodulation (*nod* genes); these are located on an 8.7-kb segment of the *R. meliloti* megaplasmid. Following saturation Tn*5* mutagenesis and complementation analysis we were able to identify four genes required for nodulation. The cloned *nod* genes from *R. meliloti* were able to restore nodulation ability to Nod$^-$ *R. trifolii* mutants on its host plant, clover. In addition, cloned *R. trifolii nod* genes restored Nod$^-$ *R. meliloti* to Nod$^+$ on alfalfa. These genes thus represent common nodulation functions and indicate an allelic relationship between the *nod* genes of *R. meliloti* and *R. trifolii*. We have determined the DNA sequence of the four *R. meliloti nod* genes, the organization of which are schematized below. The *nodD* and *nodA* genes, which are transcribed from divergent promoters, are separated by approximately 300 bp. We are currently investigating the nature of the regulatory signals which govern their expression.



0814 REGULATION OF HUMAN KERATIN GENES, Elaine Fuchs, Douglas Marchuk, Angela Tyner, Amlan Ray Chaudhury, Marjorie Lindhurst, Sean McCrohan, and Meg Eichman, University of Chicago, Chicago, IL. 60637

The keratins are a family of 20 different polyeptides (MW40-70kd) that comprise 8nm cytoskeletal filaments in virtually all epithelial cells. These proteins can be divided into two distinct classes on the basis of the ability of their mRNAs to hybridize with one or the other of two different cloned keratin cDNAs. Different pairs of type I and type II keratin mRNAs are coordinately expressed in different epithelia and at different stages of differentiation and development, indicating the importance of both types of subunits in filament assembly. cDNA sequence analyses have revealed that different pairs of keratins have unique sequences in localized domains and these differences influence the properties of the resulting filaments. Through cDNA sequencing, we have learned that the two types of keratins share only about 25% homology. We have now shown that despite this divergence in sequence, the predicted structures of these proteins and also the structures of their genes are remarkably similar. We are now investigating the regulatory regions of these genes to begin to elucidate the basis for their differential expression. In this context, we are particularly interested in determining whether vitamin A plays any direct role in regulating the expression of the keratin genes. We have already demonstrated that the vitamin specifically influences the levels of certain keratin mRNAs. It has been proposed that the vitamin and its cellular receptor act in a fashion similar to that of steroid hormones.

0815

PATTERN RECOGNITION IN *E. COLI* PROMOTERS: ARE THERE OTHER SIGNALS?, David J. Galas and Michael S. Waterman, University of Southern California, Los Angeles, CA 90089-1481

The basic nature of the sequence features that define a promoter sequence for *E. coli* RNA polymerase have been established by a variety of biochemical and genetic methods. We have developed rigorous, analytical methods for finding unknown patterns that occur imperfectly in a set of several sequences, and have used them to examine bacterial promoters. The algorithm easily discovers the "consensus" sequences for the -10 and -35 regions, which are identical to the results of previous analyses (Hawley and McClure, 1983), but requires no prior assumptions about the patterns. By explicitly specifying the nature of the search for "consensus" sequences we give a rigorous definition to this concept that is widely applicable. We also provide estimates for the statistical significance of common patterns discovered.

In addition to providing a rigorous basis for defining the known "consensus" regions we have found additional features in these promoters that may have functional significance. These added features were located on either side of the -35 region. Recent results relating DNA sequence to helix conformation suggest that the upstream pattern may have a function in the promoter. Possible roles are discussed in this light.

0816

REGULATION AND EXPRESSION OF THE ENTOMOCIDAL δ-ENDOTOXIN GENE OF THE GRAM POSITIVE BACTERIUM B. THURINGENSIS VAR KURSTAKI. M. Geiser, S. Schweizer, C. Grimm and M. Kuster. CIBA-GEIGY LDT. CH-4002 Basel/Switzerland

The gram positive bacterium B. thuringiensis, var. Kurstaki produces a protein toxin which is lethal to the larvae of lepidopteran insects. The δ-endotoxin is produced during sporulation and deposed in the cell as paraspora1 crystalline protein inclusion.

The gene for the toxin has been shown to be coded by a plasmid and/or by the chromosome. The plasmid-coded gene for the B.thuringiensis, var. Kurstaki toxin has been cloned in our laboratory and its DNA sequence determined. An open reading frame for a 138 000 daltons protein has been found.

We now have constructed E. coli lac z gene fusions with the δ-endotoxin promoter in an appropriate vector system and we hope to be able to transform a plasmid-cured B. thuringiensis strain as well as a wildtype-strain and we will answer questions concerning timing, usage of the two tandem promoters and hope to get insights in the *in vivo* regulation of the δ-endotoxin gene in B.thuringiensis.

0817

EFFECTS OF DIFFERENT PROMOTERS AND TERMINATORS ON THE EXPRESSION OF pBR322 β-LACTAMASE IN BOTH E.COLI AND B.SUBTILIS - G.Grandi, A.Mele, L.Colletti, S.Campagnoli, R.Nogarotto - ASSORENI, Via Fabiani, 1 - 20097 SAN DONATO MILANESE (MI) ITALY

One important condition to garantee high levels of expression of a cloned gene is to locate it under the control of efficient promoter sequences. It has been recently shown that in E.coli the maintenance of strong promoters on plasmid vectors is dependent on the presence, on the same plasmid, of efficient terminator regions downstream from the promoter. This condition seems to prevent the transcription process from interfering with the plasmid replication and consequently allows the maintenance of the plasmid in the cells. In order to investigate on the influence of different promoters and terminators on the β-lactamase expression in both *E.coli* and *B.subtilis* we have constructed a shuttle vector in which the pBR322-coded β-lactamase is inserted in such a way to present an EcoRI site immediately preceeding the ATG of the gene and a HindIII site immediately following its stop codon. In this way we have been able to easily modify both the promoter and the terminator strenght and to follow the effects of these modifications on the expression of the gene. A comparison of the behavior of *E.coli* and *B.subtilis* under these different conditions is reported.

0821 CLONING AND CHARACTERIZATION OF A NEW FAMILY RETROVIRUS-RELATED DNA SEQUENCES FROM THE MOUSE GENOME,

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New family of murine retrovirus-related sequences (MURRS) has been described. These 5.6 kb elements are flanked by 500 bp long terminal repeats identical to previously described LTR-IS elements (1). There are about 50 - 100 of 5.6 kb elements and about 500 - 1000 of solo LTR-IS elements per mouse haploid genome. It is shown that the RNA polymerase II regulatory signals within LTR-IS elements constitute an active promoter which requires either a cis-acting enhancer element or a trans-acting transcription factor present in 293 cells. Northern blot analysis provided evidence for MURRS specific RNA in mouse cells. Sequence analysis of the MURRS DNA revealed an open reading frame and show some homology with gag and pol proteins of other retroviruses.

1) Wirth, T., Glöggler, K., Baumruker, T., Schmidt, M. and Horak, I., Proc. Natl. Acad. Sci USA, 80, 1983, 3327.

0822 A MULTICOMPONENT YEAST MITOCHONDRIAL RNA POLYMERASE,

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We have partially purified an RNA polymerase activity from yeast which synthesizes a discrete transcript from the upstream region of the yeast *GAL10* gene. The transcript initiates within a sequence, ATATAAGTA, previously described as a consensus yeast mitochondrial promoter [Osinga, DeHaan, Christianson and Tabak (1982) *Nucleic Acids Res*,]10], 7993-8006]. Although this enzyme preparation is from whole yeast cells it is probable that the activity corresponds to the yeast mitochondrial RNA polymerase [Levens, Morimoto and Rabinowitz (1981) *J. Biol. Chem.*, *256*, 1466-1473]; the activity can be chromatographically separated from the three nuclear RNA polymerases, it is not sensitive to rifampicin or α-amanitin and it selectively transcribes templates containing known mitochondrial promoters. Two separable enzyme activities exist in this preparation. One is only active on non-selective templates such as poly[d(AT)]; the second is only active on promoter containing templates. The second, selective RNA polylmerase is multicomponent (it can be chromatographically separated into two inactive fractions which can be reconstituted into selective enzyme) and it has kinetic properties similar to those of prokaryotic RNA polymerases. The two enzymes are differentially regulated in response to carbon source and mutations in the mitochondrial genome.

0823 REGULATION OF LEGHEMOGLOBIN GENE EXPRESSION,

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Leghemoglobins (Lbs) are hemoproteins synthesized exclusively in the root nodules of plants during symbiosis with nitrogen fixing bacteria. Soybean nodules contain four major Lbs components called Lba, Lbc$_1$, Lbc$_2$, and Lbc$_3$. The Lbs are encoded by a family of genes arranged in two clusters. DNA sequence analysis revealed four active genes coding for Lba, Lbc$_1$, Lbc$_2$, Lbc$_3$ and demonstrated a primary structure with three intervening sequences. Consensus CT/AG dinucleotides were found at the borders of the intervening sequences. Putative regulatory sequences (CAP site, TATA box, CAT box, poly A site) similar to other eukaryotic gene sequences were also found. Transcription of the Lb genes was measured with specific probes These probes distinguish the mRNA precursors from the different Lb genes, and it was demonstrated that the Lbc$_3$ and Lbc$_2$ genes are transcribed before the Lbc$_1$ and Lba genes, during nodule development. The Lba gene was transcribed at the highest level followed by the Lbc$_3$, Lbc$_1$ and Lbc$_2$ with the lowest level. Induction followed exponential kinetics and all four genes remained transcriptionally active. An Agrobacterium based transformation system has been modified for legumes. Tissue specific transcription, transcript processing and the Lb protein synthesis can therefore be followed after transfer of complete soybean Lb genes to other legumes. Chimeric genes that allow easy detection of Lb gene activity has been constructed. Regulatory sequences required for Lb gene activity can therefore be defined by deletion and site directed mutagenesis.

0824 SIMULTANEOUS TRANSCRIPTION OF TWO EXPRESSION SITES FOR VARIANT SURFACE GLYCOPROTEIN GENES IN TRYPANOSOMES

Patricia J. Johnson, Albert W.C.A. Cornelissen, Jan M. Kooter, Titia De Lange, Andre Bernards and Piet Borst, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Trypanosomes are protozoan parasites which are entirely coated by a single variable surface glycoprotein (VSG) that is periodically switched to overcome the host's immune response. Coat protein exchange is mediated by transcriptional activation and inactivation of VSG genes. All transcriptionally active VSG genes reside within a telomeric expression site. In T. brucei, strain 427, at least 3 chromosome ends can act as expression sites. To investigate the coordination of multiple expression sites, we have studied the inactivation of VSG gene 221a. This gene and at least 8.5 kb of adjacent telomeric sequence are deleted in 5 out of 6 independent inactivation events (Bernards, et al., Cell 36,163-70, 1984). In the one population in which the 221a gene is retained, we find a 30 kb DNA insertion upstream of the inactivated gene as the only telomeric alteration. Whereas DNA upstream of the insertion, known to be part of the transcriptional unit of this expression site, is still transcribed, transcription stops in the insertion. The newly activated gene, VSG 060, does not reside within the insertion but in another telomere. These data provide the first observation of VSG gene inactivation by insertion and show that two VSG expression sites can be concomitantly active (the 221 and 060 telomeres). This supports the hypothesis that VSG switching may result in transient expression of more than one VSG gene by a single trypanosome(Bernards, et al., NAR 12,4153-70, 1984).

0825 INVESTIGATION OF THE EXPRESSION OF CHIMAERIC GENES INTRODUCED INTO PLANTS USING AGROBACTERIUM TUMEFACIENS.

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The objective of this work was to investigate which DNA sequences are necessary and sufficient for the expression in leaves of chimaeric genes in transformed, non-hormone-perturbed whole plants. A series of transcriptional and translational fusions were constructed in which a presumed promotor region of a chlorophyll a/b binding protein (cab) gene from the dihaploid Mitchell Petunia (see Dunsmuir et al, J. Mol. App. Gen., 2, 285-300, 1983) was placed adjacent to octopine synthase coding sequences with either a short (150bp) or long (700bp) stretch of octopine synthase 3' untranslated sequence. These chimaeric genes were introduced into tobacco and petunia using an Agrobacterium strain which conferred kanamycin resistance, and whole plants were regenerated from the transformed tissue.

Analysis of these plants permits the following conclusions. (1) Expression of the chimaeric gene can be detected in the leaves of most but not all transformants. (2) The level of expression in the highly expressing transformants is 10%-100% of that of the endogenous cab gene. (3) The 5' end of transcription of the chimaeric gene is the same as for the endogenous cab gene. More studies on the regulation of these chimaeric genes and on the influence of 5' and 3'sequences on mRNA levels will be presented.

0826 ENRICHMENT OF RNA POLYMERASE III TRANSCRIPTION FACTORS BY CHROMATOGRAPHY OF AVIDIN-BIOTINYLATED DNA COMPLEXES ON BIOTIN-CELLULOSE

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A general affinity chromatography procedure for the rapid enrichment of specific DNA-protein complexes has been developed and applied to the purification of factors required for the transcription of eucaryotic class III genes. Plasmid DNA containing the Adenovirus type 2 gene for VAI RNA was linearized at a site distal to the gene, end-labeled with a biotin-nucleotide analog of TTP, and incubated with avidin to form an avidin-biotinylated DNA complex. When HeLa cell S100 extracts were programmed with a titrated amount of the avidin-VAI RNA gene, stable transcription complexes were formed and shown to have the same transcriptional activity as extracts programmed with an avidin-free VAI RNA gene. Chromatography of the programmed extract over a biotin-cellulose affinity resin resulted in the selective, and virtually quantitative, retention of the VAI RNA gene transcription complex. After washing the resin to remove non-specifically bound proteins, a transcriptionally active fraction was eluted by the addition of 1.5 M KCl. Competition and complementation assays demonstrated that this affinity selected fraction contained factors essential for transcription of the VAI RNA gene and was enriched 400-fold relative to the original S100 extract.

0827 REGULATED EXPRESSION OF GLOBIN GENES IN K562 CELLS, Khashayarsha Khazaie, Dimitris Kioussis, Frank Wilson, Frank Grosveld, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA. U.K.

We have studied the expression of globin genes in the myeloid erythroleukemic cell line K562. Hybrid genes were constructed containing the human 5' half of the ε, γ and β-globin genes and the rabbit 3' half of β-globin gene to allow a distinction between the endogenous and the constructed genes. The human sequences have respectively 0.98, 2.2 and 1.5 kilobases of normal 5' flanking sequences, whilst the rabbit has 0.7 kilobases of normal 3' flanking sequences. These were introduced into human K562 cells by calcium phosphate precipitation, together with the AGPT gene which confers G418 resistance to the harboring cells. Clones of G418 resistant cells were grown and harvested for RNA and DNA preparation. Using suitable probes the expression of both the endogenous and exogenous genes, or of more than one exogenous globin gene, can be detected in the same S1 assay.

We have found a high level of expression of the transfected γ and ε genes, and have shown that neither the endogenous nor the exogenous β gene is expressed in K562 cells. At present we are using hybrid gene constructs in these cell lines to identify sequences inhibitory to the expression of the β globin in K562 cells.

0828 ANDROGEN REGULATED GENES FROM RAT SEMINAL VESICLE AND PROSTATE SHARE AN UPSTREAM HOMOLOGY, Steve Kistler, Jagan C. Kandala and Malathi K. Kistler, University of South Carolina, Columbia, SC 29203

Based on results from other steroid regulated systems, one anticipates that androgen regulated genes will share some nucleotide homologies that serve as binding sites for regulatory proteins, such as the androgen receptor. Recent sequence analysis of the rat SVS IV gene in the -145 to -500 region has revealed a 30 base pair segment with striking homology to a comparable unit located at -197 of the rat gene for prostate steroid binding protein component 3 (Hurst, H.C. and Parker, M.G. (1983) EMBO J. 2, 769-774). The SVS IV sequence, located at -303, is: ACACTTATGCTGTGGGAAGCGTGATCACAG. Nucleotides shared with the prostate C3 gene are underlined. Functional tests are in progress to determine whether this sequence is necessary for androgenic control of the SVS IV gene. The expression of the SVS IV and C3 genes is organ specific, and it has been shown that undermethylation of the C3 gene is found in the prostate but not in seminal vesicle or other nonexpressing organs (White, R. and Parker, M. (1983) J. Biol. Chem. 258, 8943-8948). A comparable study of the SVS IV gene shows that 7 methylation sensitive restriction sites are undermethylated in the seminal vesicle compared to liver and kidney. However, all sites that are undermethylated in the seminal vesicle are also undermethylated in the prostate. Accordingly, while there may be common features to androgen regulated gene expression in the seminal vesicle and prostate, there also appear to be differences.

Supported by NIH grant HD 13472

0829 TRYPANOSOMES: ONE mRNA,TWO TRANSCRIPTION UNITS,MULTIPLE RNA POLYMERASES. Jan M.Kooter,T.de Lange, Albert W.C.A.Cornelissen, Peter W.Laird and Piet Borst. The Netherlands Cancer Institute,Amsterdam,The Netherlands.

In trypanosomes many,if not all,mRNAs contain the same sequence of 35 nt at their 5'end. This sequence is not contiguously encoded with the remainder of the gene but encoded by a mini-exon embedded in a 1.35 kb repeat.The 200 copies per nucleus of these repeats are tandemly linked in clusters of up to 15 copies. We have obtained evidence that the 35-nt sequence and the remainder of the mRNA are encoded by two separate transcription units by analysis of steady-state RNA and analysis of nascent RNA,synthesized in isolated nuclei. First,transcription of the mini-exon repeats yields a 141-nt transcript with the 35-nt sequence at its 5'end. No run through transcription was detected showing that the 141-nt transcript is not a processing product. Secondly, transcription of mini-exon genes is sensitive to a moderate level of α-amanitin while transcription of protein coding genes is inhibited by a lower level suggesting that these genes are transcribed by two different RNA polymerases.We also found that the transcription of genes coding for trypanosome surface antigens is not inhibited by up to 1000μg α-amanitin per ml; a distinct RNA polymerase may therefore exist for the transcription of these genes.

De Lange et al.(1984),Nucl.Acids Res. 12,3777-3790 ; Kooter et al.(1984) EMBO J. 3, 2387-2392 ; Kooter and Borst , in preperation.

Sequence Specificity in Transcription and Control

0830 MUNG BEAN NUCLEASE RECOGNIZES PROMOTER AND TERMINATOR REGIONS IN SUPERCOILED PBR322 DNA,

David Kowalski and Lowell G. Sheflin, Roswell Park Mem. Inst., Buffalo, NY.

Transcription initiation from certain promoters is affected by DNA supercoiling by an unclear mechanism. The effects may be mediated by changes in DNA secondary structure induced by negative supercoiling. We used mung bean nuclease, a single-strand-specific endonuclease, to determine the location and nature of nucleotide sequences involved in recognizable DNA unwinding. A variety of reaction conditions were used (all at neutral pH) since temperature and ionic environment affect enzyme site specificity by altering the conformation of supercoiled DNA (NAR *12*, 7071, '84). Three mutually exclusive sets of sites were identified. At 37°C, sites map near the end of the ampicillin-resistance (Amp-R) gene transcript. An 80bp dA+dT-rich sequence is nicked at many positions but in a non-random manner, as observed with PM2 DNA (NAR *12*, 7087, '84). With addition of Mg^{2+}, sites map at the RNA primer promoter for DNA replication, at the promoters for tetracycline-resistance (Tet-R) and Amp-R genes, and at the terminator for RNA-I. Inverted repeat sequences are cleaved in the non-base-paired loops of potential hairpin structures. At 27°C, sites map at a promoter activated by cyclic-AMP receptor protein and near the presumed end of the Tet-R gene transcript. Examination of the known sequences around the 27°C sites shows average dA+dT-content and no strong hairpin potential. Our results demonstrate that DNA unwinding detectable by mung bean nuclease occurs at promoter and terminator regions in preference to protein-coding regions in supercoiled pBR322 DNA. Thus, one way supercoiling might affect promoter function is by altering local DNA secondary structure.

0831 MATURE mRNAs OF *TRYPANOSOMA BRUCEI* APPEAR TO ACQUIRE THEIR 5' CAP BY DISCONTINUOUS RNA SYNTHESIS,

Peter W. Laird, Jan Kooter, Titia de Lange and Piet Borst, Nederlands Kanker Instituut, Amsterdam, The Netherlands

Trypanosoma brucei displays a discontinuity in RNA synthesis,in which 35 nt from the 5' end of an RNA molecule of about 140 nt (mini-exon-derived precursor RNA or medRNA) end up at the 5' end of all mRNAs analyzed to date. Whether this discontinuous transcription results from reinitiation of transcription using the medRNA as a primer or from bimolecular splicing is unknown. Recent studies with isolated nuclei indicate that mini-exon genes,VSG genes and other protein-coding genes each differ in α-amanitin sensitivity of transcription,which suggests that different polymerases are responsible for their transcription. We have investigated whether mature mRNAs in *T.brucei* contain a 5' cap and if so,whether the cap is already present on the medRNA. Chemical decapping and enzymatic recapping with α-^{32}P-GTP of total and poly$(A)^{+}$ RNA followed by either dot blot hybridization or polyacrylamide gel electrophoresis,electroblotting and mini-exon hybridization showed that both mature mRNAs and medRNA are 5' capped. We conclude that the medRNA donates its 5' cap to the mRNA molecule. These experiments also confirmed the existence of a second minor medRNA of ~125 nt and revealed other prominent small capped RNAs which do not hybridize to the mini-exon.

Laird,P.W. *et al.*,in prep.;De Lange,T. *et al.*(1984),*NAR* *12*,3777;Kooter,J. *et al.*(1984),*EMBO J.* *3*,2387;Van der Ploeg,L.H.T. *et al.*(1984),*Cell* *39*,213;Kooter,J. and Borst,P.,in prep.;Campbell,D.A. *et al.*(1984),*Nature* *311*,350.

0832 ANALYSIS OF BACTERIOPHAGE N4 EARLY PROMOTERS,

C. Malone, J. Chase*, and L. B. Rothman-Denes. Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL. 60637 and *Department of Molecular Biology, Albert Einstein College of Medicine, Bronx, N. Y.

Bacteriophage N4 has unique requirements for its early transcription. The enzyme responsible for synthesis of early RNA is a N4 encoded virion encapsulated RNA polymerase. This enzyme has been purified to homogeneity and transcribes denatured N4 DNA as template with *in vivo* specificity. Comparison of the three early N4 promoter regions shows extensive homology between -18 and +1 with a conserved G-C rich heptamer centered at -10 and two sets of inverted repeats; one upstream and one containing the site of initiation of transcription. We are analyzing the importance of both the inverted repeats and the consensus sequence by oligonucleotide directed site specific mutagenesis to create mutant promoters. Preliminary evidence suggests that the upstream inverted repeat may be essential for transcription activity.

Virion DNA, which is linear and double-stranded, is not used for transcription *in vitro*. *In vivo* experiments suggest that early transcription utilizes a supercoiled template and is completely dependent upon the presence of *E. coli* single-stranded binding protein (ssb). We are testing the activity of our mutant promoters as supercoiled templates in a transcription assay with purified ssb.

Sequence Specificity in Transcription and Control

Promoters B

0833 SPECIFICITIES OF THE BACTERIOPHAGE T3 AND T7 RNA POLYMERASES, W.T. McAllister, N.J. Horn, J.F. Klement and Claire E. Morris, UMDNJ-Rutgers Medical School, Piscataway, New Jersey 08854

The RNA polymerases encoded by bacteriophages T3, T7, and SP6 although similar in structure, exhibit nearly exclusive template specificities. Nucleotide sequences of ten promoters recognized by the T3 RNA polymerase have now been determined. Like the T7 promoters, the T3 promoters consist of a highly conserved 22 base pair sequence. Significant differences between the two kinds of promoters are localized in a three base pair region from -10 to -12. By use of synthetic promoter sequences, the relative importance of each of these three positions on promoter specificity is being explored.

The nucleotide sequence of the gene encoding the T7 RNA polymerase (and hence the amino acid sequence of the enzyme) has been determined in other laboratories. We have determined the nucleotide sequence of the T3 RNA polymerase gene. The predicted amino acid sequence of the T3 RNA polymerase exhibits very few changes when compared to the T7 enzyme (82% of the residues are identical). Significant differences appear to cluster in three distinct regions in the amino-terminal half of the protein. Analysis of the data from both enzymes suggests features that may be important for polymerase function. In particular, a region that differs between the T3 and T7 enzyme exhibits significant homology to the α_2-α_3 bihelical domain that is common to many sequence specific DNA-binding proteins. Experiments to test the importance of this region in promoter recognition are in progress.

0834 LATE TRANSCRIPTIONAL CONTROL OF THE B. SUBTILIS BACTERIOPHAGE Ø29, Rafael P. Mellado, José L. Carrascosa, Isabel Barthelemy, José M. Lázaro, José M. Sogo and Margarita Salas, Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma. Canto Blanco, 28049 Madrid. Spain.

The Ø29 DNA region coding for the viral protein p4 which controls the phage late transcription, has been cloned under the control of bacteriophage λ P_L promoter in a pBR322 derivative plasmid. Upon induction of the E. coli cell cultures harbouring the recombinant plasmid, the protein p4 produced accounted for 30% of the total de novo synthesized cellular protein.

B. subtilis RNA polymerase (either core or holoenzyme) is unable to transcribe in vitro the late region of Ø29 DNA. Protein p4 has been assayed on its capacity to address the B. subtilis RNA polymerase (either core or holoenzyme) to transcribe the Ø29 late genes in vitro. Analysis of the transcriptional products obtained using either crude E. coli cell extracts containing p4 or partially purified p4 showed that the viral DNA late region was indeed transcribed. Visualization under the electron microscope of the R-loops made in vitro in the presence of protein p4 has allowed to identify three new Ø29 promoters. An early one localized at 85% of the genome and two late ones placed at 53% and 62% of the viral DNA, respectively. The results obtained so far indicate that protein p4 functions as a σ-like factor. Experiments to identify Ø29 late promoters in vivo will be also presented.

0835 THE ROLE OF THE ntrA GENE PRODUCT IN POSITIVE CONTROL OF NITROGEN ASSIMILATION GENES IN KLEBSIELLA PNEUMONIAE, M.J. Merrick, M. Buck, R.A. Dixon, M. Drummond and W.D.P. Stewart, AFRC Unit of Nitrogen Fixation, Brighton, BN1 9RQ, U.K.

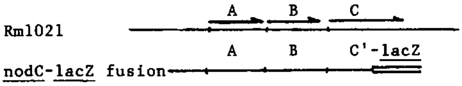
In K. pneumoniae and other enteric bacteria expression of many genes required for nitrogen assimilation is coordinately controlled by the nitrogen regulation (ntr) gene products. The ntr system can act both positively and negatively and in the case of positively controlled operons, which include the nitrogen fixation (nif) regulon, the products of the ntrA gene together with either the ntrC or the nifA product are required for transcriptional activation. The promoters of genes subject to positive ntr control are atypical and have a concensus sequence CTGGCAC N_5 TTGCA between positions -26 and -10 instead of the normal -35, -10 concensus. The absolute requirement for ntrA for transcription initiation suggests that ntrA could encode an alternative RNA polymerase sigma factor. The ntrA gene from K. pneumoniae has been cloned and Southern blotting shows no homology to rpoD (sigma gene). The gene product is a 75 kdal acidic polypeptide. NtrA::lacZ fusions show that the gene is not transcriptionally regulated in response to the N-status of the cell. Experiments with multicopy plasmids carrying ntrA or rpoD indicate competition between the two gene products consistent with an interaction between ntrA product and RNA polymerase.

0836 TRANSCRIPTIONAL REGULATORY ELEMENTS OF THE CHICKEN LYSOZYME PROMOTER, Richard J. Miksicek and Günther Schütz, German Cancer Research Center, Heidelberg, F.R.G.

Previous experiments with a lysozyme/SV40 T antigen fusion gene have shown that the chicken lysozyme promoter is expressed efficiently only in primary chicken oviduct cells and that sequences responsible for progesterone and glucocorticoid stimulated expression of this gene reside within the first 208 bp upstream of the major *in vivo* transcription start site. In order to more closely delineate the cis-acting *transcriptional* regulatory elements responsible for these effects, selected restriction fragments from the lysozyme promoter region were analyzed for their ability to confer steroid inducibility on a chimeric indicator gene consisting of the HSV thymidine kinase promoter fused to the coding region of the bacterial chloramphenicol acetyl transferase gene (pTK·CAT). In agreement with *in vitro* DNA binding experiments using purified receptor preparations, sequences between *-208 and -162* and between -94 and -47 upstream of the lysozyme cap site render the TK promoter inducible by progesterone and dexamethasone, respectively, when analyzed following transient expression in chick oviduct cells. Promoter hybrids containing both receptor binding sites (-208 to -47) fail to show regulation in non-oviduct cells, but in some cases evidence a strong constitutive enhancer activity which is both distance and orientation independent. This enhancer activity has been observed in three mammary cell lines (MCF7, NMuMG, and T47D) and a feline kidney line, but is not observed in HeLa cells or in non-epithelial cell lines (Ltk$^-$ and XC cells). The significance of these observations with respect to the narrow host specificity of the lysozyme promoter is currently under investigation.

0837 REGULATION OF NODULATION GENES IN *RHIZOBIUM MELILOTI*, John T. Mulligan and Sharon R. Long, Stanford University, Stanford, CA 94305

The bacterium *Rhizobium meliloti* invades alfalfa root hairs and induces the plant to form symbiotic nodules in which nitrogen fixation occurs. The genes in the *nodABC* region of the *R meliloti* pSym megaplasmid are required for this invasion. These genes are closely linked, translated in the same direction and may be coordinately regulated. I have constructed a derivative of a broad host range plasmid containing these genes in which a *nodC*-*lacZ* gene fusion has been substituted for the *nodC* gene. Beta-galactosidase activity in this fusion is induced in the presence of a plant product. This product has a low molecular weight and is produced by both host and non-host plants. We are using RNA transcript mapping to determine the start site for transcription and genetic analysis to characterize the regulation of this locus.



0838 SEQUENCES REQUIRED FOR CIS AND TRANS ACTIVATION OF ADENOVIRUS IVa$_2$ GENE TRANSCRIPTION. Venkatachala Natarajan and Norman P. Salzman, NIH, Bethesda, MD 20205

The transcriptional control region of the adenovirus IVa$_2$ promoter was analyzed by cloning this promoter in front of a gene coding for chloramphenicol acetyl transferase (CATase) and estimating CATase and IVa$_2$ promoter specific RNA synthesized after transfection. Detectable amounts of IVa$_2$ specific RNA was synthesized only when an enhancer (SV40 72 base pair repeats) was present in *cis* or when the adenovirus EIA gene was present in *trans*. Estimation of IVa$_2$ specific RNA synthesis using various deletion mutants having an enhancer in *cis* shows that sequences between -38 and -64 of the RNA initiation site are necessary for efficient transcription. In contrast, sequences present between -242 to -179 are necessary for *trans* activation of this promoter by adenovirus E1A gene. E1A gene in *trans* and an enhancer in *cis* have an additive effect on RNA synthesis. Measurements of CATase activity show that in order to produce measureable amounts of CATase with the IVa$_2$ promoter, an enhancer has to be present in *cis*. CATase activity was not reduced significantly even after deletion of all sequences upstream of the RNA initiation site. The discrepancy observed in CATase and RNA can be explained based on the activation of transcription from cryptic promoters present in the plasmid.

0839 *Transactivation of γ_2 promoter-linked genes in viral genome, transformed lines and transient assay.* P.M. Nazos, S.Silver and B.Roizman. The Kovler Viral Oncology Laboratories, The University of Chicago.

The HSV-1 genes form three major groups, designated as α, β and γ, whose synthesis is coordinately regulated and sequentially ordered in a cascade fashion. During productive infection, the α genes are expressed first and the products of $\alpha 4$ gene are required for the expression of β genes. The β polypeptides turn off the synthesis of α genes and enable the expression of γ genes. The γ_1 gene expression is semi-dependent on DNA replication whereas the expression of γ_2 genes requires sustained viral DNA replication. To determine how infected cells differentiate between β and γ promoters, a γ_2 promoter was fused with the structural sequences of thymidine kinase (TK) gene. In the viral genome, both β-TK and γ_2-TK chimeras require $\alpha 4$ gene products, but γ_2-TK required in addition viral DNA synthesis. In transformed cells β - and γ_2-TK chimeras required for induction functional $\alpha 4$ genes, but were not induced by $\alpha 0$ gene products. In transient expression systems, the chimeric γ_2TK gene and βTK gene were induced by co-transfection with DNA fragments carrying the $\alpha 4$ and $\alpha 0$ genes, but not the DNA fragments carrying α genes 22 or 27. These results demonstrate that in transient assay systems both γ_2- and β- gene expression require only $\alpha 4$ or $\alpha 0$ gene products. The requirements for induction of β- and γ_2 genes in transient assay system differ therefore from those observed in trans-activation of resident genes in transformed cells and those observed in productively infected cells.

0840 MUTATIONS THAT AFFECT *nifA*- AND *ntrC*-MEDIATED ACTIVATION OF THE *KLEBSIELLA PNEUMONIAE* NITROGENASE PROMOTER. David Ow *, Yue Xiong, Wing Gu, San-chiun Shen. Academia Sinica Institute of Plant Physiology, Shanghai, China. * Present address: Dept. of Biology C-016, UC San Diego, La Jolla, CA.

Nitrogen regulated (*ntr*) and nitrogen fixation (*nif*) gene promoters are structurally similar to each other, but bear little resemblance to canonic *E. coli* promoters. *ntr* promoters are normally activated by the *ntrC* (also known as *glnG*) product, but can also be activated by the *ntrC*-related *K. pneumoniae nifA* product. In contrast, *nif* promoters of *K. pneumoniae* such as the nitrogenase (*nifH*) promoter can only be *nifA*-activated. Sequence comparisons have shown that *nifA*-regulated promoters share the consensus sequence CTGG-6bp-TTGCA between -26 and -10, whereas the consensus sequence TTTTGCA was found centered at -14 among several *ntr*-activated promoters. We analyzed 2 classes of mutants isolated after sodium bisulfite site-directed mutagenesis of the *K. pneumoniae nifH* promoter, which contains the sequence CTGG-4bp-CCCTGCA. Class A mutants failed to respond to *nifA*-mediated activation while class B mutants acquired the ability to be activated by the *ntrC* product. With class A mutants, we found that a transition at any of 4 different bases of the consensus sequence reduced *nifA*-mediated regulation (underlined where transitions were found: CT*GG*-4bp-CT*GC*A). With class B mutants, a change from CCCTGCA to either TCCTGCA, CCTTGCA, or TCTTGCA was sufficient to confer *ntrC*-mediated transcription.

0841 REGULATION OF $^{I}\alpha$ AND $^{II}\alpha$ GLOBIN GENES OF THE GOAT. Jacqueline W. Pierce, Anil G. Menon and Jerry B Lingrel, Department of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Sequences involved in regulated expression of α globin genes have not been defined. We are studying differential expression of two functional α globin genes of the goat; $^{I}\alpha$ and $^{II}\alpha$. These genes are highly homologous (99%) throughout their coding regions, IVS and immediate 5' and 3' flanking regions extending 122 bp 5' to the initiation codon and 139 bp 3' to the stop codon (Schon *et al.*, 1982). Both $^{I}\alpha$ and $^{II}\alpha$ contain all signals known to be important for the transcription including CCAAT, ATA box, cap site and polyA addition site; however, $^{I}\alpha$ protein is about 3-fold more abundant than $^{II}\alpha$ in both fetal and adult goats. Sequences upstream of CCAAT or downstream of polyA may dictate different levels of expression. Notably, the repeat CACCCTACACCCT which is important in the transcription of adult β globin genes is found upstream of $^{I}\alpha$ but not $^{II}\alpha$. In order to delineate sequences which are involved in differential expression of $^{I}\alpha$ over $^{II}\alpha$, we have assayed the transient expression of these genes in HeLa cells. The $^{I}\alpha$ and $^{II}\alpha$ genes were transfected separately into HeLa cells and α mRNA levels were measured by S1 nuclease assay. As observed for human α genes, both goat α genes are expressed in the absence of cis enhancer sequences. We found that $^{I}\alpha$ is expressed at a higher level than $^{II}\alpha$. These results suggest that linkage of $^{I}\alpha$ and $^{II}\alpha$ is not required to achieve differential expression of these genes. In addition, sequences dictating the different levels of expression are located within 500 bp 5' and 200 bp 3' of the α structural genes. We are currently examining the expression of a fusion gene $^{I}\alpha/^{II}\alpha$ in order to further localize sequences which determine the level of α globin gene expression.

Sequence Specificity in Transcription and Control

0842 DNAase PROTECTION OF ADENOVIRUS TYPE 2 PROMOTER SEQUENCES IN HeLa CELL TRANSCRIPTION EXTRACTS, Leonora Poljak[1], Roberto Weinmann[2], and Jay Gralla[1], [1]University of California,Los Angeles, CA 90024; [2]The Wistar Institute of Anatomy and Biology, Philadelphia, PA, 19104.

We have applied the technique of DNAase footprinting to identify sequences of adenovirus type 2 (Ad2) promoter DNA which are protected by factors in a whole-cell transcription extract. Using a soluble HeLa cell extract and a restriction fragment containing the promoters for both the major late transcription unit and the IVa_2 gene, three regions of protection were observed. Two of the protected regions are located directly upstream of the Ad2 major late promoter. The first extends from approximately -25 to +1 and the second from -48 to -67 nucleotides relative to the mRNA start site. These protected regions correspond to sequences which affect the efficiency of transcription of the major late promoter *in vitro*. A third region of protection is observed from approximately -46 to -90 nucleotides upstream of the IVa_2 mRNA start site.

0843 MODULATION OF THE EXPRESSION OF A BACTERIAL GENE BY INSERTIONS OF IS1 AT OR NEAR ITS PROMOTER, Pierre Prentki*, Bruce D. Teter*, David J. Galas*, and Michael Chandler+, University of Southern California, Los Angeles, CA 90089-1481*, and CNRS, Toulouse, France+.

Transposable elements are known to be able to alter the expression of genes adjacent to their insertion points. For IS1, however, little is known other than that it is often polar. We have isolated a large collection of insertions of IS1 into pBR322. Some of these are located near or within the promoter for the ampicillin resistance (*bla*) gene. In none of the inserts, however, was resistance to ampicillin abolished. The Ω transcription/translation termination module (Prentki and Krisch, Gene 29, 303-313, 1984) was inserted in several locations in these pBR322::IS1 plasmids to localize the origins of transcription, and *in vitro* analysis was carried out on some. The results show that IS1 can influence the expression of the *bla* gene in at least three ways. i) Hybrid promoters: novel promoters are created in which the IS1 provides the -35 region for the original -10. Both ends of IS1 may contribute to this effect. ii) Internal promoters: readthrough transcription originating inside the element drives the *bla* gene. iii) Finally, the determinants of polarity of IS1 have been localized. Our studies have suggested the possibility that the control of transposition and the modulation of adjacent genes may be related.

0844 RNA POLYMERASE II TRANSCRIPTION SYSTEM. DISECTING THE INITIATION REACTION AT $TATA^+$ PROMOTER AND AN EXAMPLE OF TRANSCRIPTION TERMINATION, Danny Reinberg and Robert G. Roeder, The Rockefeller University, New York, NY 10021.

We have developed an *in vitro* assay that allowed us to quantitate each of the factors involved in the initiation of transcription at $TATA^+$ promoter sequences. We have isolated to or near homogeneity three of the four factors required in this reation. We have also extended our studies to look for auxiliary proteins that may stimulate RNA polymerase II activity at specific promoters. The mechanism of action of these proteins will be presented. In addition, we are studing the mechanism of transcription termination. We have constructed a DNA molecule containing a putative "terminator" sequence downstream from the adenovirus major late promoter. By using this template we have been able to show that the adenovirus DNA encodes a termination sequence. This sequence is a very strong pausing site for RNA polymerase II and the process of termination is dependent upon the addition of a protein fraction.

0845 ELEMENTS OF TRANSCRIPTIONAL CONTROL AT THE *E. coli gal* AND *lac* OPERONS,

A. Revzin, S.H. Shanblatt and D.D. Lorimer, Michigan State University, East Lansing, MI 48824

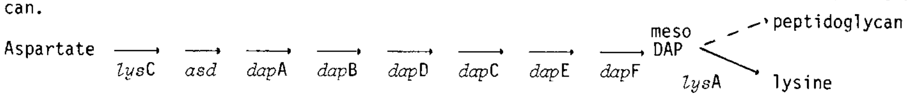
The gal operon contains overlapping promoter regions (Musso *et al.*, Cell *12*, 847 (1977)). The P2 promoter is used in the absence of the catabolite activator protein (CAP); if the CAP/cAMP complex is active then RNA polymerase (RP) initiates at P1, five base pairs downstream from P2. We have shown that transcription from P1 involves the adjacent binding of two CAP molecules (Shanblatt and Revzin, PNAS *80*, 1594 (1983)). Our current work is aimed at elucidating the function of the second CAP. To this end we do *in vitro* experiments with purified proteins and DNA fragments. We study DNA-protein binding by means of gel electrophoresis as well as doing a variety of nuclease protection and transcription assays. Studies using both wild-type and mutant promoters, and with truncated DNA fragments, show that the second CAP molecule has dual functions: it stabilizes the "one CAP/one RP" intermediate at P1, thus excluding P2 binding; in addition, it is involved in interactions which aid RP in "melting in" at P1. The entire process is exquisitely dependent on the cAMP level, at µM concentrations likely to be encountered *in vivo*.

The *lac* control region also has overlapping promoters. Our data reveal a marked competition between the P1 and P2 sites for available RP molecules. We find that although a single CAP/cAMP entity binds at the *lac* promoter, the role of CAP is similar to that at *gal*. It can stimulate transcription both by excluding RP from P2 (so that more initiation complexes can form at P1) and also by enhancing the ability of RP to form stable complexes at P1.

0846 MOLECULAR STUDY OF THE E. COLI DIAMINOPIMELATE-LYSINE GENES.

C. Richaud, J. Bouvier, and P. Stragier, Institut de Microbiologie, Université Paris-Sud, 91405 - Orsay Cedex (France).

Nine genes, scattered on the *E. coli* chromosome, are involved in the biosynthesis of lysine and its direct precursor, the diaminopimelate (DAP), component of the cell-wall peptidoglycan.



All these genes are currently studied in our laboratory by cloning, DNA sequencing and promoter mapping. Most of them are submitted to regulation by the internal lysine pool. Our ultimate goal is to elucidate the molecular mechanism of their expression by analyzing their regulatory sequences. No common element was so far identified to account for the repressive effect of lysine ; furthermore no attenuation-like structure can be found.
Several arguments lead us to propose an activatory mechanism of expression for some of these genes, lysine excess antagonizing this positive effect.

0847 IDENTIFICATION OF A STREPTOCOCCAL M PROTEIN PROMOTER SEQUENCE,

John Robbins, Jonathan Spanier and Patrick Cleary, University of Minnesota, Minneapolis, MN 55455.

S. pyogenes, varies in its expression of the antiphagocytic M protein and other potential virulence factors. The aim of our study is to characterize the molecular switch responsible for the "on-off" states of M protein synthesis. Previous studies have shown prophage activation of M protein synthesis and others have identified similar 50 bp deletions upstream from the structural gene. A 1700 bp segment of DNA able to express M protein synthesis in *E. coli* has been used in hybridization experiments. The "off" state although not absolute, clearly reflects transcriptional control. This segment of DNA has been sequenced and an 80 bp fragment, 5' to the putative structural gene, has been inserted into the promoter detection plasmid pCM1 where it activates chloramphenicol resistance. Preliminary mapping studies suggest that this fragment overlaps sequences known to contain deletions in some M^- cells.

0848 CONSENSUS SEQUENCES AT THE 5' END OF LATE VACCINIA VIRUS GENES.

Johannes L. Rosel, Jerry P. Weir and B. Moss, NIH, Bethesda, MD 20205

Vaccinia virus gene expression is temporally regulated. Early genes are expressed before DNA replication while late genes are expressed during and after that event. In order to identify possible cis-regulatory signals, we have mapped three late genes and compared the nucleotide sequences to those of previously mapped early genes. Regions of the vaccinia virus genome that encode late polypeptides were located by hybridization of mRNA to cloned DNA restriction fragments followed by cell-free translation of the selected mRNA. Specific antiserum was used to identify the products of two of the late genes as major virus core polypeptides. The RNA start sites of each gene were located by nuclease S1 protection and polyacrylamide gel electrophoresis next to a sequence ladder. A comparison of the sequences at and preceding the RNA start sites of early and late genes revealed differences that may provide the basis for cis-regulation. Computer analysis revealed two late gene consensus sequences: one was a perfectly conserved hexanucleotide TAAATG located very close to the RNA start site and the other was a TGTAAC located approximately 50 base-pairs upstream. The former includes both stop and start codons and is followed by a long open reading frame.

0849 ANALYSIS OF THE HUMAN INTERFERON α1 PROMOTER, John Ryals, K.-Dietmar Kuhl, Peter Dierks and Charles Weissmann, Institut für Molekularbiologie I, Universität Zürich, Hönggerberg, 8093 Zürich, Switzerland.

We previously showed that induction of interferon is due to activation of transcription and that a segment of 117 nucleotides preceding the cap site suffices for inducibility of the IFN-α1 gene. To identify the minimal sequence sufficient for induction, we constructed a set of hybrid promoters in which 3' truncated IFN promoters and 5' truncated β-globin promoters were combined in various fashions and joined to the β-globin transcription unit. Mouse LMTK⁻ cells were permanently transformed with the modified genes by TK-linked transformation and correctly initiated β-globin transcripts from induced and uninduced cells were determined by quantitative S1 mapping. The results show that an IFN promoter segment extending from position -675 to -64 (relative to the IFN cap site) is sufficient to mediate viral induction of transcription when placed 56, 78 and even 109 nucleotides upstream of the β-globin cap site. A 5' deletion analysis of the hybrid promoter with a junction at -64 of the IFN promoter and -56 of the β-globin promoter identified, as the minimal sequences required for induction, the following 46 nucleotides located between -109 and -64:

$$\text{GAGTGCAT}\overset{1}{\underline{\text{GAAGGAAAG}}}\text{CAAAAA}\overset{2}{\underline{\text{CAGAA}}}\text{A}\overset{1}{\underline{\text{TGGAAAG}}}\text{TGGCC}\overset{2}{\underline{\text{CAGAA}}}$$

The first 8bp direct repeat $\text{A}\substack{\text{T}\\\text{A}}\text{GGAAAG}$, shows a strong homology to the core enhancer sequence $\text{GTGG}\substack{\text{AAA}\\\text{TTT}}\text{G}$ present in the polyoma, SV40, MSV and Ig enhancers. Experiments are now underway to determine if this sequence can confer inducibility to the β-globin promoter in an orientation and distance independent manner.

0850 ISOLATION OF STABLE RNA POLYMERASE II PREINITIATION, INITIATION AND ELONGATION COMPLEXES. IDENTIFICATION OF 60,000 AND 80,000 DALTON POLYPEPTIDE COMPONENTS,

Brian Safer, Linda Yang, H. Eser Tolunay, and W. French Anderson, National Heart, Lung, and Blood Institute, NIH, Bethesda, MD 20205

Distinct RNA polymerase II transcription preinitiation, initiation and elongation complexes can be formed in HeLa whole cell extracts on cloned Ad2 sequences containing the major late promoter. These transcription complexes are stable and can be rapidly isolated by gel filtration. In the absence of exogenous nucleotides, a limiting transcriptional component and 60 and 80 kD polypeptides are sequestered into a stable, but transcriptionally incomplete, preinitiation complex. In the presence of dATP, dissociation of the 80 kD polypeptide occurs and RNA polymerase II is added. This forms a stable initiation complex capable of forming, but prior to the formation of, the first phosphodiester bond. When this complex is isolated and incubated in the presence of all four nucleoside triphosphates, the correct runoff transcript is generated. The abundant 60,000 dalton polypeptide associated with the transcription preinitiation and initiation complexes remains stabily associated with the DNA template during elongation. In contrast, the dATP-independent binding of the limiting transcription component sequestered in the pre-initiation complex is reversed upon elongation. This factor can reassociate with new DNA templates during subsequent rounds of initiation. Class II genes, therefore, do not appear to form activated transcription units stable for multiple rounds of transcription; rather, their transcriptional activity may be controlled by regulating each initiation event.

0851 CONTROL OF SHORT CHAIN FATTY ACID DEGRADATION IN *E. COLI*. Lauren Sallus and W.D. Nunn, U.C. Irvine, Irvine CA 92717

The β-keto short chain fatty acid (SCFA), acetoacetate, can be utilized by wild type *Escherichia coli* as a sole carbon and energy source. The structural and regulatory genes responsible for the degradation of SCFAs are encoded by the *ato* system. Three structural genes and a regulatory gene have been identified on a 6.4 kb fragment cloned into pBR322, resulting in the recombinant plasmid pATO. pATO contains *atoA* and *atoA'* which encode the and subunits of acetyacetyl CoA transferase, *atoB* which encodes thiolase II, and *atoC* which encodes a positive controlling element that regulates the activity of the *ato* structural genes. These four genes have been mapped and are closely linked within the 47 min region of the *E. coli* chromosome and may comprise an operon.

We have identified the proteins encoded by pATO via the maxicell procedure and as expected (Frerman *et al.* Archives Biochem and Biophy 171:14) have found that *atoA* (the α subunit) codes for a 28 kd protein, *atoA'* (the β subunit) codes for a 26 kd protein, and *atoB* codes for a 42 kd protein. We have also identified the *atoC* gene product as a 48 kd protein. Preliminary evidence has shown that the regulatory properties of the *ato* system deviate from other well characterized positive regulatory systems (i.e. the *ara*, *dsdC*, *mal* systems).

0852 UPSTREAM REGION MEDIATES GLUCOSE-REGULATED EXPRESSION OF THE *SUC2* GENE OF YEAST, Laura Sarokin and Marian Carlson, Columbia University, New York, N.Y. 10032

The *SUC2* gene produces two mRNAs with different 5' ends. The 1.9-kb mRNA encodes a secreted form of invertase and is regulated by glucose repression. The 1.8-kb mRNA encodes the intracellular invertase and is produced constitutively at low levels. To identify 5' regulatory sequences, we constructed *in vitro* a series of deletions and inserted them into the yeast genome. Analysis of the effects of each deletion on *SUC2* expression identified an upstream region required for derepression of secreted invertase synthesis. The 3' boundary of this region is near -418. The 5' boundary is not sharply defined, but lies ~100 bp upstream. No essential sequences lie between this region and the TATA box at -133. Sequences between -1900 and -86 are dispensable for the expression of the 1.8-kb mRNA.

To prove that the upstream region mediates regulation by glucose repression, the sequence from -390 to -900 was placed upstream from the promoter of a *LEU2-lacZ* fusion. Expression of β-galactosidase became glucose regulated and was appropriately affected by unlinked mutations that alter the regulation of *SUC2* gene expression.

We then selected mutations suppressing the defect in invertase expression caused by partial deletion of the upstream region. Both dominant, *cis*-acting mutations at *SUC2* and recessive, unlinked mutations were recovered. These mutations may provide insight into the protein-DNA interactions involved in regulation of *SUC2* gene expression.

0853 TRANSCRIPTION FACTOR SP1 BINDS TO THE PROMOTER OF THE MOUSE DIHYDROFOLATE REDUCTASE GENE AND STIMULATES TRANSCRIPTION IN VITRO. Shelley Sazer*, William S. Dynan°, Robert Tjian°, and Robert T. Schimke*, *Stanford University, Stanford CA 94305 and °University of California, Berkeley, CA 94720.

The enzyme dihydrofolate reductase (DHFR) is necessary for the production of tetrahydrofolate, an essential cofactor in the biosynthesis of thymidylate, purines and glycine. We have demonstrated that a factor present in HeLa whole cell extract binds to the 5' flanking region of the DHFR gene and stimulates transcription in vitro. SP1 has previously been shown to recognize and interact specifically with the sequence motif CCGCCC located in the 21 base pair repeats of the SV40 early promoter and to activate transcription. We have now shown by DNase footprint analysis that four regions, each approximately 20 nucleotides long, located near the start of DHFR transcription, are also protected from DNase digestion in the presence of SP1. The CCGCCC hexanucleotide lies within each of the protected regions of the DHFR promoter, on the non-coding strand. In addition, there are 6 copies of this hexanucleotide, three of which bind SP1, in a region approximately 700 nucleotides 5' of the start of translation which has previously been shown to act as a promoter in transfection assays. Competition footprint analysis has demonstrated that the same transcription factor, SP1, binds to these two DHFR promoters and to the 21 base pair repeats of SV40. Preliminary evidence from in vitro transcription assays in which each of these DHFR promoters was inserted 5' of the herpes thymidine kinase gene indicates that transcription from these promoters is activated in the presence of SP1.

Sequence Specificity in Transcription and Control

0854 TRANSCRIPTION OF THE E. COLI M1 RNA GENE, Francis J. Schmidt and Younghoon Lee, C. U. Park and Stephen J. Johnson, University of Missouri-Columbia, Columbia, MO 65212

The leader sequences of the E. coli M1 RNA gene includes three putative promoter homologies. P-1, P-2, and P-3. The P-1 sequence if nearest to the 5'-end of mature M1 RNA and most active in vitro. The promoter sequences were cloned singly and combination into the Galk expression vector, pKO-100. Only the P-1 promoter directed the synthesis of galactokinase while the P-2 and the P-3 promoters gave the basal levels of activity in this assay. DNAase I footprinting experiments showed that sequences at P-1 were protected by RNA polymerse to a greater extent than those at the P-2, while the P-3 was not observed to be specifically protected by the enzyme. Bal 3I nuclease digestion experiments were used to examine whether upstream sequences affected the level of galactokinase made from the M1 RNA promoter(s). The presence of the P-2 promoter caused a decrease of galactokinase synthesis from the P-1 promoter. We show that there exist one or more sites in the M1 RNA structural sequence between nucleotides +22 and +168 which reduce synthesis of galactokinase. This result implies that these sites can control gene expression of M1 RNA, perhaps by transcription termination.

0855 IDENTIFICATION OF THE PROMOTER FOR infC, THE GENE FOR E. COLI TRANSLATION INITIATION FACTOR 3, I. Schwartz, A. Pramanik, J. Schwartz, Dept. Biochem., N.Y. Med. Coll., Valhalla, NY

infC, the gene which codes for translation initiation factor 3 (IF3) is clustered with several other genes for translational components in the genome of E. coli. Of particular interest is the close physical relationship between infC and the upstream gene thrS, which codes for threonyl-tRNA synthetase. Sequencing of this region has revealed that only three nucleotides separate the termination codon of thrS from the AUU initiation codon of infC [Sacerdot et al (1982) EMBO J., 1,311]. This implies that infC is either co-transcribed with thrS from a thrS promoter sequence or that the transcriptional signals for infC are embedded within the upstream thrS. Previous in vitro studies [Mayaux et al (1983) Proc. Nat. Acad. Sci., 80, 6152; Wu et al (1984) J. Mol. Biol, 173, 177] did not definitively resolve these two possibilities. In the present work, several different plasmids have been constructed which encompass infC and varying amounts of upstream thrS sequences. The ability of the plasmid DNA, or restriction fragments derived therefrom, to direct the synthesis of IF 3 was tested in an in vitro DNA-dependent coupled transcription-translation system and in plasmid-transformed maxicells. The results indicate that IF 3 is synthesized equally well in the presence or absence of the thrS promoter. A promoter whose presence is sufficient for expression of infC has been localized to an 89 bp region which occurs 178-267 bp upstream of the infC start codon and includes sequences with a high degree of homology to the -35 and -10 consensus sequences. At this point it cannot be concluded whether this is the only promoter for infC or if additional promoters exist further upstream in thrS. It is clear, however, that infC can be transcribed from a promoter within the thrS coding sequence. (Supported by NIH grant GM 29265).

0856 REGULATION OF INTACT AND HYBRID α AND β ACTIN GENES INSERTED INTO MYOGENIC CELLS. S. B. Sharp, Caltech, Pasadena, CA 91125; T. A. Kost, Norden Labs, Lincoln, NB 68501; S. H. Hughes, Frederick Cancer Research Facility, Frederick, MD 21701; C. P. Ordahl, UCSF, San Francisco, CA 94143; N. Davidson, Caltech, Pasadena, CA 91125.

In order to determine which regions of cytoplasmic and skeletal muscle chicken actin genes contain cis-acting sequences important in regulating their developmentally timed expression, we have transferred intact and hybrid genes into a myogenic cell line and monitored their expression. Each of four genes, intact β-actin, intact skeletal α-actin, a 5'α-3'β hybrid, and a 5'β-3'α hybrid, was inserted into a plasmid containing the gene for G418 resistance, and transferred into the mouse myoblast-like cell line, BC3H-1. Transformant colonies from each of the transfers were pooled and expanded. Gene expression in the undifferentiated and differentiated states was monitored by RNA gel blots using gene specific probes from the 3' untranslated regions of the chick α and β genes. The intact α and β genes were differentially regulated. β mRNA was present in uninduced cells, and just as for the endogenous mouse β mRNA, was substantially reduced in differentiated cells. Chicken α message was not appropriately upregulated upon induction. It, like β, was present in uninduced cells, but unlike β, showed no dramatic decrease in abundance upon differentiation. Preliminary results with the hybrid genes indicate that the β promoter is stronger than the α promoter in undifferentiated cells, and that most of the information for down-regulation upon differentiation resides in the 3' half of the β gene.

0857 THE PROMOTER OF THE RIBOSOMAL RNA GENE IS LARGE AND CONTAINS MULTIPLE DOMAINS

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We have examined the nucleotide sequences involved in directing transcription of mouse and frog rRNA genes by RNA polymerase I. Only a minimal promoter domain is detected at optimal initiation conditions. In mouse in vitro systems this corresponds to rDNA residues -35 to +9. However, as reaction conditions are made more stringent by varying ionic, template or extract conditions, the 5' border of the region necessary to promote efficient mouse rDNA transcription moves progressively upstream to residues -39, -45, ~-100, and ~-140. All of these domains act by contributing to the formation of the stable transcription complex.

Similar results are observed with the Xenopus leavis rRNA gene. A small proximal promoter domain specifies effecient transcription under the optimized conditions of microinjecting oocytes with relatively high concentrations of rDNA template. However, a promoter extending out to residue ~-140 becomes increasingly obvious under the less effecient conditions of transcribing in vitro or injecting oocytes with low concentrations of template. The polymerase I enhancer sequences which reside still further upstream in the X. leavis rDNA spacer function in concert with the upstream promoter domains. This enhancer only acts on promoters with intact upstream domains and only under injection conditions where these domains significantly affect the level of synthesis. Thus the rDNA promoter consists of a minimal essential domain flanking the initiation site and additional domains that extend in the 5' direction and act to augment the process of initiation.

0858 PROMOTER ELEMENTS OF THE MOUSE METALLOTHIONEIN GENE IDENTIFIED BY IN VIVO ASSAY OF SYNTHETIC SEQUENCES,

Gary W. Stuart, Peter F. Searle and Richard D. Palmiter,
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Recent studies have demonstrated that the transcriptional response of human and mouse metallothioneins to heavy metals is mediated by two or more short (<12 bp) homology units repeated several times in metallothionein (MT) promoters. Synthetic copies of five such homology units found within the first 200 bp's of the mouse MT-I promoter are being tested for their ability to induce transcription from a heterologous gene. In our assay, HSV-TK genes containing these potential metal regulatory elements (mre's) inserted within the promoter are tested for their ability to produce metal regulated TK activity after DNA-mediated transfection of TK$^-$ BHK cells. Two separate mre's have been unambiguously identified using this approach. At least one other synthetic homologue acts as a basal promoter element that is relatively unresponsive to the presence of metal. Distance effects and promoter element interactions are also being investigated. Single mre's do not allow induction while duplicate mre's can give up to a 5 fold response. In contrast, a single copy of the identified basal promoter element appears fully functional. Both types of elements exhibit reduced efficiency at greater distances from the TATA box. In vivo assay of synthetic promoter elements provides a novel and relatively unambiguous method of analyzing the structure of inducible promoters and the mechanisms by which they operate.

0859 CHICKEN U2 AND U1 RNA GENES SHARE A REGION OF HIGH SEQUENCE HOMOLOGY APPROXIMATELY 200 BASE PAIRS UPSTREAM OF THE RNA CAP SITE,

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Transcription of the small nuclear RNA genes is regulated by unusual promoter sequences in the 5'-flanking DNA. Although these genes are transcribed by RNA polymerase II, they do not possess TATA boxes at the canonical position. We have recently cloned and sequenced four chicken U1 RNA genes (Earley et al., Nuc. Acids Res. 12 [1984] in press). In the 5'-flanking DNA, these four U1 genes share a 40-50 base pair region of high sequence homology located approximately 200 nucleotides upstream of the U1 RNA cap site. We have now sequenced a chicken U2 RNA gene and compared its 5'-flanking DNA sequences to those of the U1 RNA genes. An outstanding feature is that the chicken U2 gene likewise shares this conserved upstream sequence at approximately the same location relative to the RNA cap site. This sequence homology is shown below (the letter N stands for non-conserved nucleotides, Y for pyrimidine, and R for purine):

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   CHICKEN U2              -239  GGGCGGGGCCATGCAAATCGGAG-CCGCGGGG-GCG-GCCGGGAGC  -197
   CHICKEN U1 (consensus)  -236  GGGYGGGGAYATGCAAATNRANNNCCGCGCGCTGCATGCCGGGAGC  -191
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In the region shown, 35 out of 46 nucleotides (76%) are held in common between the U2 and U1 gene sequences. This finding implies strongly that this region is a necessary sequence element required for chicken snRNA gene expression. These results complement the recent studies of Skuzeski et al. (J. Biol. Chem. 259, 8345-8352 [1984]) which demonstrate a functional requirement for sequences at this same location for human U1 RNA gene expression.

0860 SEQUENCE DETERMINANTS OF *E. COLI* PROMOTER STRENGTH *IN VIVO*,

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We have cloned random fragments of *E. coli* DNA and analyzed their ability to direct the expression of the chloramphenicol acetyl transferase (CAT) gene carried on the promoter selection plasmid, pKK232-8 (Brosius, Gene 27, 151, 1984). We found two fragments that direct a greater level of CAT activity than the strong hybrid promoter, tac (deBoer et al., PNAS 80, 21, 1983, as constructed by Amman et al., Gene 25, 167, 1983). The relative promoter strengths were confirmed with independent constructions. The DNA sequences of these two randomly cloned fragments reveal multiple, overlapping regions homologous to the -35 and -10 consensus sequences. In one case, these regions extend beyond the unique transcription start site, mapped by S1 analysis. The tac promoter constructed by Amman et al. is three times stronger in our CAT assay system than the version of Russell and Bennett, Gene 20, 231, 1982. These promoter fragments are identical up to two bases beyond the -35 region, where the Amman et al. tac promoter preserves the natural trp promoter upstream sequences, while the Russell and Bennett tac promoter substitutes non-promoter pBR322 sequences. The influence of upstream sequences on the tyrT promoter has been observed by Lamond and Travers, Nature 305, 248, 1983. Using one of the two randomly cloned promoter fragments, we have begun to define the sequences upstream from the -35 region that influence promoter strength.

0861 TRANSCRIPTIONAL STATES OF THE RAT METALLOTHIONEIN-1 GENE AND TWO PSEUDOGENES,

Susan Taplitz[1,3], Robert Andersen[3], Bruce Birren[2,3], Harvey Herschman[1,2,3], 1Department of Biological Chemistry, 2 Molecular Biology Institute, 3 Lab of Biomedical and Environmental Sciences, *University of California, Los Angeles.*

The metallothionein gene is transcriptionally regulated by both glucocorticoid hormones and by metal ions such as cadmium and zinc. Karin and co-workers have identified separate regulatory sequences which control a metallothionein (MT) gene's response to metal and glucocorticoids respectively. We have isolated a rat MT-I gene and two pseudogenes and have characterized the pattern of DNase I hypersensitive sites prior to and after induction. Both pseudogenes are cDNA copies of the MT-I gene. Pseudogene 21 contains homologous sequences only as far 5' as the cap site, but pseudogene 27 contains homologous sequences 133 b.p. further 5'. This pseudogene contains all sequences required for metal regulated expression of the MT gene and probably arose from a transcript initiated at an upstream promoter. DNase I hypersensitivity studies show a single hypersensitive site upstream from the MT-I gene. Pseudogene 21, which lacks all sequences required for expression, lacks this hypersensitivity.Pseudogene 27, which contains sequences required for regulated expression, also does not have a hypersensitive site, leading us to predict that this gene is not expressed. This prediction is substantiated by our failure to detect transcripts corresponding to this pseudogene by a variety of experimental approaches. These sequences which are required for expression of the MT-I gene, are therefore not sufficient to direct transcription of this MT-I pseudogene.

0862 STEROID HORMONE REGULATION OF THE GENE ENCODING THE CHICKEN PROGESTERONE RECEPTOR B ANTIGEN,

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Using a previously isolated cDNA clone coding for progesterone receptor B (PR^B) antigen as probes, we have studied the hormonal regulation of its expression in chick oviduct as well as other tissues. We observed a 50-fold increase of PR^B antigen upon secondary stimulation with either estrogen or progesterone for 16 hrs. Testosterone and dexamethasone also increase PR^B antigen mRNA but to a much lesser degree. Nuclear "run-off" assay indicated that only part of this increase (2-5 fold) was due to regulation at the transcriptional level. Therefore, both transcription of the gene and stability of mRNA are affected by the presence of steroid hormones. In addition, prolonged administration of progesterone and estrogen resulted in a reduced level of mRNA sequences in the oviduct tissue while ovalbumin mRNA sequence continued to increase. Therefore, the PR^B gene was apparently self regulated. When the other tissues were examined, we were surprised to detect the mRNA sequence for PR^B antigen, although some of these tissues have not been reported to bind progesterone. This suggests that progesterone target tissues may require a redefinition to those which can produce the hormone binding isoform.

0863 SEQUENCES UPSTREAM FROM THE TATA BOX ARE REQUIRED FOR EFFICIENT TRANSCRIPTION OF OVALGLOBIN FUSION GENE IN VITRO, Sophia Y. Tsai, Martine Pastorcic, Ming-Jer Tsai, and Bert W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

The promoter elements necessary for the initiation of transcription of the ovalbumin gene have been localized previously by transfer of an ovalglobin fusion gene into HeLa S_3 cells. A region containing the "TATA" box, a highly conserved sequence located around 25 to 30 base pairs upstream from the cap site of most mRNA-encoding genes, is required for initiation of transcription. A second region spanning between -95 to -48 upstream from the cap site is also required for efficient initiation of transcription. Although the requirement of the "TATA" sequence for initiation of ovalbumin gene is well demonstrated *in vitro*, the importance of the upstream sequence is not yet defined. Here we show that in addition to the "TATA" box, DNA sequences located between positions -95 and -77 upstream of the cap site are also essential for efficient *in vitro* initiation of ovalbumin gene using various deletion mutants of ovalglobin fusion gene as template. The enhancement of initiation of transcription by the upstream sequence can be demonstrated using circular or linear DNA as template and total cell or nuclear extracts from HeLa cells as source of transcription factors. In addition, the upstream sequence dependency is markedly influenced by the transcription factors to DNA ratio. These results are consistent with the notion that two promoter elements, the "TATA" box and the upstream sequences are necessary for efficient initiation of transcription *in vitro* as well as *in vivo*.

0864 TRANSCRIPTION OF ATP SYNTHASE GENES OF PHOTOSYNTHETIC BACTERIA, V.L.J. Tybulewicz, G. Falk and J.E. Walker, MRC Laboratory of Molecular Biology, Cambridge, England.

Rhodopseudomonas blastica and *Rhodospirillum rubrum* are purple non-sulphur photosynthetic bacteria that can grow either by photosynthesis or by respiration. Under both growth conditions their ATP synthase uses the energy of a transmembrane proton gradient to synthesize ATP. The enzyme consists of an extrinsic membrane sector (F_1) with five polypeptides (α, β, γ, δ and ε) and an intrinsic membrane sector (F_0) with at least three subunits.

The genes for the five F_1 subunits of the enzyme from *Rps. blastica* and *R. rubrum* have been cloned and sequenced. Analysis of transcription by S_1 nuclease mapping and primer extension techniques showed that the five F_1 genes form an operon in both organisms. The order of genes is conserved in both bacteria and is the same as that in the *Escherichia coli unc* operon except that genes for F_0 subunits are not associated with the F_1 genes (as they are in *E. coli*). In *Rps. blastica* a sixth gene of unknown function was found within the operon and further experiments showed the existence of a second promoter internal to the operon which transcribes only the two distal genes. Signals involved in initiation of translation (Shine & Dalgarno sequences) and termination of transcription in the photosynthetic bacteria resemble those in *E. coli*. On the other hand, in *Rps. blastica* no *E. coli*-like promoter sequences can be identified in regions adjacent to sites of initiation of transcription, and in *R. rubrum*, though there is a "Pribnow" box at -10, no -35 sequence can be seen. It is clear that promoter sequences in these photosynthetic bacteria are rather different from those in *E. coli* and thus probably reflect divergence in the sigma factor of RNA polymerase.

0865 REGULATION OF BACILLUS AMYLOLIQUEFACIENS PROTEASES IN BACILLUS SUBTILIS, N. Vasantha and L.D. Thompson, Genex Corporation, Gaithersburg, MD 20877

Bacilli secrete two major proteases, an alkaline protease (subtilisin) and a neutral protease (metalloprotease). We have cloned the genes for alkaline protease (*apr[BamP]*) and neutral protease (*npr[BamP]*) from *B. amyloliquefaciens*. Expression of each gene on a multicopy plasmid in *B. subtilis* in either synthetic or complex media reveal that they are regulated differently. Under these conditions, the amount of neutral protease made is maximal during exponential growth. In contrast, the amount of alkaline protease made is maximal after the end of the exponential growth. The possible reasons for this difference in regulation will be discussed.

0866 IS PROMOTER RECOGNITION FOR THE GONADOTROPIN β SUBUNIT GENES TISSUE-SPECIFIC?, J.B. Virgin and J.H. Nilson, Case Western Reserve University, Cleveland OH, 44106

Glycoprotein hormones, including gonadotropins, are composed of two non-covalently associated subunits; a common α subunit and unique β subunit which confers biological specificity. To study the regulation of gonadotropin gene expression, we have isolated and characterized cDNA and genomic clones for the β subunit of bovine lutropin (bLH). The bLHβ gene is expressed in the pituitary and is present as a single copy spanning less than 1.1 kbp, containing three exons encoding an mRNA of 550 nucleotides. The mRNA for bLHβ contains an unusually short 5'-untranslated region of only 6-11 nucleotides; an unexpected finding since the highly conserved β subunit genes of the human LHβ/CGβ gene family (expressed in pituitary and placenta) have 5'-untranslated regions in excess of 350 nucleotides. Further comparison between the bLHβ and hLHβ/hCGβ genes reveals that both have consensus TATA sequences in identical positions. However, when the human gonadotropin β subunit gene family is expressed in placenta, transcription starts at an upstream promoter which bears no homology to the consensus TATA sequence. Recently, we transfected the bovine LHβ gene into a human placental cell-line which normally expresses gonadotropin β subunits. Our preliminary data suggest that transcription of the bovine LHβ gene may begin preferentially at an upstream promoter site, closely related to that normally used by the human genes. Additional experiments in progress should reveal whether promoter recognition for the gonadotropin β subunit genes is tissue-specific.

0867 GENERATION OF POTENTIAL Z-DNA WITHIN THE METALLOTHIONEIN PROMOTER, Daniel Visentin and Calvin B. Harley, McMaster University, Hamilton, Ont. Canada, L8N 3Z5

Alternating purine-pyrimidine DNA sequences can undergo B to Z transitions when stabilized by negative supercoiling in physiological conditions. Studies with anti-Z antibody in biological systems strongly suggest a role in gene expression or DNA packaging, but there has been no direct evidence for this *in vivo*. The mouse metallothionein-I promoter has a potential Z-DNA sequence 86-102 base pairs from the transcription start site within the 216 bp Sst I-Bgl II region of the promoter thought to be involved directly with heavy metal induction. This sequence 5'-GCGCGGGTGACTATGCGTG-3' is an alternating purine/pyrimidine sequence except for the central two base pair interruption. The Sst I-Bgl II fragment was cloned into pUC 12 and shown not to flip into Z conformation by two-dimensional chloroquine gel electrophoresis. To facilitate the B to Z transition and study its effect on transcription, we cloned this fragment into m13-mp10 and inverted the central AC to CA by oligonucleotide mediated mutagenesis. This creates a 17 base alternating purine/pyrimidine sequence within the potential Z-DNA region. The wild type and mutant sequence was confirmed by dideoxy sequencing. A 216 bp region containing the mutation was re-cloned into pUC 12. Two-dimensional chloroquine gel electrophoresis of the mutant metallothionein promoter demonstrated a structural transition which may involve Z-DNA. The effect this mutation has on transcription of linked genes with respect to heavy metal induction is currently being assayed by transfection and Northern Analysis.

0868 STRUCTURE AND FUNCTION OF EXTRACHROMOSOMAL rDNA IN PHYSARUM. Volker M. Vogt, Eileen Epstein, Patrick J. Ferris, and Sheri Kostelny. Section of Biochemistry, Cornell University, Ithaca NY 14853.

The genes encoding rRNA in the acellular slime mold *Physarum polycephalum* are located on linear palindromic extrachromosomal DNA molecules of 60 kb. Transcription initiates at sites 18 kb from each end and proceeds outwards. Two introns interrupt the 26s RNA coding region. (1) We have determined the DNA sequence of the 22 kb comprising the central spacer region. The structure of this region is a complex array of palindromes within palindromes. Unlike in some other organisms, the promoter sequences are not repeated in the spacer. (2) We are characterizing a strain of *P. polycephalum* that contains a third rDNA intron about 1 kb in size. DNA sequencing shows that this intron is located exactly at the nucleotide where an intron is found in species of *Tetrahymena*. Chromosomal DNA from the strains of *Physarum* lacking the third intron contains low or single copy sequences homologous to this intron. (3) We are purifying and characterizing two nuclear proteins that bind specifically to sequences on the rDNA. One protein, purified over 100-fold, recognizes a sequence about 100 nucleotides upstream of the RNA start site. A second protein fraction recognizes sequences at or near the telomers of the rDNA.

0869 DIRECT SELECTION FOR MUTATIONS REDUCING EXPRESSION OF THE E. COLI RECA GENE,

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A λ phage carrying a recA-lacZ protein fusion grew normally on wild-type E. coli, but its growth was severely inhibited in lexA(Def) mutant strains that express recA constitutively at high levels. Mutants of the transducing phage that grew on the lexA(Def) strains were isolated and found to affect production of the RecA-β-galactosidase hybrid protein. Most mutants were phenotypically LacZ$^-$ and included a number of nonsense mutants. LacZ$^+$ mutants were also isolated and most of these expressed lower basal and induced levels of β-galactosidase activity. DNA sequence analysis revealed that some of the LacZ$^+$ mutations were in the recA promoter. Unexpectedly, three of the mutations that reduced expression were located in the recA structural gene. Further analysis showed that these most likely affected translation. These mutations also reduced synthesis of RecA protein when present in a complete recA gene. Thus, synthesis of RecA protein may be subject to translational as well as transcriptional control.

0870 SEQUENCES INVOLVED IN THE TRANSCRIPTIONAL REGULATION OF VACCINIA VIRUS GENES,

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Bal31 deletion analysis was performed to characterize the promoter region of an early vaccinia virus gene that encodes thymidine kinase (TK), and a late gene that codes for a 28K polypeptide of the virus core. Early genes such as TK are transcribed within minutes after infection by the virus-specific RNA polymerase, and their expression is not prevented by inhibitors of either protein or DNA synthesis. Late genes such as the one coding for the 28K gene are switched on during the time of DNA replication concomitant with the shutoff of most early genes. Examination of the DNA sequences preceding the start of several early genes has revealed an extremely A-T rich region that differs markedly from the upstream regulatory regions of both prokaryotic and eukaryotic genes. Several late genes were mapped and common sequences were found in their 5' ends that distinguish them from early genes. It is also notable that in most late genes examined, the first possible initiation of translation codon is located almost immediately adjacent to the mRNA start site. To determine, in a functional way, what sequences are involved in vaccinia transcriptional regulation, we have fused the promoter and regulatory regions of the thymidine kinase and 28K genes to the structural gene for the prokaryotic enzyme, chloramphenicol acetyltransferase (CAT). After a series of 5'-3' deletions was made in the promoter regions, the resulting chimeric genes were reinserted into the virus, and the effect on CAT expression was determined. In the case of both early and late vaccinia promoters, a short region upstream from the mRNA start site was necessary and sufficient for promoter and regulatory activity.

0871 THREE INTERGENIC REGIONS OF CORONAVIRUS MHV-A59 GENOME CONTAIN A CONSERVED SEQUENCE THAT IS HOMOLOGOUS TO THE 3' END OF THE MESSENGER RNA LEADER SEQUENCE,

Susan R.Weiss, Carol J. Budzilowicz, and Sharon P. Wilczynski, Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104

Mouse hepatitis virus (MHV), a positive strand RNA virus, generates a set of 7 subgenomic mRNAs overlapping at the 3' ends. Each mRNA contains a leader sequence of about 70 nucleotides, probably derived from the 5' end of genome RNA. In order to look for possible transcriptional control sequences in the MHV genome, we sequenced the regions just upstream of three MHV genes. Thus, cloned cDNAs containing intergenic regions preceding genes 5,6 and 7 were subcloned into M13 and sequenced by the dideoxy chain terminating method. A conserved 11 nucleotide sequence CUAAUCU(C)AAAC, was found preceding the coding region of each gene. This sequence is homologous to the 3' end of the leader sequence found upstream from the coding regions of mRNAs 6 and 7. These data suggest that the conserved 11 nucleotides may be a binding site for a leader RNA that primes the transcription of mRNAs. Nucleotide #7 of the conserved sequence probably marks the end of the leader sequence and the beginning of the body of the messenger. The fact that nucleotides #7-#11 are also conserved suggests they are important for the attachment of a leader RNA or primer/polymerase complex. The variation in homology between the leader and its putative binding site among the three genes examined may effect the differential efficiency of transcription of the mRNAs.

Sequence Specificity in Transcription and Control

0872 TRANS-ACTING TRANSCRIPTIONAL REGULATION OF A CLONED FROG VIRUS 3 PROMOTER BY VIRION PROTEINS, D.B. Willis and A. Granoff, St. Jude Children's Research Hospital, Memphis, TN 38101

Frog virus 3 (FV3) is a large icosahedral DNA virus whose genes are expressed in an orderly stepwise manner. Immediate-early RNAs, defined as those RNAs synthesized in the presence of cycloheximide, are not inhibited by α-amanitin in a mutant CHO cell line with an α-amantin resistant RNA polymerase II, but are inhibited by α-amanitin in wild-type CHO cells, implicating the host polymerase in the synthesis of immediate-early viral RNA. We have cloned and sequenced the 78 base pair promoter region of a major immediate-early FV3 gene and found an A-T rich region (TATTTTA) at -30 bp upstream from the transcription start site. This presumed promoter was ligated into a plasmid 5' to the coding region of the bacterial chloramphenicol acetyl transferase (CAT) gene; the FV3 promoter-CAT construct was then introduced into α-amanitin sensitive and resistant cells by $CaPO_4$ co-precipitation. After 24 hr, one set of dishes was treated with UV-inactivated FV3; extracts were prepared for CAT assay 6 hr later. CAT synthesis occurred only in UV-FV3 treated cells having a functional RNA polymerase II. Therefore, a trans-acting component of the virion was required for recognition of the promoter by the host enzyme.

0873 A NEW WAY TO THINK ABOUT tRNA GENE CONTROL, Ellen T. Wilson, Lisa S. Young and Karen U. Sprague, Inst. of Molecular Biology, Univ. of Oregon, Eugene, OR 97403

In a currently popular model of eukaryotic tRNA gene control, two small coding regions corresponding to conserved parts of tRNAs direct transcription of tRNA genes. At variance with this view are the observations that certain mutant tRNA genes do not have the expected phenotypes. In some cases, removal of one of the internal control elements does not abolish transcription; in others, mutations outside the critical regions have pronounced effects on transcriptional activity. We have shown that the requirement for particular sequences downstream from the transcription initiation site is highly dependent on certain transcription reaction parameters. The full control region for a *Bombyx* tRNA^{Ala} *gene defined by our experiments* is larger than the transcription unit itself (98 bp), extending from at least -14 to +147 bp relative to initiation (+1). The large control region is separable into two functional domains -- a region including 5' flanking sequences, and a large ($\geq$140 bp) coding and 3' flanking region that binds a necessary transcription factor.

What has complicated the analysis of tRNA gene control is a DNA-binding inhibitory substance present in the cell-free extracts typically used to catalyze transcription *in vitro*. Specifically, conditions that permit the inhibitory substance to mask the contribution of certain control elements can make the control region appear smaller. When the effects of the inhibitor are minimized by the addition of non-specific DNA to transcription reactions, assays become more sensitive to mutant phenotypes, and the full size of the Bombyx tRNA^{Ala} gene control region is observed. We propose that this finding explains much of the observed variability in the sequence requirements for transcription of different tRNA genes.

0874 A RAPID SENSITIVE TRANSIENT ASSAY SYSTEM [illegible] ANALYSIS OF PROMOTER ACTIVITY. Clive R. Wood, John H. Kenten, Paul E. Stephens, Christopher C. Hentschel, and Michael A. Boss, Celltech Ltd., 244-250 Bath Road, Slough, Berks. SL1 4DY, U.K.

Transient assays of gene expression have proved very valuable for the study of the *in vivo* function of mammalian gene sequences. Using a cDNA of human tissue plasminogen activator (tPA), in combination with the fibrin agar overlay method, we have demonstrated that we can detect the transient expression of promoter - tPA cDNA constructs when introduced into the hybridoma SP2/0-Ag 14. Furthermore, the differences in tPA activity produced by different promoters, correlates well with the differences observed between the same promoters using the less sensitive chloramphenicol acetyltransferase assay. We have used the tPA transient assay to determine the relative strengths/activities of the anti-NP Ig heavy chain gene, SV40 early and RSV LTR promoters. In addition, we have carried out experiments to examine the function of the Ig heavy chain enhancer.

0875 ALTERED SEQUENCE AND DISTANCE BETWEEN THE TWO CONSENSUS SEQUENCE BLOCKS AFFECT TRANSCRIPTION OF AD2 VA1 RNA GENE, Guang-jer Wu, Ronald E. Cannon and Johnny F. Railey, Emory University School of Medicine, Atlanta, Ga. 30322.

The boundaries of the transcriptional control region of Ad2 VA1 RNA gene have been delimited internally at about +9 to +72. However it is not known whether all the DNA sequence in the region is absolutely required for an all-or-none transcriptional control effect. To answer this question, linker-scanning mutations with Kpnl linker, dCGGTACCG, replacing the DNA sequence between the two blocks were constructed. Four mutants in which four different clusters of DNA sequence between the two blocks replaced with the Kpnl linker, respectively, were obtained. The transcription efficiencies of these mutants were about 50% that of the wild type gene indicating that the DNA sequence between the two blocks is not absolutely essential for transcriptional control, but it may be required for efficient transcription. Furthermore, three mutants with longer DNA distance between the two blocks were also constructed. The transcription efficiencies of these three mutants, (+10), (+17) and (+27), were 140, 120 and 42% that of the wild type gene, respectively, indicating that the distance between the two blocks can be extended to about 61 bp and probably more, however the optimal distance is about 44 bp. Moreover, six mutants with shorter distance between the two blocks were constructed. The transcription efficiencies of some of these mutants with a distance between the two blocks 6 bp shorter than the wild type gene were drastically abolished indicating that the minimal distance between the two blocks is about 28 bp. These results clearly prove that the control region of the VA1 RNA gene is similar to that of tRNA genes in eukaryotes.

0876 TRANSCRIPTION OF A MUTANT DIPHTHERIA TOXIN GENE (CRM228) IN CORYNEBACTERIUM DIPHTHERIAE AND ESCHERICHIA COLI, Gerd Zettlmeissl, Rolf E.Streeck, Michel Kaczorek Institut Pasteur, Groupement de Génie Génétique, F-75015 Paris

Iron plays an important regulatory role in the expression of diphtheria toxin by C.diphtheriae infected by a phage carrying the _tox_ gene (Murphy and Bacha, 1979, Microbiology, 181-186) : in the presence of iron in the medium no expression is observed, whereas _tox_ production is induced under conditions of iron limitation.

In C.diphtheriae strains carrying the mutant phage β228, toxin specific mRNA is detectable only following induction. It is thus probable that the regulation of _tox_ expression by iron occurs at the level of transcription.

The mutant _tox_ gene CRM228, together with its putative promotor have been cloned in E.coli, where the mutant protein is expressed with low efficiency (Kaczorek et al.,1983, Science, 221,855-858). S1-nuclease mapping and primer extension demonstrate that the start-sites of transcription are identical both in induced C.diphtheriae and E.coli.

Sequence Specificity in Transcription and Control

Eukaryotic Promoters

0877 INTERACTION OF RNA POLYMERASE I TRANSCRIPTION FACTOR(S) WITH rDNA PROMOTER ELEMENTS, Ingrid Grummt, Detlev Buttgereit and Joachim Clos, Institut für Biochemie der Universität, Röntgenring 11, D-8700 Würzburg, F.R.G.

The transcription of the ribosomal genes is very efficiently regulated according to the proliferation rate of the cells. A more or less efficient transcription is brought about by modulation of the initiation frequency of RNA polymerase I on the rDNA. The elucidation of the molecular mechanism of this transcriptional regulation requires both the identification of the promoter sequences and the protein factors which are required for the initiation process.
A cell-free system consisting of crude nuclear extracts from cultured Ehrlich ascites cells was used which faithfully initiates transcription on a cloned 5'terminal fragment of mouse rDNA. The following results were obtained:
(1) The cell-free transcription system reflects the rRNA synthetic activity of the cells. Only extracts from rapidly proliferating cells promote transcription of cloned rDNA; extracts from growth-inhibited cells are transcriptionally inactive.
(2) The transcription of ribosomal rDNA requires extracts from homologous cells, which indicates that species-specific factor(s) are involved in the initiation reaction.
(3) Fractionation of cell-extracts on several ion exchange columns showed that in addition to RNA polymerase I at least two proteins are required for accurate and efficient transcription initiation. TFIA is present or active only in rapidly proliferating cells and copurifies with RNA polymerase I. TFIIB is a species-specific DNA binding protein which is required for stable transcription complex formation. It is present both in growing or growth arrested cells.
(4) An RNA polymerase I control region essential for the initiation of pre-rRNA transcription has been identified by mutagenesis in vitro of mouse rDNA and transcription in cell-free systems derived from Ehrlich ascites cells. Substitution of nucleotides between -35 and -14 by foreign DNA sequences caused a loss of template activity, which indicates that an important promoter element is located within this region. At least two evolutionary highly conserved nucleotides a G at position -16 and a T at -1 play an important role in the interaction of TFIB with the rDNA promoter.

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3) Grummt, I. and Skinner, J. (1983), Proc. Natl. Acad. Sci. USA in press

0878 CHARACTERIZATION OF THE GLUCOCORTICOID RECEPTOR GENE AND TRANSCRIPTS IN WILD TYPE AND MUTANT CELLS USING CLONED cDNA, Roger Miesfeld, Sandro Rusconi and Keith R. Yamamoto, Dept. Biochemistry, University of California, San Francisco, CA 94143.

Steroid hormone effects are mediated by intracellular hormone-specific receptor proteins; the hormone-receptor interaction increases the affinity of the receptor for nuclear binding sites, thereby modulating the expression of specific genes. Although the precise mechanisms of steroid action are not understood, the glucocorticoid receptor is perhaps the most fully characterized eukaryotic positive transcriptional regulatory factor. Purified glucocorticoid receptor binds in vitro with high affinity to defined regions of DNA near regulated promoters, and sequences essential for these interactions are functional in vivo as hormone-dependent transcriptional enhancer elements. We have employed polysome immunoadsorption using glucocorticoid receptor-specific antibodies to obtain eight overlapping cDNA clones which together appear to contain the entire coding sequence of the rat glucocorticoid receptor gene. One of these clones containing a cDNA insert of 2.6 kb was used for a preliminary analysis of glucocorticoid receptor gene organization and expression. The *receptor seems to be encoded by a single copy gene which is indistinguishable in DNA* isolated from rat liver or fibroblast cell lines. Wild type rat and mouse cells accumulate a 5.8 kb receptor transcript; receptor deficient (r^-) mutants, which display reduced steroid binding activity, accumulate reduced amounts of the 5.8 kb transcript. Increased nuclear transfer (nt^i) mutants, which produce a receptor of 40 kd rather than the 94 kd protein observed in wild type cells, contain a receptor transcript of only 4.8 kb. Gene transfer experiments of receptor coding sequences into receptor mutant cell lines are being performed to assess directly the functional potential of these clones.

Sequence Specificity in Transcription and Control

0879 PROMOTER SPECIFICITY AND THE EUKARYOTIC TRANSCRIPTION MACHINERY. R. Tjian, W. Dynan, D. Gidoni, K. Jones, M. Briggs, U. Heberlein, and M. Learned. Department of Biochemistry, University of California, Berkeley CA 94720.

By fractionating various types of transcription extracts and examining different genes, we have recently identified several promoter-specific transcription factors that impart selectivity to RNA polymerases I and II. The first of these auxiliary transcription factors identified was Sp1, which binds specifically to the "GC-boxes" that are contained within the control sequences located upstream of the SV40 early promoter. More recently, this cellular transcription factor has been shown to bind and activate not only SV40 transcription, but also several other promoters, including the immediate-early promoter of the HSV ICP4 gene as well as cellular promoters such as the mouse dihydrofolate reductase gene and the promoter for a cellular monkey gene. In each of these cases, Sp1 was found to bind multiple GC-box containing sequences located upstream from the start site of transcription and that activation of RNA synthesis required the presence of Sp1. In addition to studying the interaction of specific transcription factors with viral and cellular genes, we have also begun to investigate the transcription of developmentally regulated and tissue-specific genes in Drosophila. Transcription of the alcohol dehydrogenase gene is under the control of two tandem promoters (proximal and distal) that are activated in a temporally-regulated fashion during Drosophila development. We have fractionated a Drosophila tissue culture extract system and identified a specific transcription factor, Adf-1, that binds to the upstream region of the distal Adh promoter and activates transcription. Footprint analysis also revealed the presence of additional sequence-specific binding proteins present in the extract that recognize and interact with the upstream regions of the proximal promoter of Adh.

As a third case study, we have identified a promoter-specific transcription factor, SL1, that is required to activate transcription from the human ribosomal promoter by RNA polymerase I. Analysis of mutant templates suggests that the "core" control element of the human ribosomal promoter is required for activation of transcription by SL1. We have purified SL1 approximately 200,000 fold and have shown that addition of SL1 can reprogram the otherwise nonpermissive mouse transcription system to recognize and initiate accurate RNA synthesis from the human ribosomal RNA promoters. Antibodies raised against SL1 bind preferentially to a protein in the nucleolus of primate cells but not rodent cells. Moreover, anti-SL1 specifically inhibits in vitro transcription initiating from the human ribosomal promoter but not the mouse promoter. These findings suggest that SL1 is a nucleolar factor that imparts promoter recognition to RNA polymerase I, and that it can discriminate between promoters from different species. DNA binding studies suggest that, unlike the RNA polymerase II factors, SL1 is not a sequence-specific DNA binding protein.

Terminator Selection

0880 ELEMENTS OF THE N TRANSCRIPTION ANTITERMINATION REACTION: STUDIES ON THE E. COLI nusA AND nusE GENES AND λ nut REGION. D.I. Friedman, A.T. Schauer, and E.R. Olson. Department of Microbiology and Immunology, The University of Michigan, Ann Arbor, Michigan 48109.

Effective action of the N transcription antitermination function of λ, gpN, requires the participation of a number of bacterial functions, Nus, as well as nut, where the transcribing RNA polymerase is modified to a form that can overcome many downstream termination signals (1). We report studies: 1) defining, in part, domains of the nut region and 2) analyzing two Nus functions, the products of the nusA and rpsJ (nusE) genes.

nut: Three regions of potential or proven importance have been identified in the nut region: BoxA, pyGCTCTT(T)A, the nut stem-loop structure and boxC. Two mutations have proven that boxA is important in the gpN reaction, boxA1 and boxA5. The boxA1 (2) mutation results in a transversion that substitutes an A-T bp for a T-A bp. This change from CGCTCTTA to CGCTCTTT is necessary for the λ N product to function with the NusA protein from Salmonella typhimurium. Two other related phages with different N products that can function with the NusA of Salmonella, phages 21 and P22, have boxA sequences with the three T's. The boxA5 mutation was synthesized and results in the change of one bp in the boxA sequence so that it now reads CTCTCTTA. A nut region with the boxA5 mutation no longer will support the N-directed modification leading to a termination-resistant polymerase. Manipulation of the translation reading frame upstream of nutR shows that if translation of the promoter-proximal cro gene extends 4 bp towards nutR, there is interference with the modification reaction (3). This steric hindrance by ribosomes suggests an interaction between protein(s) and RNA.

nusA: Comparison of the nusA sequences of S. typhimurium and E. coli (4) reveals that the two genes are very similar. Most of the nucleotide differences are in wobble positions and thus do not result in amino acid heterogeneity. The most significant difference is in one codon resulting in a basic amino acid in the place of an acidic amino acid.

nusE: The nusE-71 (5) mutation was mapped to the rpsJ gene (ribosomal protein S10). In order to assess the nature of the role of the S10 protein in the N modification reaction, we have selected for mutations that suppress the effect of the nusE mutation. Mapping and complementation studies place one suppressor in the rplP gene (ribosomal protein L16). This observation is consistent with ribosomal involvement in the N modification reaction.

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(3) Olson, E.R. et al. (1982). Cell 31: 61-70.
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0881 REGULATION OF TRANSCRIPTION BY THE NUS PROTEINS OF E. COLI, Jack Greenblatt, Yukiko Goda, Robert Horwitz, Joyce Li, and Linda Peritz, Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada M5G 1L6

Antitermination of transcription by the N protein of bacteriophage λ requires several *E. coli* proteins, one of which is the NusA transcription termination factor. We have used photolabeled NusA to study the interaction of NusA with other proteins. Free NusA is a dimeric protein. When NusA dimers bind to multimers of the core component of RNA polymerase, both disaggregate to monomers. In standard transcription conditions NusA binds tightly to RNA polymerase only after the initiation subunit σ^{70} has disociated from the enzyme and RNA polymerase has paused at a NusA-sensitive pause site. However, the NusA-binding site on RNA polymerase is different from the σ^{70}-binding site. σ^{70} stabilizes a conformation of RNA polymerase R_e which has low affinity for NusA and which is different from the conformation R_p stabilized by NusA. We suggest that NusA extends transcriptional pause times at NusA-sensitive sites by stabilizing the R_p conformation of RNA polymerase.

We have cloned two NusA-dependent terminators. One is the Rho-dependent terminator in orientation II of the insertion element IS1. The terminal octanucleotide, CUCAAAAU, in IS1 (II) is identical to the one in the Rho- and NusA-dependent terminator *trpt'*. We will argue that UCAA is a signal for Rho action and that the terminal As signal NusA involvement. IS1 (II) and the other cloned NusA-dependent terminator also have the perfect upstream match GCTGTTTTA, and this is a *boxA* sequence believed to be a signal for NusA action. A model for regulation by NusA and N at the nucleic acid level will be presented.

N cannot function in a strain with the *nusE71* mutation in ribosomal protein S10. We have purified two proteins that restore N function *in vitro* when added back to a reaction containing S100 extract from a *nusE71* mutant. One is probably S10 itself functioning in transcriptional control as an extraribosomal protein. The other is a new *E. coli* transcription termination factor that we have called Zeta. Some of the properties of these proteins will be discussed.

0882 RHO FACTOR, TANDEM TERMINATION SITES, AND RNA PROCESSING ARE ALL REQUIRED TO GENERATE THE MATURE 3' END OF E. COLI TRYPTOPHAN OPERON mRNA, Terry Platt, John E. Mott, Jill L. Galloway, and Raymond A. Grant, Department of Molecular Biophysics & Biochemistry, Yale University, 333 Cedar Street, New Haven CT 06510

Tandem sites, *trp t* and *trp t'*, are involved in transcription termination at the end of the E. coli *trp* operon. In *vitro*, *trp t* is only 25% efficient and unaffected by termination factor rho while *trp t'*, 250 bp downstream in an AT-rich region with little secondary structure potential, is *virtually* 100% efficient, but requires rho factor both in vivo and in vitro. In vivo, the 3' end of *trp* mRNA coincides with the RNA hairpin of *trp t*. Paradoxically, readthrough transcription at *trp t* is observed in vivo in *rho*$^-$ *strains*, or in strains with deletions of the *trp t'* *region*. Three lines of *evidence* support a processing model to explain these observations.

(1) When cloned between the *gal* promoter and the galactokinase gene (*galK*), the terminator efficiencies in vivo, *either* singly or in tandem configuration, *agree* with those observed in vitro. Thus termination alone cannot account for the puzzling results.

(2) In vitro, transcripts terminating at the distal rho-dependent *trp t'* site can be trimmed by the 3' exonuclease RNase II into shorter species similar to *those* terminating at the proximal *trp t* site.

(3) With vectors carrying *trp t'* alone distal to *galK*, there is 3-fold less *galK* activity than with both sites *present*; experiments in *an RNase* II$^-$ strain are underway.

Analysis of the *trp t'* region shows that an important component (if not all) of the rho recognition region *resides* in RNA sequences considerably upstream from the points of termination. These sequences are presumably required for binding and/or activation of the NTPase activity of rho factor essential for its ability to catalyze termination. Substantial 3' degradation would remove this RNA recognition region and reduce the deleterious RNA-dependent NTPase activity of rho factor in the cell. In summary, as transcription proceeds beyond the *trp* operon structural genes, 20-40% of the polymerase molecules terminate at the *trp t* hairpin site. The remaining 60-80% read through to the distal *trp t'* region, where *rho dependent* termination occurs. Subsequently, the long untranslated and *unstructured* trailer region is degraded by a 3' exonuclease back to the RNA hairpin (corresponding to *trp t*), yielding a 3' end identical to that produced by the low level termination at *trp t*. *Thus*, *trp t'* is the major terminator of the operon, and *trp t* has a dual function, *as a minor terminator*, and as a protective barrier to degradation *of* upstream structural mRNA. The interplay between these two sites suggests a mechanism and rationale for the evolution of two distinct classes of termination site, in the context of a model linking requirements for operon expression to the configuration of the termination sites themselves.

0883 ANTITERMINATION BY PHAGE LAMBDA Q PROTEIN IN VITRO

Jeffrey W. Roberts, Elizabeth J. Grayhack, Xianjie Yang, and Jeffrey A. Goliger, Section of Biochemistry, Molecular & Cell Biology, Wing Hall, Cornell University, Ithaca, N.Y. 14853

Gene Q of phage lambda, and corresponding genes of related phages, are positive regulators of phage late gene expression. They encode antiterminator proteins whose role is to allow transcription through terminators that precede and block expression of the phage late genes. We have purified the late gene regulators of phage lambda and its relative phage 82. They are active as antiterminators in transcription by purified RNA polymerase, and each is specific for the late gene promoter of its own phage. NusA protein, a transcription factor required for function of the lambda gene *N* antiterminator, greatly stimulates antitermination by Q protein but is not absolutely required. The sequences that encode specificity for lambda Q extend from within the promoter to about nucleotide 20 of DNA encoding the late transcript, although the antitermination activity is expressed at sites far distant from the promoter. Lambda Q protein can act after initiation of RNA synthesis: transcription from the lambda late promoter pauses at nucleotide 16 of the late transcript, and addition of Q protein to the paused transcription complex drives RNA polymerase out of the pause. NusA protein also is required for this activity of Q protein; thus NusA can act as early as nucleotide 16, and, in this case, does not bind free RNA as it acts. A BoxA sequence, suggested by Friedman to be the target of NusA protein, is present in DNA just after the pause site. We suggest that the capacity of Q protein to inhibit transcriptional pausing accounts for its action as an antiterminator.

0884 MECHANISMS OF RHO-DEPENDENT TRANSCRIPTION TERMINATION

Peter H. von Hippel, James A. McSwiggen, Johannes Geiselmann, Thomas Yager, David G. Bear* and William D. Morgan†, Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403

A three-step model has been proposed to account for transcription termination at rho-dependent termination sites in *E. coli*. This model is based on *in vitro* studies of rho-dependent termination of the transcript initiated at the P_R promoter of phage λ (1,2) and on physical chemical studies of rho-polynucleotide interactions (papers in preparation); it is supported by analyses of the sequence and secondary structure of transcripts subject to rho-dependent termination that have been reported in the literature (Morgan *et al.*, submitted). (See ref. 3 for a summary and complete literature review.)

The steps involved in the proposed model are as follows. (i) Transcribing RNA polymerase pauses at or near potential termination sites. This pausing does not require rho, shows an *in vitro* relaxation time of at least 10 seconds, and depends only on local DNA template composition and sequence. (ii) At the point of pausing a potential binding site for a rho hexamer must exist on the proximal portion of the nascent transcript. This putative binding site is proposed to be 70 to 90 nucleotides in length, to be relatively unencumbered by stable secondary structure, and (other than a requirement for some cytosine residues) to be non-sequence-specific. (iii) Binding to this site activates the RNA-dependent ATPase of rho, leading to termination at the site of polymerase pausing by presently unknown processes.

In this lecture recent studies bearing on the mechanism of each of the above steps will be presented, and the present state of our overall knowledge of rho-dependent transcription termination will be summarized. (These studies have been supported by USPHS research grants GM-15792 and GM 29158 and by USPHS research training grant GM 07750.)

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Terminator Selection; Transcriptional Controls A

0885 TRANSCRIPTION OF HISTONE H5 GENE DOES NOT TERMINATE AT A UNIQUE SITE. Markus Affolter and Adolfo Ruiz-Carrillo, Cancer Research Center and Department of Biochemistry, School of Medicine, Laval University, Quebec, Canada

Control of transcription termination has been shown to be an important mechanism in procaryotic gene expression. However, much less is known whether a similar regulation exists in eucaryotic gene transcription. To define the transcriptional unit of the H5 gene, DNA fragments covering the whole genomic locus were subcloned into M13. Single stranded recombinants were then fixed on nitrocellulose filters and hybridized with *in vitro* nuclear transcripts of anemic chicken red blood cells. The results obtained showed that 1) the sequences immediatly upstream of the 5' end of H5 mRNA are not transcribed, 2) only the coding strand serves as template for RNA polymerase II 3) sequences as far as 470 bp downstream of the polyadenylation site are actively transcribed whereas no transcriptional activity is detectable 80 bp further downstream. To identify more precisely the region of transcription termination, overlapping single-stranded probes of high specific activity were annealed to total anemic chicken RNA and digested with S1 nuclease. The results obtained with this method showed that transcription *in vivo* continues beyond the site of polyadenylation, but no unique termination site was found. Interestingly, a 150 bp region situated 100 bp beyond the polyadenylation site was found to be extremely sensitive to DNase I digestion in erythrocyte chromatin. Nucleotide sequence analysis of the same region revealed a stretch of 200 nucleotides which is capable of forming stable secondary structures. We are now examinating whether these features influence the process of termination of transcription.

0886 EXPRESSION OF PRO α2(I) COLLAGEN MINIGENE. Sirpa Aho, Mitchell Finer and Helga Boedtker. Harvard University, Cambridge, MA 02138

We have constructed a minigene of chicken pro α2(I) collagen gene to study a) the formation of the 3' end of the mRNA and the use of the four different AAUAAA signal sequences, b) the correct splicing of the 1.9 kb intron 1 and 0.6 kb intron 2 resulting in the addition of the 11 bp exon 2 to the mRNA, and c) the role of GC-rich intron 1 in the regulation of gene expression.

The minigene extends from BamHI site at -1086 to the HindIII site in intron 3, which is ligated to HindIII site in intron 51, including exons 1, 2, 3 and 52 and introns 1 and 2, 200 bp of intron 3 and 400 bp of intron 51 and 500 bp of DNA sequences after the last poly-A addition site. It is ligated to NdeI-BamHI fragment (from 2297 to 375) of pBR322.

Preliminary experiments including transient transfection of human 293 cells with the minigene, indicate that this gene may be accurately transcribed and results mainly in fully spliced transcripts, 680 and 1150 bp in size, with the 680 bp species predominating. Small quantities of unspliced transcripts 3800 and 4250 bp, could also be detected. To determine sequences necessary for correct generation of each 3' end and to study the use of poly-A addition sites in 3' end deletion mutants, DNA sequences from the 3' end of the gene have been inserted into SP64 vector to make anti-RNA probes.

0887 cAMP-CRP AS A NEGATIVE REGULATOR FOR TRANSCRIPTION, Hiroji Aiba, Kyoto University, Kyoto 606, Japan

Studies on the regulation of *E. coli* adenylate cyclase gene revealed that cAMP-CRP acts as a transcriptional repressor for the *cya* expression both *in vivo* and *in vitro*. Quantitative analyses of *cya* mRNA by a dot blot and an S1 digestion assays indicated that crp⁻ cells produce about 5-fold more *cya* mRNA than do wild type cells. The level of *cya* mRNA in cells was dramatically reduced by introducing a *crp* plasmid and by adding cAMP exogenously. *In vitro* transcription of purified DNA fragments containing the *cya* promoter region gave direct evidence that the transcription of *cya* gene is specifically inhibited by cAMP-CRP. DNAase footprinting showed that cAMP-CRP interacts with a unique site, containing a consensus CRP binding sequence, which overlaps with RNA polymerase binding region. In addition, it was shown that the RNA polymerase-promoter interaction is altered in the presence of cAMP-CRP. It is concluded that cAMP-CRP inhibits the *cya* transcription by preventing the functional binding of RNA polymerase to the promoter.

0888 INSULIN REGULATION OF PROTEIN BIOSYNTHESIS IN 3T3 ADIPOCYTES: REGULATION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE mRNA LEVELS, M. Alexander & H.M. Goodman, Massachusetts General Hospital, Boston, MA 02114

Enzymes whose relative rate of synthesis is altered by insulin are being identified to serve as markers to define how gene expression is altered by insulin (i.e., translation or transcription). Once the locus of insulin's action is identified, one could identify the enzymes which regulate these insulin-sensitive pathways and define areas in the purified DNA of these marker enzymes which might confer inducibility on constitutive genes. To identify enzymes modulated specifically by insulin as the sole hormonal pertubant, insulin's effect on biosynthesis was examined in differentiated 3T3-L1 and 3T3-F442A adipocytes. Independent of insulin's ability to hasten expression of the adipocyte phenotype, insulin altered the relative rate of synthesis of specific proteins. We identified a M_r 33,000 protein as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) by purification of activity and comparison of peptide maps with that of rabbit skeletal muscle enzyme. Exposure of 3T3 adipocytes to insulin specifically increases GAPDH activity, protein content and synthetic rate 2-3 fold, accounted for by an increase in relative rate of synthesis of GAPDH within 4 hr of insulin exposure, preceded by a proportionate increase in hybridizable GAPDH mRNA. These responses could not be demonstrated in unconverted 3T3 preadipocytes even at 250 mU/ml. The increase in GAPDH mRNA (20 fold at 7 hr) as well as GAPDH biosynthesis (2-5 fold at 24 hr) in 3T3 adipocytes could be demonstrated with physiologic concentrations of insulin. Thus insulin can increase the synthesis of certain 3T3 adipocyte proteins by altering the cellular content of a specific mRNA.

0889 ATTENUATION IN SV40 AS A MECHANISM OF TRANSCRIPTION-TERMINATION BY RNA POLYMERASE II, Yosef Aloni, Nissim Hay and David King, Weizmann Institute of Science Rehovot. Israel & Princeton University, Princeton, N.J. 08544.

In eukarvotes, despite several attempts, transcription-termination sites of RNA polymerase II transcripts have not been clearly identified. Consequently, the regulatory elements involved in the process of transcription-termination and antitermination could not be properly investigated, and they are almost unknown.

We have developed a system of isolated nuclei in which an efficient transcription-termination at the SV40 attenuation site is occuring. This system allows to start defining the regulatory elements involved in the mechanism of termination and antitermination.

Experiments will be described indicating the involvement of the SV40 agnoprotein in enhancing attenuation, of a heat labile nuclear factor and RNA secondary structure involved in the process of transcription-termination and of an heat stable nuclear factor involved in the process of antitermination.

0890 REGULATION OF THE GENES RESPONSIBLE FOR PARTITION OF P1 PLASMID DNA, Stuart J. Austin and Ann L. Abeles, NCI-Frederick Cancer Research Facility, LBI-Basic Research Program, Frederick, MD 21701

The *par* region of the P1 plasmid acts rather like the centromere of a eukaryotic chromosome, ensuring that one of the two products of DNA replication is placed in each daughter cell during cell division. In addition to containing the apparent recognition site for this process, the *par* region contains an operon that encodes two proteins that are essential for partition. The balance between the concentrations of the *par* region components is critical and is maintained by a tight autoregulatory loop. The complete sequence of the region has been determined. There is only one probable candidate for the *par* operon promoter. Between this sequence and the putative Shine-Dalgarno sequence for the first *par* cistron lies an extremely AT-rich stretch that includes a 20-bp palindrome. A similar AT-rich region is present in the partition site, downstream of the operon. We present evidence that *par* proteins interact at the upstream site to effect autoregulation and at the downstream site to promote partition.

Research sponsored by the National Cancer Institute, DHHS, under contract No. N01-CO-23909 with Litton Bionetics, Inc.

0891 SEGMENTAL DIFFERENCES IN STABILITY WITHIN THE POLYCISTRONIC *rxcA* TRANSCRIPT RESULT IN DIFFERENTIAL EXPRESSION OF PHOTOSYNTHESIS GENES

Joel G. Belasco, J. Thomas Beatty, Camellia W. Adams, Alexander von Gabain, and Stanley N. Cohen, Stanford University, Stanford, CA 94305

The molecular basis for the 10- to 25-fold difference in expression of the photosynthesis genes within the *rxcA* operon of *Rhodopseudomonas capsulata* has been investigated. Differential expression has been found to result largely from the disparate stabilities of the component segments of the polycistronic *rxcA* transcript. This 2.7 kb transcript encodes the light-harvesting B870 polypeptides and the reaction center L and M subunits. The 3' portion of the *rxcA* transcript is quickly degraded to produce two slowly decaying 5' mRNA remnants (0.50 and 0.49 kb in length), both of which encode only the B870 polypeptides. The greater stability of the 5' segment of the transcript accounts for most of the excess of B870 polypeptides over reaction center proteins found in vivo. Degradation of the 3' portion of the *rxcA* transcript stops at either of two alternative stem-and-loop structures, which apparently act as barriers to 3' exoribonucleases and thereby protect the upstream RNA segment.

0892 EXPRESSION OF HUMAN TISSUE PLASMINOGEN ACTIVATOR IN MOUSE CELLS

Mary M. Bendig, Paul E. Stephens, Christopher C. Hentschel, Celltech Limited, Slough SL1 4DY, U.K.

We have evaluated the usefulness of different promoters and terminators for expressing the human tissue plasminogen activator (tPA) gene in mouse C127 cells. The tPA cDNA gene was linked by transcriptional fusion to promoters such as the mouse metallothionein (MMT) promoter, the Rous sarcoma long terminal repeat (LTR), or the mouse Moloney leukaemia virus LTR. Terminators were provided by linking the 3' end to DNA fragments containing the MMT polyA site or the SV40 early polyA site. The reconstructed tPA genes were inserted into bovine papilloma virus (BPV) vectors containing the BPV genome, the MMT gene, and bacterial plasmid DNA. Cells were transfected with the DNA constructs and tPA-producing foci were easily identified using a novel application of the fibrin-agarose assay method for detecting tPA activity. Stably-transformed, tPA-producing cell lines were analyzed in detail for the physical state and copy number of the tPA gene in the cells, the level and authenticity of tPA transcription, and the amount of active tPA protein being secreted.

0893 SYNTHESIS OF MOUSE ANTIBODY PEPTIDES IN *ESCHERICHIA COLI*

R.E. Bird, D.J. Graham, L. Martarano, M. Mattingly, S. Pope and D. Scandella, Genex Corporation, Gaithersburg, Maryland 20877

We have studied the expression of a γ1 (heavy) chain and a κ (light) chain from a mouse monoclonal antibody (IgG) to bovine growth hormone in *Escherichia coli*. The two chains were expressed as *met*-mature peptides in pBR322 derived plasmids. We inserted the sequence ATCGATG at the 5' end of the mature sequences to provide an ATG for translation initiation and a *ClaI* site. The *ClaI* site was used to position a hybrid λ phage regulatory region, O_L/P_R, in front of the coding sequence. Strains carrying the λ C_I857 mutation in the host chromosome were used to induce synthesis of the IgG peptides by raising the culture temperature of 42°. The κ chain is synthesized as 12-15% of the total cell protein and the γ chain as 0.1-0.3%. The difference appears to be the rate of translation initiation. Experiments to alter the rate of translation initiation by changing the sequence of the 5' end of the mRNA for the κ chain are in progress. In addition we will discuss the effects of removing the 3' untranslated region of the clones on their expression.

0894 COMPLEMENTATION OF SV40 AGNOPROTEIN MUTANTS BY AGNO-EXPRESSING CELL LINES. Susan Carswell and James C. Alwine. Department of Microbiology/G2, Univ. of Penna., Philadelphia, PA 19104.

We are studying the function of the agnoprotein of SV40, which is encoded in the leader region of late viral mRNA. Previous studies indicated that agnoprotein plays a role in late transcriptional regulation, although the precise mechanism of this control is not understood. In order to define the specific role of agnoprotein in the SV40 lytic cycle, we have constructed monkey CV-1P cell lines in which the agnogene, under the control of a retrovirus LTR, is stably integrated and constitutively expressed. Viruses with point and deletion mutations in the agnogene, which make small plaques in normal CV-1P cells, produce wild-type sized plaques in the agnoprotein producing cell lines. Southern blot analysis of the viral DNA extracted from agno-producing cell lines infected with agno mutants demonstrates that the agnogene has not recombined into the mutant viruses to reconstruct wild-type virus. Thus, the agnoprotein from the cell lines restores wild-type plaquing size in trans, indicating a positive role of the agnoprotein with respect to plaque size. Transcriptional studies will be discussed.

0895 SPECIFICITY OF THE DAUNOMYCIN-DNA INTERACTION. J. B. Chaires, The University of Mississippi Medical Center, Jackson, MS 39216-4505.

The anthracycline antibiotic duanomycin is widely used in cancer chemotherapy. The drug is a potent inhibitor of both transcription and DNA replication. Previous results from this laboratory have shown that duanomycin will bind preferentially to alternating purine-pyrimidine DNA sequences. Since these are the sequences able to undergo the transition from B form DNA to the left handed Z form, the effect of daunomycin on the B to Z transition has been examined. Daunomycin inhibits the rate B to Z transition. Binding of the drug to poly d(G-C) under solution conditions that favor the Z form is cooperative, a finding consistent with a model in which the drug preferentially binds to the B form, and allosterically converts Z DNA to an intercalated B form. The allosteric conversion of Z DNA back to the B form has been directly demonstrated by circular dichroism, sedimentation, and enzymatic methods. The conversion of Z DNA to the B form is strongly dependent on ionic strength, and under some conditions as little as one drug molecule for every 25 b.p. is sufficient to completely convert the polymer to the B form. This is a striking demonstration of how a small molecule may exert long-range allosteric effects on DNA conformation. These observations are important for understanding the molecular mechanism by which daunomycin acts, and may be of interest as an indication of long-range conformational effects of molecules on DNA that may be important in transcriptional control. Supported by NCI Grant CA 35635-01A1.

0896 AMS INHIBITION OF AN EARLY ESTROGEN INDUCED mRNA SPECIES IN HUMAN BREAST CANCER CELLS. Jolanta J. Cholon, Lee F. Allen, Department of Pathology, UMDNJ, New Jersey Medical School, Newark, New Jersey 07103.

Aminonucleoside of puromycin (AMS), a selective inhibitor of the growth of estrogen-responsive as opposed to estrogen-unresponsive mammary tumor cells, has been found to inhibit a particular class of poly(A)-containing mRNA species in estrogen-responsive cells *in vitro*. Estrogen-responsive, MCF-7 cells and estrogen-unresponsive, BT-474 cells were maintained for one day prior to hormonal stimulation on media supplemented with hormone-stripped serum. In a parallel series of experiments, the stripped media was replaced for both cell lines with media supplemented with a physiological concentration of estradiol (10^{-9} M), and simultaneously labeled with [^{3}H]-adenosine for 30 minutes. Cytoplasmic RNA was extracted from magnesium precipitated polysomes by SDS-phenol-chloroform extraction procedures, and poly(A)-containing species were separated by poly(U)-sepharose column chromatography, eluting with increasing concentrations of formamide. The mRNA species were fractionated by sucrose gradients, and assayed for radioactivity through liquid scintillation counting. Inclusion of AMS (100 ug/ml) in the stimulating medium resulted in inhibition of a particular class of poly(A)(+) mRNA in estrogen-responsive cells, within 1/2 hr, while this class was resistant to AMS in estrogen-unresponsive cells. These results indicate some alteration in the mRNA metabolism of estrogen-unresponsive mammary tumor cells, and point to a possible growth regulatory function for this AMS resistant class of mRNA in these cells. (Supported in part by a grant from the Foundation of UMDNJ.)

Sequence Specificity in Transcription and Control

0897 NERVE GROWTH FACTOR INDUCES ORNITHINE DECARBOXYLASE BY CHANGING THE mRNA LEVEL,

P. Coffino, S. Dana, L. McConlogue, E. Shooter* and S. Feinstein*, UCSF, San Francisco, CA and Stanford U., Stanford, CA*

The activity of mammalian ornithine decarboxylase (ODC) changes significantly and promptly in response to multiple effectors of cell growth and differentiation. The availability of a cDNA probe for mouse ODC has facilitated study of the mechanism of induction (PNAS 81:540 (84)).

Nerve growth factor (NGF) causes differentiation of cultured rat PC12 pheochromocytoma cells and induces ODC activity about 10-fold within 4 hours. Both time-course and dose-response experiments indicate an excellent concordance between ODC activity and the level of ODC mRNA. A substantial (but lesser) degree of induction of mRNA by NFG is seen when protein synthesis is inhibited by cycloheximide. We conclude that most or all of the induction lies at the level of mRNA and that the action of NGF is not dependent on protein synthesis.

0898 TARGET SITES FOR POSITIVE AND NEGATIVE REGULATORY ELEMENTS CONTROLLING EXPRESSION OF AN INDUCIBLE EUCARYOTIC GENE.

R.A. Sumrada & T.G. Cooper, Department of Microbiology & Immunology, University of Tennessee Center for the Health Sciences, Memphis, Tennessee 38163.

Transcription of the **CAR1** & **CAR2** genes in **S. cerevisiae** is induced by arginine. Both positive and negative (**CAR80**) regulatory elements have been demonstrated to control expression of the genes. Our objective was to localize the upstream target sites that interact with these elements. The negative element target site was identified by a cis-dominant mutation causing constitutive **CAR1** expression. This mutation was shown to be a single C to G transversion at position -153. A 13 bp deletion covering position -153 also generates the constitutive phenotype. Since **CAR1** and **CAR2** are similarly regulated, the sequence containing the C-G transversion should be present in the 5' flanking regions of both genes. It is. Deletion analysis identified a positive element target site required for expression of **CAR1**. Deletion of this site, situated 5' to position -200, resulted in complete loss of **LACZ** expression in a **CAR1-LACZ** fusion. These data suggest that **CAR1** expression is regulated by positive and negative control elements whose targets are situated in the order: positive site-negative site-TATAT, and raise the possibility that action of the positive element is blocked or neutralized by interaction of the negative element with its corresponding site.

0899 Termination of the E1A Adenovirus Transcription Unit by Insertion of the Mouse β-Major Globin Terminator Element.

E. Falck-Pedersen[1], J. Logan[2], T. Shenk[2] and J. E. Darnell, Jr.[1]. [1]Rockefeller Univ. New York, N.Y. 10021, [2]Princeton Univ., Princeton N.J.

In induced erythroleukemia cells, transcription of the β globin gene terminates in a region 600-1500 nucleotides downstream from the poly(A) site (Citron et al. Nuc. Acids Res. in press). To determine whether this region of the mouse DNA functions to terminate transcription when moved to another genomic site, portions of the putative termination region have been inserted into the adenovirus (type 5) chromosome. The present series of viral insertions were made within the second exon of the E1A transcription unit. Analysis of RNA labeled either in isolated nuclei or in whole cells early after infection with reconstructed viruses indicated that transcription is terminated if the inserted DNA contains the globin poly(A) site plus an additional 1395 nucleotides downstream. The orientation of the insert must be in same direction with respect to transcription as in the β globin transcription unit. Insertion of this segment of DNA in the reverse orientation does not result in transcriptional termination. In addition to halting transcription within the E1A transcription unit, the insertion of the terminator region had a negative cis effect on the E1B transcription unit which begins 363 base pairs downstream from the globin insert. The E1B transcription unit was the only early gene affected, and complementation of the virus containing the functioning terminator region within a functional E1A did not restore transcription of the E1B gene. Late in infection following DNA replication and amplification of the number of functional transcription units, the terminator efficiency is slightly reduced, and E1B transcription is recovered.

Sequence Specificity in Transcription and Control

0900 PURIFICATION AND STRUCTURE OF A CHROMATIN ASSOCIATED PROTEIN,

Rodrigo Franco, M.G. Rosenfeld, University of California, La Jolla ,CA 92093

Our lab is working in the general area of peptide hormone regulation of gene expression. We have identified a 25,000 Dalton chromatin associated protein whose increased phosphorylation correlates with the increase in prolactin gene transcription seen upon treatment of GH4 rat pituitary tumor cells with thyrotropin releasing hormone and certain other hormones and pharmacologic agents. I have purified this protein (BRP for basic regulated phosphoprotein) and obtained a partial amino acid sequence. Search of protein sequence libraries indicate that BRP is a previously uncharacterized protein with no significant homologies with any other classes of proteins. Current work is focusing on obtaining further amino acid sequence and synthesizing one or more long oligonucleotides with which will be obtained a cDNA clone which will be sequenced to obtain the entire structure of the protein. Subsequently, monoclonal antibodies currently being raised to BRP and anti-sense message derived from the cDNA clone will be used to try to link this protein directly to transcriptional regulation of the prolactin gene.

0901 MUTATIONAL ANALYSIS OF THE ANTITERMINATION N PROTEIN OF COLIPHAGE LAMBDA,

Naomi C. Franklin, University of Utah, Salt Lake City, Utah 84112

The N protein of coliphage lambda is being extensively mutagenized in an effort to understand the structure of the protein in relation to its known functions. Interacting with the E. coli transcription complex subsequent to transcription initiation, the small N protein (107 amino acids) functions as an antiterminator, allowing polymerase to ignor most termination signals. N protein may interact with polymerase through intermediary host transcription proteins: a mutation in host nusA protein blocks N function, but can be compensated by mutation within N (D. I. Friedman laboratory). N protein also needs to recognize specific genome sequences in object operons. So far, regions essential for total function or for nusA interaction have been identified by mutation within N.

Franklin, N. C., J. Mol. Biol. in press, 1984: The "N" transcription-antitermination proteins of bacteriophages lambda, Ø21 and P22.

0902 *IN VITRO* AND *IN VIVO* MRNA SPLICING OF THE ADENOVIRUS 2 LATE TRIPARTITE LEADER,

Greg A. Freyer, Kathy E. O'Neill, Kathleen A. Tohill, and Richard J. Roberts, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Our *in vitro* mRNA splicing studies have concentrated on using a substrate that contains the first and second leader sequences of adenovirus 2 (Ad2) late mRNA separated by a truncated version of the first intron containing only 86 nucleotides. This substrate is synthesized from a plasmid in a HeLa whole cell extract. This transcript when spliced in a "Dignam" extract and analyzed on a 10% acrylamide urea gel produces the following: the 41 nucleotide-long first leader, final splice product, a 118 nucleotide-long fragment, and a band whose mobility depends on the length of the runoff transcript. The 118 nucleotide band behaves like a circular RNA and, by fingerprint analysis, has been shown to be contained within the intron. This RNA comprises the circular portion of the lariat that has been described by others, but does not contain the handle. No other circular molecules are detected. The band whose mobility depends on the length of the runoff corresponds in size to the distance from the point at which the circle forms to the end of the runoff. This implies that linear RNAs combine to form a final splice product and that lariats are not obligate intermediates in splicing.

Our *in vivo* studies have examined the fate of the first and second introns from the Ad2 tripartite leader from HeLa cells during late infection. Northern analysis of nuclear and cytoplasmic RNAs has shown that both introns accumulate in the cytoplasm, are not detected in the nucleus, do not contain poly(A) and are seen as multiple bands, suggesting either different forms of the RNA, i.e. linear, circular or branched molecules, or processed intermediates. Structural analysis of these RNAs is currently in progress.

0903 EXPRESSION OF A HUMAN DIHYDROFOLATE REDUCTASE MINIGENE, Merrill E. Goldsmith, Carolyn A. Beckman, and Kenneth H. Cowan, Clinical Pharmacology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

DNA sequences from the human dihydrofolate reductase (DHFR) gene were isolated from an MCF-7 breast cancer cell line containing amplified DHFR genes. These genomic sequences in conjunction with human DHFR cDNA sequences were used to construct a functional minigene. Calcium phosphate mediated transfer of the minigene into DHFR gene deleted Chinese hamster ovary cells converts the cells to a DHFR$^+$ phenotype with a frequency of 0.12%. This transfection frequency was obtained without added enhancer sequences. Individual clones of minigene transfected cells contain 20-30 minigene copies. Analysis of mRNA from transfected cells shows that a 3.8 kb DHFR mRNA is synthesized. The DHFR gene in the MCF-7 human cells synthesizes several mRNAs including a 3.8 kb species. These mRNAs are produced by multiple polyadenylation sites in the 3' nontranslated region of the gene. The amount of DHFR protein produced in the transfected cells is similar to that produced by the wild type MCF-7 cell line. DHFR expression is controlled by modulators which function at transcriptional or post-transcriptional levels. As in normal cells, DHFR levels in minigene transfected cells are regulated in response to growth modulation by serum and amino acids. Analysis of a series of minigene deletion mutants has begun. Our results suggest that DHFR regulation by these growth modulators is caused by DNA sequences 5' to the start of transcription or in intron 1 and not by sequences in the long 3' nontranslated portion of the gene.

0904 MODEL SYSTEM FOR *IN VITRO* PROCESSING OF tRNA PRECURSORS FROM *BACILLUS SUBTILIS*, Christopher J. Green and Barbara S. Vold, SRI International, *Biomedical Research*, Menlo Park, CA 94025

A cluster of 21 tRNA genes from *B. subtilis* has been cloned into pSP64. This plasmid has the strong, highly specific promoter for SP6 RNA polymerase. Transcripts have been produced *in vitro* from this construct containing all 21 tRNA genes. The template can also be shortened by cleavage with appropriate restriction enzymes to produce transcripts containing either 1 or 6 tRNAs. These transcripts have been processed *in vitro* by RNase P and the catalytically active component of the enzyme, P-RNA. A transcript of the same gene cluster cloned into pSP65, in the reverse orientation, produces a transcript which is not cleaved by either P-RNA or the holoenzyme attesting to the specificity of the reaction for tRNA precursors. The reaction products from the 6 tRNA containing precursor have been characterized by chemical sequencing analysis and shown to be processed correctly at the 5'-terminus of each tRNA.

0905 MAPPING OF THE 5' TRANSCRIPTION INITIATION SITE IN THREE INSULIN-EXPRESSING TISSUES. Nancy Z. Guggenheim and M. Alan Permutt, Washington University School of Medicine, St. Louis, Missouri 63110.

Proinsulin mRNA was analyzed by RNA blot hybridization in three insulin-expressing tissues from the rat, adult pancreas, an insulinoma cell line and fetal pancreas. The proinsulin mRNA transcripts from the tumor cell line and fetal pancreatic tissue were estimated to be respectively 100 bases and 50 bases larger than the adult pancreatic transcript. It has been shown that glucose is an important regulator of proinsulin mRNA *in vivo*. There is known to be a marked increase in the concentration of proinsulin mRNA and insulin in the developing rat neonate although plasma glucose levels are quite low. Expression of proinsulin mRNA independent of glucose levels is also found in insulinoma tissue. In addition, there is a second TATA sequence upstream of the putative start site in rat insulin gene II. These observations, including the fact that the transcription initiation site(s) has never been mapped in these tissues, suggested that alternative promoter sites may be important in control of initiation of gene transcription. To map the 5' end of the gene, primer extension was performed using a synthetic oligonucleotide primer complementary to the first twenty bases of the coding portion of the two rat insulin genes. The extended products of the proinsulin mRNAs from the three tissues were identical indicating that at least 95% of proinsulin mRNA transcription occurs at the putative start site. The 3' ends of the proinsulin mRNA transcripts were evaluated by ribonuclease H digestion and it was shown that the noted size differences could be accounted for by different length poly A tails. Experiments are currently in progress to define the potential role of poly A tails in stability and physiologic regulation of proinsulin mRNA.

0906 *IN VITRO* MUTAGENESIS OF THE THREONINE REGULATORY REGION OF *ESCHERICHIA COLI*,

R.I. Gumport, Ali Roghani, and J.F. Gardner, University of Illinois, Urbana, IL 61801

We have incorporated a dG residue in place of a dA residue at position -82 of the leader sequence of the threonine operon of *E. coli*. This mutation destroys the translation stop codon of the putative leader peptide. The stop codon resides in a region of RNA that is thought to be involved in the formation of an antiterminator stem-loop structure when the cell has ample Thr and Ile. The consequence of translation of the leader peptide to the stop codon is to prevent the antiterminator from forming thereby allowing the transcription terminator stem-loop structure to form and prevent the expression of the operon structural genes. In the mutant, translation of the leader peptide would be expected to continue to the next downstream inframe stop codon. Such a codon occurs 36 nucleotides away and lies in the region of RNA that is thought to form the transcription terminator. An octadecamer was used as a primer on a template of *thr* control region DNA that had been cloned in M13mp9. The point mutant was transferred onto a mini-ColE1 plasmid containing the *thr* regulatory region fused to the *lac Z* gene. The mutated *thr-lac Z* fusion was cloned into λgt4 so that lysogens could be formed. Assays of β-galactosidase in single-copy lysogens indicate that the mutation causes a 2- to 3-fold increase in the expression of the enzyme in the presence of Thr and Ile. These data may be explained by an attenuation model in which the leader peptide coding region is translated and, in the case of the mutant, translation to the new stop codon precludes the transcription terminator structure from forming thus leading to expression of the gene. This work was supported in part by NIH GM 28717.

0907 THE DNA SEQUENCE OF THE *lacA* GENE AND THE 3' END OF THE LACTOSE OPERON OF *Escherichia coli*.

Matthias A. Hediger, David F. Johnson, Donald P. Nierlich and Irving Zabin, University of California, Los Angeles, CA 90024

The lactose operon of *Escherichia coli* has been one of the most intensively studied genetic systems and yet until now its DNA sequence has not been completed. Furthermore, there are still important features to be understood concerning the transcriptional and translational regulation of the *lac* genes. The DNA sequence of the *lacA* gene and the 3' end of the *lac* operon is presented here. DNA sequencing was performed using the Sanger dideoxy sequencing technique. DNA fragments were produced by cleaving plasmid pGM 8, a PBR322 derivative which contains a *lac* DNA segment. The fragments were separated using a novel apparatus for preparative gel electrophoresis and then used for cloning into the M13 vectors mp 18 and 19. The DNA sequence at the 3' end of the *lac* operon also includes a fragment downstream from the *lacA* gene. This region has been analyzed for secondary structures of the mRNA which may represent signals for termination of transcription. A stable hairpin structure may form 15 nucleotides downstream from the 3' end of the *lacA* gene.

0908 TRANSCRIPTIONAL CONTROL OF GENE EXPRESSION BY OLIGODEOXYNUCLEOTIDES COVALENTLY LINKED TO INTERCALATING AGENTS,

Claude Hélène[*], Tula Behmoaras-Saison[*], Aurelio Zerial[∞] Ulysse Asseline[×] and Nguyen T. Thuong[×], [*]Laboratoire de Biophysique, Muséum National d'Histoire Naturelle, INSERM U.201, CNRS UA 481, 75005 PARIS, [∞]Centre de Recherche de Vitry, Rhône-Poulenc Santé, 94400 VITRY, [×]Centre de Biophysique Moléculaire, 45045 ORLEANS, France.

We have recently synthesized a new family of molecules in which an oligodeoxynucleotide is covalently linked to an intercalating agent(1-3). These ONBI (oligodeoxynucleotide-bridge-intercalator) bind selectively to the complementary sequence. The intercalating agent provides an additional binding energy thereby stabilizing the mini-duplex structures. ONBIs can be used to selectively control gene expression at the transcriptional or translational level. Using ONBIs with sequences complementary to the open region of a RNA polymerase-promoter complex it is possible to block gene transcription. Experiments carried out with the amp^R gene of pBR 322 will be presented.

The transcription of influenza virus RNAs can also be blocked by ONBIs whose sequence is complementary to the 3' sequence common to all eight viral RNAs.

(1) U. Asseline, N.T. Thuong & C. Hélène (1983) C.R. Acad. Sci. Paris, 297, 369-372.
(2) U. Asseline *et al.* (1984) EMBO J., 3, 795-800.
(3) U. Asseline *et al.* (1984) Proc. Natl. Acad. Sci. USA, 81, 3297-3301.

Sequence Specificity in Transcription and Control

0909 TRANSCRIPTIONAL ACTIVATION OF CELLULAR DNA ADJACENT TO RANDOMLY INTEGRATED RETROVIRUS DNA,

Steven A. Herman and John M. Coffin, Tufts U. Sch. of Med., Boston, MA

The long terminal repeats (LTRs) that flank integrated retrovirus DNA contain transcriptional control elements in an unusual arrangement. The polyadenylation signal is between the promoter "TATAA" box and the start site of initiation, and the polyadenylation site is at base 21. Normal viral transcripts are initiated in the upstream LTR and polyadenylated in the downstream LTR. Two other types of viral transcripts can be imagined: (i) downstream transcripts, initiated in the downstream LTR and elongated into the adjacent cellular DNA and (ii) readthrough transcripts initiated within the upstream LTR, but elongated past the normal polyadenylation site in the downstream LTR. Downstream transcripts have been shown to activate the c-*myc* gene in retrovirus induced lymphomas of chickens.

We used nuclease mapping techniques to further characterize viral transcripts that are elongated into adjacent cellular DNA in clonal and non-clonal populations of infected cells. We found readthrough RNA in tumors and fibroblasts infected in culture at relatively high levels, approximately 5% of viral transcripts. Downstream RNA was detected at lower levels in tumors, but not in infected tissue culture cells. Readthrough and downstream RNAs were polyadenylated.

These results indicate that a fraction of normal viral RNA escapes processing at the downstream LTR, but is elongated and polyadenylated at a downstream site, and suggest that DNA adjacent to proviruses could be transcribed by readthrough RNA.

0910 THE 3' ENDS OF U1 AND U2 SMALL NUCLEAR RNA ARE GENERATED BY TWO STEPS OF RNA PROCESSING,

Nouria Hernandez, Chung-Yee Yuo, Manuel Ares, Jr. and Alan M. Weiner, Molecular Biophysics and Biochemistry, Yale Medical School, New Haven, CT 06510.

Histone mRNA and small nuclear RNAs such as U1 and U2 are the only stable products of RNA polymerase II lacking a 3' terminal poly(A) tract. Work in other laboratories has shown that the 3' end of histone mRNA is formed by mRNA processing; the reaction requires a stem and loop structure as well as a CAAGAA signal located at a fixed distance downstream. Like histone mRNA, U1 and U2 have a 3' terminal stem and loop structure, but the stem and loop stuctures of the two genes differ in size and in sequence. Sequences downstream from the stem and loop are somewhat conserved among U1 and U2 genes from different species, but do not strongly resemble the histone CAAGAA signal. We have assayed normal and mutagenized human U1 and U2 genes using a variety of heterologous and homologous systems, including microinjection of the genes or labeled SP6 runoff transcripts into Xenopus oocytes, and transient expression in HeLa cells using SV40-based vectors. We can show that the 3' end of U2 RNA is generated by RNA processing in two distinct steps: cleavage of a longer precursor to an intermediate known as U2+10 (about 198 nt) and maturation of U2+10 to U2 (188 nt). In contrast to histone mRNA processing, we find that the 3' terminal stem and loop structure of U2 is not required for processing, and that an RNA sequence located between +6 and + 23 downstream from the mature 3' end of U2 is independently capable of directing an RNA cleavage at the U2+10 site. The processing of U1 snRNA appears to be substantially similar to that of U2.

0911 STRUCTURE AND EXPRESSION OF THE *NUSA* AND *NUSB* GENE IN *E.COLI*, AND FUNCTION OF THE *NUSB* PROTEIN,

Fumio Imamoto, Shunsuke Ishii, Toshio Maekawa and Takahiro Nagase, RIKEN (The Institute of Physical and Chemical Research), Wako-shi, Saitama, 351-01, Japan

The *nusA* and *nusB* genes of *E.coli* have been cloned in plasmid pBR322 by using genetic complementation as assay for the genes. The nucleotide sequence of these genes and their flanking regions were determined. The *nusA* gene consists of an operon including the genes for $tRNA_{f2}^{Met}$, a 15 kDa protein whose function is unknown, *nusA* protein and *IF2α* in this order from the promoter. In the DNA region between genes for the $tRNA_{f2}^{Met}$ and the 15 kDa protein, there are two inverted sequences followed in each case by a run of thymidines. These are the typical ρ-independent transcriptional termination signals. Expression of this operon is possibly regulated autogenously by the *nusA* protein. The *nusA* protein has significant similarity to the sigma protein in the chemical natures such as composition and distribution of amino acids in the polypeptide. The number of the *nusA* or *nusB* protein in a cell is suggested to be comparable with that of sigma protein. The structure of the flanking region and mode of expression of the *nusB* gene, and function of the *nusB* protein are also presented.

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0912 GENETIC ANALYSIS OF TWO S. CEREVISIAE RNA POLYMERASE GENES

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A genetic analysis of mRNA synthesis in S. cerevisie would be facilitated by the availability of mutant strains with defects in RNA polymerase II. The conservation of the structure of this enzyme through evolution enabled us to use the DNA of a D. melanogaster RNA polymerase II gene to identify and clone two related genes from yeast (Ingles et al. PNAS 81, 2157, 1984). One of these genes RPO21, encodes the largest, Mr 220,000, polypeptide of RNA polymerase II, the other RPR, encodes a smaller polypeptide. DNA sequence analysis of each of these genes has confirmed that both RPO21 and RPR share extensive sequence homology.

These DNAs are being used to create conditional lethal mutants by a technique of mutagenesis and gene replacement. By integrating mutagenized RPO21 plasmids bearing part of the structural gene and its 5' flanking sequence at the RPO21 chromosomal locus, several Ts RNA polymerase II strains have been isolated. One of these Ts RPO21 mutants was then used as parent in the selection of Ts^+ revertants. These Ts^+ revertants include several classes of second site suppressors, some which confer unique pleiotropic phenotypes on the cells. Some suppressor strains are unable to undergo meiosis and sporulation while others have acquired new Cs phenotypes. The characterization of these mutants may identify novel RNA polymerase-transcription factor interactions. (Supported by MRC, Canada).

0913 MITOCHONDRIAL TRANSCRIPTS ARE DIFFERENTIALLY EXPRESSED BETWEEN LIFE CYCLE STAGES OF TRYPANOSOMA BRUCEI

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Mitochondrial genes of the African trypanosome Trypanosoma brucei are differentially expressed between the procyclic and bloodstream stages. Several classes of mitochondrial gene transcripts were observed: 1)those present in the procyclic stage only (COX II), 2) those more abundant in the procyclic stage (apocytochrome b, COX I), 3) those more abundant in the bloodstream stage (mammalian URF4/5, other transcripts), and 4) those with similar abundance in both stages (mammalian URF1 and several small transcripts). Class 1 and 2 transcripts all correspond to the respiratory proteins. Many but not all transcripts exist as pairs, and differential expression is usually evident in only one transcript of the pairs. Large transcripts, presumably precursors for contiguous genes, are observed for some but not all transcribed regions. Small transcripts corresponding to flanking sequences of identified genes have in common purine rich sequences.

0914 MODULATION OF YEAST RNA POLYMERASE III TRANSCRIPTION BY 5'-FLANKING SEQUENCES.

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The intragenic promoter elements found in class III genes of all eukaryotes are not sufficient for efficient transcription by yeast RNA polymerase III. This conclusion is drawn from results of deletion analyses of a yeast $tRNA^{Leu}$ gene and comparisons of template activities of cloned Xenopus tRNA and 5S RNA genes as well as an adenovirus VA RNA gene. The heterologous genes all contain functional internal control regions, as evidenced by their transcription in Hela cell-free extracts, but are very weak templates for the yeast transcription apparatus. Yeast $tRNA^{Leu}$ genes with deletions in the 5'-flanking regions can form stable transcription complexes but apparently do not initiate transcription efficiently in a cell-free extract. The in vitro results have been verified in vivo using an amber suppressor form of $tRNA^{Leu}$ and several yeast strains bearing amber mutations. The ability of cloned $tRNA^{Leu}$ genes to effectively suppress mutants correlates well with the efficiency of transcription measured in vitro. Heterologous tRNA, 5S RNA and VA RNA genes vary in their ability to compete with yeast tRNA or 5S RNA genes in an assay designed to meaure stable complex formation. A conserved sequence has been identified in the 5'-flanking region of some, but not all, yeast tRNA genes. We postulate that this sequence may be used to enhance expression of specific tRNA species which are abundant in yeast. The sequence may act by facilitating initiation by the yeast form of RNA polymerase III.

0915 REGULATION OF p15A PREPRIMER RNA SYNTHESIS BY A PLASMID ENCODED PROTEIN, Syd Johnson, Vanderbilt University, Nashville, TN 37235

A gene has been identified on the plasmid p15A which is involved in regulating the level of transcription of the p15A preprimer RNA. The gene is contained on a 330 bp *RsaI* fragment of the plasmid. This fragment contains an open reading frame which is 63 percent homologous to that of the ColE1 *rop* gene. The 62 amino acid protein predicted by this open reading frame is 60 percent homologous to the 63 amino acid ColE1 Rop protein. When the region containing the p15A primer promoter and RNAI coding region is fused to the galactokinase gene, expression of galactokinase is decreased greatly by the presence of the p15A *rop* gene on a second plasmid in the same cell. The copy number of a p15A derivative in which the *RsaI* B fragment is deleted is increased only 50 percent over its *rop*$^+$ parent. We conclude that the p15A Rop protein regulates transcription of the preprimer RNA at a level after initiation of transcription of the preprimer. We propose a model in which RNAI inhibition of primer formation is mediated by two distinct modes of binding to the nascent preprimer transcript, one of which requires the Rop protein. In the absence of Rop, RNAI binding alters the secondary structure of the preprimer RNA in such a way that hybridization to the template at the origin is prevented. In the presence of Rop, RNAI binds in such a way as to lead to attenuation of the transcript.

0916 CELL CYCLE REGULATION AND GENE AMPLIFICATION OF DIHYDROFOLATE REDUCTASE, Randal N. Johnston and Robert T. Schimke, University of Calgary, Canada, and Stanford University, California

The frequency of amplification of the gene for dihydrofolate reductase (DHFR) is elevated when DNA synthesis is transiently inhibited (by chemotherapeutic agents such as methotrexate, hydroxyurea and aphidicolin) in cells *in vitro*. The inhibition of DNA synthesis results in a block in the progression of cells through the cell cycle, and cells therefore accumulate in S phase and at the G_1-S boundary. During the block, RNA and protein synthesis continue, and S phase specific enzymes, including DHFR, accumulate within the cell. When the metabolic block is removed, DNA synthesis resumes at levels in excess of controls. This is associated with the damage and fragmentation of chromosomes, and the generation of extrachromosomal DNA. In addition, such cells show an elevation in DNA content, and an enhancement of DHFR gene amplification and of MTX drug resistance. The magnitude of this response increases with duration of the block, and is in accordance with the accumulation of DHFR enzyme. We suggest, therefore, that treating cells with chemotherapeutic agents such as methotrexate prolongs the period of transcription of genes for S phase specific enzymes, including DHFR, and that this contributes to aberrant patterns of DNA replication and to gene amplification during recovery from drug inhibition.

0917 A NEW FAMILY OF ANDROGEN-INDUCIBLE RNAs IN MOUSE, Donna King, Lauren Snider and Jerry B Lingrel, Department of Microbiology and Molecular Genetics, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

We have identified a family of messenger RNAs whose abundance in mouse kidney and liver is regulated by testosterone. Some or all of these RNAs are present at constitutive levels in various other tissues. Northern blots of kidney RNA show six species ranging in size from 1350 to 2500 nucleotides. Characterization of cDNA clones and preliminary results of genomic DNA blots indicates that some of these mRNAs may be transcribed from the same gene, with the use of alternative polyadenylation sites explaining the observed differences in size. A single polypeptide of mol. wt. 42,000 daltons is translated from these pooled RNAs. Amino acid sequence as deduced from the cDNAs does not correspond to any known sequenced protein. Hence, we have termed this family of RNAs "MAK" (mouse-androgen-kidney). A polymorphic difference exists between some mouse strains. A B1 repetitive element is present in the 3' untranslated region of large MAK transcripts in DBA/2J mice. This repeat is precisely missing in the corresponding regions of C57BL/6J and BALB/cJ transcripts.

0918 MOLECULAR EVENTS FOLLOWING TRANSFECTION OF MONKEY CELLS BY CLONED c-DNA OF POLIOVIRUS TYPE-1.

KOPECKA,H., KEAN,K., CIRINIAN,S. and GIRARD,M. Institut Pasteur

Genomic c-DNA of poliovirus type 1 cloned in bacterial plasmids can induce a viral cycle when introduced into the monkey cells.However the infectivity is very low.
One can essentially increase the infectivity of such sequences by inserting them downstream of regulatory sequences of SV40. Eftect of these signals on the replication and transcription of poliovirus sequences and the molecular events following the transfection of monkey cells bu sych plasmids will be discussed .

0919 HORMONAL AND TISSUE SPECIFIC CONTROL OF THE EXPRESSION OF RAT α_{2u} GLOBULIN,

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My laboratory is investigating the hormonal, developmental, and tissue-specific control of the synthesis of a rat protein called α_{2u} globulin. This protein is synthesized in the liver, lachrymal gland, submaxillary gland, mammary gland and preputial gland of rats. α_{2u} is encoded by a multigene family and it appears that different gene sets are being transcribed in the different tissues. Further, the hormonal regulation in each tissue is distinct. We are investigating the hormonal control of α_{2u} synthesis by introducing cloned α_{2u} genes into tissue culture cell, which contain the appropriate hormone receptors, and we are studying the tissue specificity of α_{2u} gene expression in cell lines which are analagous to the tissues which express α_{2u} in vivo, and by purifiying tissue-specific transcription factors.

0920 TRANSLATION RELEASES PAUSED RNA POLYMERASE DURING TRANSCRIPTION OF THE trp ATTENUATOR REGION,

Robert Landick and Charles Yanofsky, Stanford University, Stanford, CA 94305

Expression of the trp operons of *E. coli* and other enteric bacteria is regulated by transcription attenuation. According to the current model of attenuation, formation of alternative secondary structures in the trp leader transcript controls termination at the attenuator. Translation of a leader peptide coding region containing tandem Trp codons governs formation of the alternative secondary structures. Ribosome stalling on the Trp codons promotes formation of a secondary structure that allows transcription of the operon, while ribosome movement to the stop codon causes transcription termination. For attenuation to be efficient, translation of the leader peptide coding region must be coupled to transcription of the leader region. A transcription pause site in the leader region may accomplish this synchronization by halting transcription until the translating ribosome releases the paused RNA polymerase. We have demonstrated that the translating ribosome does indeed release the transcription pause in the trp leader region during coupled transcription/translation. Plasmid DNA templates with wild type and mutant sequences incapable of directing leader peptide synthesis were analyzed for the quantity of steady state pause RNA during coupled transcription/translation reactions. The mutant templates produced levels of pause RNA 10-fold greater than wild type. All templates gave nearly identical pausing kinetics in a purified transcription system lacking translational machinery. Release of the paused transcription complex by translation of the leader peptide coding region was confirmed by adding the translation inhibitor kasugamycin to coupled reactions with the wild type template.

Terminator Selection; Transcriptional Controls B

0921 CYCLOHEXIMIDE SELECTIVELY ENHANCES INTERFERON α-INDUCED TRANSCRIPTION OF TWO GENES IN HUMAN FIBROBLASTS, BUT NOT IN HELA CELLS, A. C. Larner, A. Chaudhuri and J. E. DARNELL, Jr., The Rockefeller University, New York, N. Y. 10021

The treatment of cultured cells with interferons results in the synthesis of a number of unique polypeptides. We have recently characterized two cDNA's (pIF-IND1 and 2) which hybridize to two mRNA's whose transcription is activated when human diploid fibroblasts or HeLa cells are treated with type 1 interferons (Larner et al. PNAS, in press). In fibroblasts, enhanced transcriptional activity of the genes corresponding to IF-IND1 and 2 occurs with less than 30 min of interferon (IFN) treatment, and the transcriptional activation of these genes declines to basal levels after 8 hrs of IFN treatment. Treatment of fibroblasts with cycloheximide prior to the addition of IFN-α both increases the rate and the period during which these genes are transcribed compared to cells incubated with only IFN-α. The action of cycloheximide is specific, since it has no effect on the transcription rates of other genes not induced by IFN such as β-tubulin, arginine tRNA, or 28S ribosomal RNA. HeLa cells treated with IFN-α demonstrate a similar time course for the initial transcriptional activation of IFN1 and 2. However, these genes are actively transcribed for 24 hrs in the continuous presence of interferon. In addition, treatment of HeLa cells with cycloheximide prior to the addition of IFN is without effect on the rate of IFN-α-induced transcription of these genes. These results suggest that a factor(s) is present in fibroblasts, but not in HeLa cells that selectively prevents the transcription of the IFN-induced genes. Protein synthesis appears to be required for this factor(s) to exert its action in human fibroblasts.

0922 REGULATION OF THE SYNTHESIS OF BACTERIORHODOPSIN, Diane U. Leong, Mary Betlach, Felicitas Pfeifer and Herbert W. Boyer, Univ. California, San Francisco, CA 94143

We are investigating the regulation of the gene encoding bacterio-opsin (bop) which is the protein moiety of bacteriorhodopsin in the purple membrane of the archaebacterium, Halobacterium halobium. On limitation of the oxygen concentration, the level of bacteriorhodopsin in the cell increases up to five-fold (Sumper, M. & Herrmann, G., FEBS Lett 69:149-152, 1976). A second putative gene affecting the synthesis of bacteriorhodopsin has been identified as the site of insertions resulting in a Bop$^-$ phenotype. This gene (designated brp) is located 526 bp upstream of the bop gene and is transcribed in the opposite direction (Betlach, M. et al., Nucleic Acids Res., in press). The brp mRNA is complementary to the bop mRNA for 13 residues near the 5' terminus. As with the bop gene, translation begins at or near the 5' terminus of the mRNA and the 5' terminus of the mRNA is capable of forming a hairpin loop. The DNA sequences immediately upstream of the bop and brp genes have significant homology suggesting that they have common promoter and/or regulatory signals. Currently studies on the effect of oxygen tension on the levels of bop and brp mRNA indicate a significant increase under low oxygen tension, suggesting that the bop and brp genes are regulated to some extent at the level of transcription. The brp gene product was further characterized by constructing fusions of portions of the brp gene to the ompF and lacZ genes using an open reading frame vector. The tribrid proteins expressed from gene fusions can be used as an antigenic source for antibodies to the exogenous gene product (Weinstock, G., et al., PNAS 80:4432-4436, 1983). The use of the antibodies to isolate the brp gene product and to localize the protein in H. halobium cells is underway.

0923 A STUDY OF *pyrBI* REGULATION IN *E. coli* WITH DELETION MUTATIONS, Henry L. Levin and H. K. Schachman, University of California, Berkeley, 94720.

Aspartate transcarbamylase in *E. coli* is encoded by the *pyrBI* operon. The DNA sequence 5' to the beginning of the *pyrB* gene contains a short open reading frame and a G-C rich region of dyad symmetry followed by a string of eight T's, suggesting that an attenuation mechanism may be responsible for the 70-fold increase in expression observed upon pyrimidine starvation. This hypothesis was tested by subcloning the promoter region of the *pyrBI* operon so as to be immediately upstream of the *E. coli galK* coding sequence in the plasmid pKO1. Cells containing this plasmid, pPYRB10, exhibit 70-fold galactokinase regulation characteristic of *pyrBI* operon expression when starved for pyrimidines. Deletions constructed in the promoter region of pPYRB10 from the 3' side produced one plasmid that exhibits wild-type regulation and several plasmids that overexpress *galK* even in the presence of large pyrimidine pools. The functionally wild-type plasmid was sequenced and found to contain the entire region of dyad symmetry, including the 8 T's. The overexpression deletions lack the region of DNA with dyad symmetry or the 8 T's. However, all deletions of this kind still exhibit residual levels of regulation, even though one deletion extends past the entire sequence coding for the putative leader peptide up to the major promoter. These results support an attenuation model, but suggest that other mechanisms may also participate in the regulation of the *pyrBI* operon.

0924 EFFECT OF A GRAM-NEGATIVE BACTERIAL TRANSCRIPTION TERMINATOR IN THE GRAM-POSITIVE BACTERIUM BACILLUS SUBTILIS,

Chih-Kai Lin, Laurie A. Quinn and Raymond L. Rodriguez, U.C. Davis, Davis CA 95616.

In the last few years, a number of viral and bacterial terminators have been cloned, sequenced, and analyzed. The majority of these studies concern transcriptional termination in gram-negative bacteria. In contrast to the extensive study of termination in *Escherichia coli*, very little information is available on transcriptional termination in *B. subtilis*. A number of *B. subtilis* genes have been sequenced and reports indicate that some of these genes have terminator-like sequences similar to those observed in gram-negative bacteria. To discern whether *B. subtilis* in fact uses sequences similar to gram-negative terminators, we have used well-characterized *E. coli* terminators, T1T2, located at 3' end of rrnB gene to investigate this problem. Previous studies indicated that the promoterless *cat* cartridge derived from the transposon Tn-9 lacks transcriptional terminators. We fused T1T2 terminator to the 3' end of these cartridges to study the *in vivo* function of prokaryotic terminators in *E. coli* and *B. subtilis*. Northern blot and S1 nuclease mapping of recombinant plasmids carrying this terminator in *B. subtilis* demonstrate that the T1T2 terminator derived from *E. coli* rrnB operon functions in *B. subtilis*. Moreover, in *B. subtilis* cells harboring the plasmids carrying T1T2 terminator exhibited a two-fold increase in *cat* expression.

0925 CO-ORDINATE REGULATION OF A SET OF EARLY GENES IN *DICTYOSTELIUM DISCOIDEUM*,

Sandra K. O. Mann and Richard A. Firtel; University of California, San Diego; La Jolla, California 92093

We are examining a set of co-ordinately regulated genes expressed early in the developmental cycle of *Dictyostelium*. These genes are not expressed in vegetative cells and transcripts are first detectable at about 4 hours during normal development on filter pads. The complementary mRNA levels peak around 6 hours and then decrease. The expression of these genes is stimulated by pulsing cells with low levels of cAMP, a condition that mimics the *in vivo* pulsing during aggregation (4 to 8 hours into development). Expression is inhibited by high, continuous levels of cAMP, a condition found later in the developmental cycle at a time when the expression of these genes decreases *in vivo*.

We have examined the structure of these co-ordinately regulated genes, particularly the sequences at the 5' ends, and have identified regions of homology that may be involved in their regulation. We are attempting to better determine the function of these regions using the DNA-mediated transformation system developed in our laboratory.

0926 ANALYSIS OF NORMAL AND TRANSDUCED GENES OF CATECHOL METABOLISM IN DROSOPHILA DEVELOPMENT,

J. Lawrence Marsh, M. Erfle, D. Eveleth, P. Gibbs, D. Haymer and C. Leeds, University of California-Irvine, Irvine, California 92717

Although the foundation of our understanding of gene regulation comes from studies of the biochemical genetics of enzymes in a variety of metabolic pathways in prokaryotes and lower eukaryotes, most of the gene systems studied in metazoans have been non-catalytic structural genes which are terminal differentiation products. The 37B,C region on chromosome II in *Drosophila* contains a cluster of genes which affect cuticle formation and at least three genes which affect catechol and biogenic amine metabolism directly. At least one of these genes (dopa decarboxylase, *Ddc*) passes through five cycles of expression and repression in response to developmental and hormonal cues. The *l(2)amd* gene has been localized immediately adjacent to *Ddc* (0.002 map units) and like *Ddc* appears to affect cuticle formation and catechol metabolism. Genetic evidence suggests that *l(2)amd* and *Ddc* are required at similar stages of development. The gene structure, organization and transcription pattern of *l(2)amd* and *Ddc* are being examined in order to identify developmental regulatory sequences which they may share in common. These are being compared at the DNA sequence and functional levels. The functional autonomy of closely linked control sequences of *Ddc* was determined by examining 15 new transformed chromosomal locations of *Ddc* with respect to tissue and temporal regulation. The putative functional significance of the organization of this cluster of related genes is discussed.

0927 **An Indirect DNAase Protection Assay for Determining Specific Protein Binding Sites on DNA.** Glenn A. McConkey and Daniel F. Bogenhagen. State University of New York at Stony Brook, Stony Brook, NY, 11794.

An indirect footprinting assay has been devised based on the method of Galas and Schmitz (NAR 5:3157-3170, 1978). The modified technique detects the specific binding of a protein to unlabeled DNA following partial DNAase treatment of the complex as in a typical direct footprinting experiment. A specific radiolabeled primer is hybridized to DNAase treated templates. The annealed primer is extended to the DNAase generated termini of the fragments with AMV reverse transcriptase. This indirect footprinting method can detect protein binding to unlabeled supercoiled DNA and and is well suited to screening protein binding to large numbers of mutant DNAs without the requirement for end-labeling each DNA. The indirect footprinting assay has been used to survey TFIIIa binding to mutants of a 5S RNA gene. Sodium bisulfite was used to induce transitions in a 5S RNA gene. Mutants with transitions in the 3'end of the intragenic control region were scanned for TFIIIa binding ability by the indirect footprinting method. Additional studies using indirect footprinting have been conducted to study the binding of TFIIIa to supercoiled and relaxed circular DNA.

0928 MECHANISMS OF RHO-DEPENDENT TRANSCRIPTION TERMINATION, J.A. McSwiggen, J. Geiselmann, T. Yager, D.G. Bear* and P.H. von Hippel, Institute of Molecular Biology, University of Oregon, Eugene, OR 97403

We are investigating, by a variety of biophysical and biochemical approaches, the following three-step mechanism for rho-dependent transcription termination in *E. coli*. (i) Termination is initiated by the rho-independent pausing of RNA polymerase in regions of the template that are GC-rich or contain palindromic sequences. (ii) This is followed by the relatively non-sequence-specific binding of rho hexamers to a site on the nascent RNA that is largely devoid of secondary structure. (iii) This binding, in turn, activates the RNA-dependent ATPase of rho, which leads to termination.

We will report studies on: (i) the exact DNA sequence-dependence requirements for pausing, based on analyses of template constructions in which specific double-stranded sequences have been inserted immediately downstream of the pXJ002 consensus promoter; (ii) the stoichiometry and binding affinity of ATP and ATP-analogues to rho hexamers with and without bound RNA; and (iii) the binding affinity and resultant ATPase activity of rho hexamers by RNA oligomers of defined length, to establish binding mechanisms and inter-subunit cooperativity in rho ATPase activation. (Supported by USPHS Research Grants GM-15792, GM-29158 and GM-32055)

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0929 THE EFFECT OF CYCLIC-AMP RECEPTOR PROTEIN ON THE OVERLAPPING PROMOTERS OF THE *LAC* OPERON, Anita L. Meiklejohn and Jay D. Gralla, UCLA, Los Angeles, CA 90024

The effect of *E. coli* cyclic AMP receptor protein (CRP) on the regulation of transcription from the *lac* operon was studied using a purified *in vitro* transcription system. DNA fragments containing the wild-type *lac* regulatory sequences were used to confirm the existence of a second productive but inefficient promoter (P2) which overlaps both the normal *lac* promoter (P1) and the CRP binding site. In the absence of CRP, RNA polymerase forms a stable open complex at P2 effectively blocking P1 utilization. The addition of CRP subsequent to open complex formation at P2 actively removes RNA polymerase from this promoter and redirects it to the primary promoter. In the presence of CRP the half-life of open complex at P2 decreases from two hours to several minutes. The dependence of this process on cyclic-AMP indicates that P2 destabilization is due to site specific CRP binding. The short distance between the transcriptional start sites of the two promoters might suggest sliding as a model for this redirection. However, competition experiments suggest that only a small portion of the polymerase molecules redirected by CRP do so without dissociating from the DNA. We interpret these results as indicating that CRP is acting as a clearing protein to remove polymerase from a nonproductive competitor site.

Sequence Specificity in Transcription and Control

0930 ALTERNATIVELY PROCESSED CHICKEN TK TRANSCRIPTS ARE STABLY EXPRESSED IN NON-REPLICATING CELLS,

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The enzyme thymidine kinase is preferentially expressed in replicating cells. Tissues from ten-day chicken embryo, which contain many actively proliferating cells, express a readily detectable TK messenger RNA 2 kb in length. Tissues from hatching chicks, which contain relatively few proliferating cells, exhibit greatly reduced levels of this message. Using SP6-generated RNA transcripts as hybridization probes and longer autoradiographic exposure times, Northern blot analyses revealed a second, less abundant species of TK mRNA approximately 4.5 kb in length. Interestingly, levels of the 4.5 kb message are not markedly reduced in tissue from hatching chicks. Preliminary results suggest that the molecular basis for variation in TK mRNA size is differential splicing in the 3' non-translated portion of the transcript. Whereas the 2 kb mRNA is colinear with genomic sequences for at least 800 bp downstream from the translation stop codon, the 4.5 kb mRNA is colinear for only 73 bp and is then spliced to sequences 937 bp downstream from the translation codon. It is intriguing that removal of this 864 bp intron results in a transcript that is stably expressed in post-replicative cells.

0931 NUCLEOTIDE SEQUENCE AND EXPRESSION OF A MOUSE INTERLEUKIN-2 RECEPTOR cDNA.

Jim Miller, Thomas Malek, Warren Leonard,* Warner Greene,* Ethan Shevach, and Ronald Germain, LI, NIAID, and *MB, NCI, NIH, Bethesda, MD 20205

Interleukin-2 (IL-2) is a hormone-like growth factor, secreted by T lymphocytes following antigenic or mitogenic stimulation. In order for IL-2 to stimulate proliferation in T cells, it must first interact with a high affinity receptor, which is expressed on activated, but not resting lymphocytes. Therefore, IL-2 mediated growth of T cells is unique in that both the ligand and the receptor need to be induced. The IL-2 receptor is a membrane bound, cell surface glycoprotein with an apparent molecular weight of 55 kD. Using cross species hybridization with cDNA for the human IL-2 receptor, we have isolated and sequenced a 1.3 kb cDNA, which contains the entire 804 bp coding region of the murine IL-2 receptor, 92 bp of 5'UT, and a complete 3'UT extending to the poly-A tail. Inserted into the expression vector pcEXV-3, this cDNA directs functional membrane expression of the IL-2 receptor on transfected COS cells. Analysis of cytoplasmic RNA from IL-2 receptor bearing T cells reveals five distinct polyadenylated transcripts ranging in size from 1.7 to 4.7 kb. Some of the size heterogeneity is due to differential polyadenylation, resulting in mRNAs differing in the 3'UT region. Although the differential splicing within the coding region of the human IL-2 receptor gene cannot occur in the mouse (the downstream acceptor splice site is absent), we cannot exclude other alternative splicing patterns or different upstream initiation sites from contributing to the size heterogeneity in murine mRNA. Determination of the transcriptional processing pattern and its possible role in regulation awaits analysis of additional cDNA clones and elucidation of the genomic organization of the IL-2 receptor gene.

0932 SITE-SPECIFIC CLEAVAGE AND POLYADENYLATION OF EUKARYOTIC mRNA IN A CELL-FREE SYSTEM,

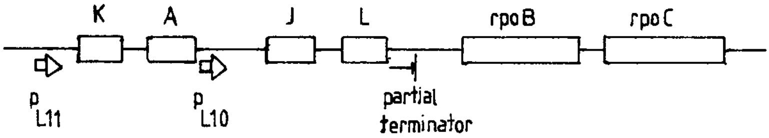
Claire L. Moore and Philip A. Sharp, Massachusetts Institute of Technology, Cambridge, Mass. 02139

We have developed a cell-free system using HeLa nuclear extract which accurately cleaves exogenously-added RNA at the L3 (hexon) site of adenovirus RNA, and adds a tract of approximately 200 adenine residues. The reaction requires ATP and exhibits a 15 minute lag before appearance of product. The reaction proceeds in the presence of 1 mM EDTA, and under these conditions, an RNA species corresponding in size to sequences downstream of the p(A) site can be detected, suggesting that an endonuclease rather than an exonuclease is responsible for the site-specific cleavage. Under normal conditions, polyadenylation is tightly coupled to cleavage. There is no accumulation of cleaved but not polyadenylated product unless the α-β analog of ATP is added to the reaction. The structure of the ends of these two cleavage products is now under investigation and should yield insight into the molecular mechanisms of this process. The *in vitro* system will be used as an assay in the purification of the enzymatic activities involved and in characterization of the specific RNA sequences which serve as signals for cleavage-polyadenylation *in vitro*.

0933 TRANSCRIPTIONAL REGULATION OF THE *rpo BC* OPERON IN *ESCHERICHIA COLI*,

Brian A. Morgan and Richard S. Hayward, Dept. of Molecular Biology, Edinburgh University, Edinburgh, Scotland.

The RNA polymerase subunits β and β' of *Escherichia coli*, encoded by the genes *rpoB* and *rpoC*, are co-transcribed with four 50s ribosomal protein genes, *rplKAJL*, in a rather interesting operon. Under various constraints, such as challenge with the antibiotic rifampicin, or partial amino acid starvation, a partial uncoupling of *rpoBC* from *rplKAJL* transcription occurs.



We are investigating the roles played in uncoupling by various transcriptional signals present in the operon, especially two strong promoters and a partial terminator of transcription. In particular we are applying SI-nuclease mapping to examine transcription *in vivo* through the DNA regions carrying the above signals. We have also examined the possibility that the dominance of the *rpoB3* (λrif^{d18}) allele has a regulatory basis.

0934 STUDIES OF NATURAL POLARITY OF *lac* OPERON EXPRESSION,

George J. Murakawa, Catherine Kwan, and Donald P. Nierlich, University of California, Los Angeles, LA, CA 90024.

It has been known for many years that although the *lac Z*, *Y*, and *A* genes of *Escherichia coli* are transcribed polycistronically, the distal, *A*, gene is expressed at a much lower level than the *Z* gene. We have studied the transcription of the *lac* operon by "Northern" blot hybridization and found first, as seen earlier by others, that a *lacZ* messenger RNA species, approximately 3000 b.p. long, is present in cells, and second, that in *E. coli* CR63, it is several fold more abundant than the *ZYA* species (5000 b.p.) and *YA* containing fragments together. Moreover, analysis of the *lacZ* mRNA 3' terminus by S1 nuclease mapping identifies a chromosomal site in the intercistronic space between the *Z* and *Y* genes that is homologous with transcriptional termination sequences found in bacterial attenuation and termination sites. Thus, the polarity of gene expression of the *lac* operon may be mediated by intercistronic termination. We are examining this possibility further by characterization of the transcript by recombinant DNA techniques using the pKO *gal* kinase vector system.

0935 NUCLEOTIDE SEQUENCE ANALYSIS OF WILD TYPE AND MUTANT FORMS OF THE BIOTIN OPERON REPRESSOR GENE FROM *ESCHERICHIA COLI*,

Anthony Otsuka, Peter Howard, Janet Shaw and Martin Buoncristiani, University of California, Berkeley, Ca. 94720

The biotin biosynthetic operon of *Escherichia coli* is under the negative transcriptional control of the *birA* protein. The bifunctional *birA* protein contains both corepressor synthesizing (biotin holoenzyme synthetase or BHS) and biotin operon repressing activities. Our DNA sequence analysis of the wild type *birA* gene reveals an open reading frame capable of coding a 35,318-dalton protein. We have determined the DNA sequences of mutations that strongly affect either the BHS or repressor activities or both. Fine-structure mapping and DNA sequence studies of the *birA* mutations indicate that regions near the middle of the protein sequence are necessary for BHS activity and sequences near the amino terminal two-thirds of the protein are required for repressor function. Changes in repressor activity can result from defective corepressor binding, altered DNA binding, inefficient aggregation of *birA* protein subunits or inability to synthesize corepressor. Mutations that result in decreased sensitivity to biotin repression map near the middle of the protein, suggesting that this region may be involved with biotin binding. The mapping of partially complementing *birA* alleles near the middle of the protein sequence suggests that this region is also important for subunit interaction.

Sequence Specificity in Transcription and Control

0936 DEVELOPMENTAL REGULATION OF RIBOSOMAL RNA TRANSCRIPTION: MOLECULAR MECHANISM AND TEMPLATE SEQUENCES INVOLVED, M. R. Paule, C. Iida, P. Kownin and D. Knoll, Colorado State University, Fort Collins, CO 80523.

Ribosomal RNA transcription is regulated by a stable modification of RNA polymerase I(RNAP I). rRNA transcription is down regulated in response to starvation in Acanthamoeba. This regulation can be reproduced in a faithful in vitro transcription initiation system: S100 extracts from vegetative cells actively transcribe rRNA while extracts from starved cells cannot unless they are supplemented with RNAP I purified from vegetative cells. The levels of both the transcription initiation factor(s) [TIF I] and the levels of RNAP I assayed on nonspecific templates (i.e. calf thymus DNA) are constant, but the RNAP I from the starved cell cannot specifically initiate transcription. This property is retained in RNAP I purified to near homogeneity from the starved cell even though its specific activity on calf thymus DNA matches that of the vegetative polymerase. RNAP I from starved cells has the same subunit composition as that from vegetative, but is 5X more heat labile than vegetative enzyme when assayed on nonspecific template. The small amount of enzyme active in the specific initiation assay has heat denaturation properties of vegetative polymerase. BAL-31 deletion mapping has shown that the sequence from -47 to +8 is necessary and sufficient to promote rRNA transcription - no far upstream sequence requirement can be detected in vitro. This sequence region has been shown to contain two domains involved in TIF-I binding plus a third required motif, and to be sufficient to demonstrate regulation in vitro. Therefore, the steps in transcription involving this DNA sequence and RNAP I are impaired by the modification of the enzyme from starved cells. Supported by NIH GM26059 and GM22580.

0937 DEGRADATION OF THE ESCHERICHIA COLI LEXA41 REPRESSOR BY THE LON PROTEASE AND EXPLANATION OF THE "SPLIT-PHENOTYPE" ASSOCIATED WITH THIS lexA(Ts) ALLELE. Kenneth R. Peterson and David W. Mount, Univ. of Arizona, Tucson, Arizona. 85724

The SOS regulon in E. coli is induced in response to treatments that damage DNA or interrupt its synthesis. At least seventeen operons negatively regulated by LexA protein are derepressed following RecA protein-promoted inactivation of LexA repressor. The lexA41 (formerly tsl-1) mutant, a lexA(Ts) allele, is a UV-resistant, temperature-sensitive pseudorevertant of the lexA(Ind⁻) allele lexA3. Cells exhibit a so-called "split-phenotype", a phenomenum in which only a subset of the SOS responses can be detected physiologically following inducing treatments. lexA41 has been cloned and sequenced; it retains the lexA3 mutation and has a second mutation downstream. We show that LexA41 protein is not cleaved by the RecA protein-catalyzed route in vivo, but is degraded by the Lon protease at both permissive and non-permissive temperatures. β-galactosidase activities of fifteen SOSop::lac fusions were measured at 30° and 42°C to determine levels of expression; the differential expression of SOS functions gives a plausible explanation for the "split-phenotype" associated with lexA41.

0938 REGULATION OF EXPRESSION OF MOUSE TYPE 1 INTERFERON GENES, P.M. Pitha-Rowe and K.A. Kelley, The Johns Hopkins University School of Medicine, Balto., MD 21205 USA

The cluster of four α interferon genes was identified in the 28kb long fragment of mouse genomic DNA. The DNA sequence analysis indicated high homology (90-95%) on nucleotide levels among three of these four genes. The fourth gene (α_4), which showed only about 80-85% homology to the others, contained an internal deletion of 15 nucleotides in the coding region; all of these genes coded for biologically active interferons when expressed in E.coli, however, the activity of the α_4 peptide was 100-fold lower than the activity of the other α interferons. Three of the four genes were expressed in virus (Newcastle disease virus - NDV) infected, but not in the uninfected L-cells as found by S-1 nuclease analysis. The relative levels of the α_4 mRNA was, however, about 10-fold higher than that of the other α genes. The DNA sequences of the promoter region of these four genes were compared and it was found that the 5' flanking region of the α_4 gene contains a number of point mutations and the insertion of a 17 nucleotide long G rich repetitive sequence not present in the 5' flanking region of the other genes.

In addition to α interferon genes, NDV induces in L-cells also the expression of β_1 interferon gene, while in poly rI.rC induced cells, the β interferon gene was expressed predominantly. Surprisingly, however, it was found that in poly rI.rC induced cells both positive and negative DNA strands of the β interferon gene were transcribed, while the virus induction led to the transcription of the proper strand only. This data are the first indication that the induction of β interferon gene by virus and dsRNA may not be identical and suggest a novel mechanism for the regulation of β interferon synthesis in the induced cells.

Sequence Specificity in Transcription and Control

0939 CHROMOSOMAL MUTATIONS THAT ALLOW READTHROUGH OF THE *rrnB* T1T2 TERMINATION REGION, E. Sarubbi, K. Rudd, and M. Cashel, LMG, NICHD, NIH, Bethesda, MD 20205

The T1T2 dual terminators of the *E. coli*, *rrnB* operon are able to stop anti-terminating rRNA transcripts. We have obtained *E. coli* mutants that allow RNA polymerase to read through the TIT2 region. We have isolated four independent host mutations with this phenotype, which result in expression of a downstream gal K gene on a plasmid. Two of these mutations map in or near *rpo B*; the remaining two map elsewhere.

None of the mutants reads through T1T2 unless an 80 bp fragment required for anti-termination is present. In the absence of this 80bp fragment T1 alone (rho-indep.) or the Is2 terminator (rho-dep) will stop transcription rendering the cells Gal-

Therefore, the defect in the mutants may specifically affect anti-terminating transcripts.

In addition, about 50% of spontaneous rifampin resistant (*rpo B*) mutants allow read through of T1T2. Two of these mutants have been examined and turned out to be defective also in normal rho-indep. termination at T1 alone, yet terminate of the IS2 rho - dep terminator.

These data suggest that the mechanism of termination of anti-terminating transcripts may be distinguished from those yielding normal termination. We propose a special term, super-termination, for the ability to terminate an anti-terminating transcript.

0940 A TIGHTLY-REGULATED, MODULAR CLONING AND EXPRESSION SYSTEM, D. Scandella, P. Arthur, M. Mattingly, and L. Neuhold, Genex Corporation, Gaithersburg, MD 20877

We have constructed a plasmid cloning and expression system for *E. coli* in which direct attachment of a gene to sequences regulating transcription and *translation* can be achieved rapidly and efficiently. The system includes the following main features: 1) A multi-copy cloning plasmid contains a bank of 10 unique restriction sites which are flanked by transcriptional terminators. A gene (clockwise 5' to 3') can be inserted at one of the restriction sites. It is protected by the 5' terminator from unregulated transcription which prevents its establishment. The gene can be conveniently modified in the cloning vector to contain a restriction site at the desired 5' end. 2) In a similar vector, a tightly regulated promoter is positioned to initiate transcription clockwise. This variant of λP_R contains O_{L2} and O_{L3} in place of O_{R2} and O_{R3}. This operator-promoter region and the the *cro* Shine-Dalgarno sequence are followed by either of two restriction sites which can be used to provide or omit the initiating ATG codon to a gene. 3) Three restriction sites in the *bla* gene are unique in the vector family. The promoter can be attached to a gene by digesting each plasmid within *bla* and at the desired gene or promoter end. Only correctly ligated isolated fragments will yield transformants on ampicillin plates. This ensures a high frequency of correct recombinants. 4) Other unique restriction sites are conveniently placed to allow replacement of the translation initiation region and the 3' transcriptional terminator.

0941 DEFINITION OF THE 5' AND 3' STRUCTURAL BOUNDARIES OF THE OVOMUCOID DNase I SENSITIVE CHROMATIN DOMAIN, Maxwell J. Scott, Ming-Jer Tsai, and Bert W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030.

Although it is well documented that actively transcribed genes are contained within a more open, DNase I sensitive chromatin conformation, little is known of how this structure is established. One model suggests that the DNA sequence at the transition from the inactive to active chromatin, through the binding of a sequence specific protein, causes the disruption of the highly compact closed chromatin structure. By defining the DNase I sensitive domain around the ovomucoid gene we hope to identify such a sequence by comparison to the previously defined ovalbumin chromatin domain. From a cosmid library we constructed a clone containing the ovomucoid gene and 16 kb of 5' and 18 kb of 3' flanking DNA has been isolated. The DNase I sensitivity of DNA fragments around the ovomucoid gene has been determined via a novel probe-excess solution hybridization assay using SP6 RNA polymerase synthesized RNAs as probes. The DNase I sensitive region extends approximately 15 kb beyond the 5' end of the ovomucoid gene and about 10 kb from the 3' end of the gene. Further analysis should precisely define the boundaries of this domain. As was the case with the ovalbumin DNase I sensitive domain, members of the CRI family of middle repititive sequences are found near the transition from an open to a closed structure. This is in agreement with the proposal that these CRI sequences may act through the binding of a sequence specific protein these open chromatin structures during differentiation of the chicken oviduct cell.

Sequence Specificity in Transcription and Control

0942 COOPERATIVE INTERACTIONS OF METAL REGULATORY ELEMENT-a OF THE MOUSE METALLOTHIONEIN -I GENE. Peter F. Searle, Gary W. Stuart and Richard D. Palmiter. HHMI, Dept.Biochem SL-15. University of Washington, Seattle Wa98195.

A fusion gene consisting of the promoter region from the mouse metallothionein-I (MT-I) gene joined to the coding region of the HSV thymidine kinase (TK) gene is efficiently regulated by zinc when transfected into baby hamster kidney cells in a transient assay. Analysis of similar plasmids containing 5' deletions, internal deletions and linker scanning mutants of the MT-I promoter region confirmed the function of the previously recognized metal regulatory element-a (MRE-a), sequence CCTTTGCGCCCG, between -55 and -44 bp from the cap site, and further localized upstream sequences with regulatory function to short regions of DNA containing sequences related but not identical to MRE-a. In total we recognize five MRE's within 180 bp of the MT-I cap site. To explore further the function of the multiple MRE's we have taken the approach of trying to build a metal-responsive promoter by inserting a 17-bp synthetic DNA fragment containing the MRE-a sequence into the non-responsive promoter of the TK gene. No induction by zinc was observed with single insertions of the regulatory sequence, whereas many different constructions with two copies of MRE-a were inducible. The precise position of the two MRE's relative to each other or to other promoter elements had relatively little effect upon the efficiency of induction, but the inducibility could be further increased by introduction of a third and fourth MRE-a sequence. The MRE's act cooperatively with the TK distal promoter elements, but in the presence of the TATA-box alone they function as zinc-dependent positive promoter elements.

0943 GLUCOCORTICOID REGULATION OF HUMAN GROWTH HORMONE EXPRESSION IN TRANSGENIC MICE, Richard F Selden, Thomas E. Wagner*, and Howard M. Goodman, Massachusetts General Hospital, Boston, MA 02114 and *Ohio University, Athens, Ohio 45701

Transgenic mice were generated by microinjection of a mouse metallothionein I/human growth hormone fusion gene into fertilized mouse eggs followed by implantation of the embryos into pseudopregnant foster mothers. Five of twenty-six mice born after one series of injections contained from 1 to 8 copies of the fusion gene stably integrated into their genomes and expressed human growth hormone in their serum. When several of these transgenic mice and transgenic offspring were treated with glucocorticoids, serum growth hormone levels were induced from 1.5- to 6.3-fold. A 4-fold induction in fusion gene mRNA in the liver of one of the five mice was also observed after treatment with glucocorticoids. We conclude that DNA sequences responsible for the observed inductions must be present in the human growth hormone portion of the fusion gene since other fusion genes containing the mouse metallothionein I promoter were not glucocorticoid inducible in previous gene transfer experiments. In particular, a recently identified glucocorticoid receptor binding site within the first intron of the human growth hormone gene might be able to confer inducibility on otherwise uninducible mouse metallothionein I gene constructs.

0944 AN INTRON SCANNING MECHANISM FOR SPLICING mRNA PRECURSORS, David Solnick, Yale University, New Haven, CT

With mRNA precursors containing multiple introns, what pairs the splice sites on opposite sides of an intron, to the virtual exclusion of all other possible combinations? Two models have been proposed. One assumes that a secondary or higher-order structure brings the opposing splice sites together; the other postulates that after one splice site is recognized the splicing apparatus scans the RNA until the opposing splice site is found. To test the scanning model, I introduced into a splicing substrate a structure that might act as a roadblock to scanning. The substrate was synthesized by transcription of an adenovirus template containing the first two late leaders and the intron between them. The roadblock took the form of an RNA-DNA hybrid, and was generated by annealing DNA fragments to various parts of the intron and exons. The hybridized substrates were incubated in a HeLa cell extract containing splicing activity, and the products were assayed by primer extension. Intron fragments arrested splicing, regardless of their length or location in the intron. Exon fragments did not. Further, deletion of most of the intron did not inhibit splicing, yet splicing was arrested when a DNA fragment complementary to this dispensable region was annealed to wild type precursors. With an RNA substrate containing the three adenovirus late leaders and the two introns between them, a DNA fragment annealed to one intron arrested the excision of that intron, but had little or no effect on excision of the other. These results suggest that some event required for splicing involves movement along the intron, presumably during a search to bring splice site partners together, and that the search goes on independently in different introns.

0945 *LAC* REPRESSOR PUNCTUATES *IN VIVO* TRANSCRIPTION OF *LAC* CONTROL REGION DNA.

Marguerite A. Sellitti and Deborah A. Steege, Duke University, Durham, NC 27710.

When the *lac* repressor gene is transcribed *in vivo*, the products are *lacI* mRNAs with discrete 3' ends in the *lac* control region. These endpoints are positioned just upstream from the CAP site (I), the -35 region (II) and the -10 region (III) of the *lacZ* promoter. From an analysis of *in vivo* transcription patterns, the factors which influence movement of RNA polymerase through the *lac* control region and generation of *lacI* mRNA 3' ends emerge. S1 nuclease mapping data and fingerprints of RNA hybridizable to control region DNA indicate that DNA sequences at the *lacI* gene end do not themselves provide an efficient signal for termination. RNAs with endpoints I, II, and III are present, but are minor components compared to readthrough transcripts. Of the proteins that have binding sites in the *lac* control region, only the repressor has a dramatic influence on the outcome of transcription. S1 nuclease mapping and Northern blot analysis show that RNAs with endpoints II and III are the major mRNA species generated by transcription of a repressor-bound template, together comprising more than 50% of the products. Repressor binding to the operator thus has a dual function. It blocks initiation of transcription from the *lacZ* promoter. In addition, it serves as a transcription factor to set the length of its own transcript, separating the *lacI* and *lacZYA* genes into distinct transcription units.

0946 DIFFERENTIAL TRANSCRIPTION OF MINICHROMOSOMAL VSG GENES IN *TRYPANOSOMA BRUCEI*.

Stuart, K., Scholler, J., Rothwell, V., and Aline R. Jr.
Issaquah Health Research Institute, Issaquah, Washington. 98027

African trypanosomes contain numerous variant surface glycoprotein (VSG) genes. Each trypanosome expresses these genes only during restricted stages of the life cycle, expresses only one VSG gene at a time, and can switch expression among the VSG genes. We have found that the IsTat 1.1 VSG gene is located near the telomere of a stable minichromosome (~100 kb) which lacks restriction sites over most of its length and lacks a spliced leader coding sequence. We have cloned and sequenced the expressed 1 VSG gene and have found characteristic telomeric sequences downstream. The 1 VSG gene is transcribed in variants of the 1 variant antigenic type but not in other variants from the same trypanosome clonal lineage that contain this gene nor upon conversion to another life cycle stage. The transcriptional activation and inactivation of the 1 VSG gene does not involve its duplication nor sequence alterations detectable by restriction enzyme mapping. Using probes cloned from the 5' flank of the expressed 1 VSG gene we have demonstrated apparent VSG mRNA precursors and a stable processing product. Transcription appears to be initiated near the beginning of the region lacking restriction sites. Supported from NIH and WHO.

0947 MOLECULAR ANALYSIS OF THE DROSOPHILA LOCUS *GLUED*

Anand Swaroop and Alan Garen, Yale University, New Haven, CT 06511

The mutant allele G1 of the *Drosophila* locus Glued has a *dominant* effect primarily on the development of the visual system, including the eye and its neural connections to the optic lobe of the brain. Restriction site mapping of genomic DNA clones from the normal G1$^+$ and mutant G1 locus showed that G1 contains a 9 kbp insertion of a retroviral-like *transposon* B104 into a transcribed region. The insertion causes formation of a truncated polyadenylated G1 transcript which terminates within the 5' terminal repeat of B104.
We are focussing on three aspects of this finding. One is the mechanism of the dominant effect of G1, and the role of transposon-induced mutations in producing such dominant effects. Another is the regulation and function of the normal Glued locus during Drosophila development. The third is the remarkable cis interaction between the B104 insertion in G1 and nearby insertions of the P-element transposon, which results in the excision of B104 and partial restoration of normal Glued function.

I would like to present a poster showing our results on the sequences involved in the regulation of the Glued locus.

0948 REGULATION OF MOUSE rRNA TRANSCRIPTION BY A SHORT LIVED SPECIFIC FORM OF RNA POLYMERASE I,

John Tower, Valeria Culotta and Barbara Sollner-Webb, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Phosphocellulose chromatography of an S-100 extract of mouse tissue culture cells resolves three fractions reported nessecary for in vitro rRNA transcription (Mishima, et al, Nuc. Acids Res. 10:6659). We have further purified two of these activities ('C' and 'D') and find that they are sufficient for accurate initiation. D forms a sequence-specific stable complex with the rRNA promoter. C appears to be a subpopulation of RNA polymerase I nessecary for specific rRNA synthesis, which stably associates with the D/promoter complex in vitro. This complex precipitates from an S-100 transcription reaction, and while the polymerase I molecules which remain soluble (~95%) catalyze non-specific RNA synthesis, they are incapable of specific rDNA initiation when supplemented with D. The 'specific-form' polymerase and D are efficiently recovered from the DNA dependant precipitate and resolved by chromatography. The resultant specific-form polymerase fraction (~100-200 fold purified) is capable of restoring transcriptional activity to extracts from both quiescent cells and cells in which protein synthesis has been inhibited by a one hour cyclohexamide treatment. While these extracts contain normal amounts of D and 'non-specific form' polymerase I, (and no detectable additional inhibitors) they are inactive for specific transcription unless so supplemented. This suggests that, in vivo, rRNA transcription may be regulated by the availability of a subpopulation of RNA polymerase I, distinct in its capability for specific initiation at the rRNA promoter.

0949 A POLYMORPHIC FAMILY OF BRAIN mRNA

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In studying the cDNA clones of rat brain messages, we identified a cDNA clone (0-44) that hybridized to a group of heterogeneously sized transcripts (100-1300 nts) which varied in abundance (0.01-0.1%) in brain, liver and kidney, most of the large species were brain specific. We isolated 33 independent cDNA clones with sequence homology to 0-44 from an Okayama-Berg rat brain cDNA library, and found three sites of structural variability. There are two 3' ends of the message, utilizing different polyadenylation (AATAAA) signals 88 nts apart. Site selection appears to be equal according to the 33 clones examined. The messages were polymorphic at their 5' ends as revealed by the presence of more than 20 bands in primer extension assays using an internal DNA fragment of 0-44 as the primer. Liver and kidney mRNA gave rise to only a few bands corresponding to the shorter brain bands. This finding is further confirmed by S1 protection assay using a probe derived from the 5' portion of the longest apparently full-length brain cDNA clone. This probe protects more than 20 5' mRNA ends in brain, but only a few in liver or kidney. Apart from these structural polymorphisms in the untranslated regions of the messages, 10% of the clones contain a 19 bp insertion at the carboxyl terminus of the open-reading frame translated from the DNA sequences. The 19 bps result in a reduction of the size of the predicted protein from 127 to 116 amino acids. We are currently analyzing the tissue distribution of the messages containing these 19 bps with S1 assay and are simultaneously investigating the identity and distribution of those putative proteins using antisera to synthetic peptides derived from various regions of the proteins.

0950 TISSUE-SPECIFIC EXPRESSION OF A MOUSE γ-CRYSTALLIN GENE IN CHICK EMBRYO LENS EXPLANTS,

Lap-Chee Tsui[1], Si Lok[1], Ana Chepelinsky[2], Joram Piatigorsky[2], and Martin Breitman[1], [1]Dept. of Genetics, Hospital for Sick Children, Dept. of Medical Genetics, University of Toronto, Toronto Ont. and [2]National Eye Institute, Bethesda.

We have been studying the developmental regulation of a family of genes encoding the γ-crystallins of the mouse eye lens. Our previous nucleotide sequence analyses of cDNAs and genomic clones revealed remarkable homology among the individuals genes, each specifying a highly conserved yet distinct polypeptide of 174 or 175 amino acid residues. To investigate the elements governing the expression of these genes, we have performed DNA transfection assays using plasmids containing sequences upstream from the coding sequence of the mouse γ2 gene fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. The results of these experiments indicate that the γ-crystallin promoter is not functional in monkey COS-1, human 293 and chick embryo neural retina cells, but is active in lens epithelial explants isolated from 14-day old chick embryos. Preliminary deletion analyses indicate that sequences extending 400 base pairs upstream of the cap site are required for expression of the γ-crystallin promoter. Failure of this promoter to function in non-lens tissues suggests the presence of lens-specific transcriptional factor(s). Since chickens do not have γ-crystallin genes, these factors are presumably not γ-specific yet are able to activate transcription of crystallin genes from different species.

0951 TRANSCRIPTIONAL REGULATION OF BACILLUS STEAROTHERMOPHILUS TYROSYL-TRNA SYNTHETASE GENE,

Mary M.W. Waye and Greg Winter,Laboratory of Molecular Biology, Medical Research Concil Centre, Hills Road, Cambridge CB2 2QH, ENGLAND

The several E.coli amino-acyl tRNA synthetases studied appear to have different patterns of regulation. For example, alanyl-tRNA synthetase and phenylalanyl-tRNA synthetase appear to be autoregulated (Putney and Schimmel,1981; Springer et al, 1983), but glutaminyl-tRNA synthetase is not (Cheung and Soll, 1984). We have characterized the 5' non-coding region of the tyrosyl-tRNA synthetase gene (tyrS) of *Bacillus Stearothermophilus* and have experimentally identified a promoter and terminator. Presumably, the terminator is involved in regulation of tyrosy-tRNA synthetase levels, but our data indicates that it is not regulated directly by the enzyme. Furthermore, the terminator differs from those found in amino acid biosynthetic operons in that there is no peptide rich in tyrosine in the putative attenuator region.

References:- Putney and Schimmel (1981) Nature 291:632-635.
Springer et al (1983) J. Mol. Biol. 171:263-279.
Cheung and Soll (1984) J. Biol. Chem. 259:9953-9958.

0952 TRANSCRIPTION OF H4 HISTONE GENES IN ISOLATED SEA URCHIN EMBRYO NUCLEI,

Eric S. Weinberg, Lawrence N. Yager, and John F. Kaumeyer, University of Pennsylvania, Philadelphia, PA 19104.

Genes coding for H4 histones expressed in sea urchin embryos at different stages are highly diverged and organized in a very different way. The early H4 gene is part of the tandemly reiterated 5 histone gene unit whereas the late H4 gene occurs mostly in H3-H4 pairs present in far fewer copies per genome (~10 vs. several hundred early H4 genes). We have demonstrated that nuclei isolated from the sea urchin, *S. purpuratus*, transcribe H4 histone sequences which are specific to early and late embryonic stages, as *in vivo*. These experiments indicate that the great increase in late H4 RNA (>50 -fold in 4 hr) is the result of transcriptional activation. The 5' ends of the nuclear H4 transcripts have been mapped by an RNase T_1 protection assay. A major portion of the early gene transcripts begins at the expected cap site but there are also run-through transcripts from upstream promoters. The transcripts terminate in an area spanning the spacer sequence between the H4 and H2B genes. The late transcripts start at several closely spaced sites near the cap site. We have identified 3 upstream sequences which are present in, and unique to, all late H4 genes examined thus far. Since there is a very high level of H4 transcription in both early and late nuclei, initiation may be taking place *in vitro*. We are testing this possibility now.

0953 ISOLATION OF GENOMIC SEQUENCES ENCODING FOR THE 220 KD SUBUNIT OF HUMAN RNA POLYMERASE II:

K. Cho, K. Khalili, S. Per, L. Weiner, and R. Weinmann

Using a Drosophila Rpo II probe, kindly provided by Dr. A. Greenleaf, we have isolated the homologous human genomic sequences. Several criteria were used to establish that these genomic sequences encode for the large subunit of RNA polymerase II. First, these probes are able to hybridize to a 7.35 kb poly A^+ mRNA, which is large enough to code for the largest RNA polymerase II subunit. Second, hybrid selection (or hybrid arrest) followed by *in vitro* translation in reticulocyte lysates suggests that this 7.35 kb mRNA encodes a 220 kd polypeptide. Third, a genomic DNA fragment was inserted into the expression vector series pUR 278, 288 and 289. A fusion polypeptide, containing approximately 20 kd of eukaryotic RNA polymerase II sequences, was generated in just one orientation in pUR 289. This 140 kd fusion protein cross-reacts with anti-RNA polymerase II antibodies. In turn, antibody directed against the fusion protein reacts with the large subunit of human and calf RNA polymerase II. Fourth and last, we have independently isolated cDNA clones from a pUC 8 expression library using the anti-RNA polymerase II antibody. These cDNAs cross-hybridize to the human genomic sequences homologous to the Drosophila Rpo II sequences. Using these genomic sequences as probes, approximately 30 kb of colinear genomic recombinants from an α-amanitin-resistant mutant have been isolated. Experiments are underway to establish the ability of the recombinants to render the wild type cells α-amanitin resistant by transfection, and to study the regulation of 220 kd subunit synthesis in the presence and absence of α-amanitin.

Sequence Specificity in Transcription and Control

0954 INTERACTIONS OF N AND HOST FACTORS AT THE PHAGE LAMBDA nut LOCUS.

William Whalen and Asis Das, Department of Microbiology,
University of Connecticut Health Center, Farmington, CT 06032.

The N-dependent antitermination system of phage lambda requires a number of genetically defined host proteins, one of which is the S10 ribosomal protein. Also essential is the site at which N modifies RNA polymerase to form a termination-resistant transcription apparatus. Using gene fusions, we have shown that translation terminating 19bp upstream of nutR impairs N function, whereas termination 23bp or 97bp upstream of nutR does not (1). These results suggest that the nut site RNA is a component of the antitermination process.

To further explore the role of nutR RNA in N function, NusA, NusB, and N proteins were purified using a sensitive in vitro assay (2,3), and nutR RNA was purified using a complementary M13 probe. The biochemical analysis of interactions between N, host factors, and nutR RNA will be presented.

(1) Warren, F. and Das, A. (1984) PNAS 81:3612-3616.
(2) Das, A. and Wolska, K. (1984) Cell 38:165-173.
(3) Ghosh, B. and Das, A. (1984) PNAS 81:6305-6309.

0955 DIFFERENT Z DNA SEQUENCES IN ϕX174 GENES AND TRANSCRIPTION CONTROL REGIONS.

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The specific interaction between left-handed Z DNA sequences in negatively supercoiled ϕX174 RFI DNA and anti-Z DNA IgG (Zarling *et. al., J. Mol. Biol.* (1984) **176**, 369-415) was investigated by high resolution darkfield immuno-EM. DNA-IgG complexes were formed under optimal binding conditions, purified by column chromatography, and visualized after uranyl acetate staining without use of aldehyde fixation, shadowing, or second antibody. Bivalent anti-Z IgGs bound to RFI DNAs, thus forming intramolecular bridges. They could also oligomerize separate molecules by intermolecular linking of Z sequences. Under low ionic and thermal conditions high affinity anti-Z IgG was retained at certain loci even after restriction endonuclease cleavage of the DNA. In these cleaved molecules some superhelices could be preserved in the loops generated by the bivalent IgG. This is the first example of IgG stabilization of local superhelical strain in a cut molecule. Z sequences in ϕX174 RFI DNA were mapped. Alternating tracts of purines and pyrimidines at nucleotides 763, 1027, 1714, 2146, 2363, 3504, 4161, 4911, and 5345 occur within the 9 different anti-Z IgG binding sites, which were expressed with varying frequencies (3-53%) on the molecules. Only a limited number of sites (generally < 2) exist on any one molecule. The formation of multiple Z sites in a given molecule, at the extracted superhelix density, was probably non-cooperative due to Z DNA's relaxation of torsional stress. Different Z sites can occur within several different genes, including regions where transcription is attentuated and in one case was located in front of a promotor of transcription.

Transcriptional Repressors and Activators

0956 NEGATIVE CONTROL OF THE GAL OPERON BY REPRESSOR REQUIRES TWO OPERATOR ELEMENTS,

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Analysis of constitutive mutations that derepress the gal operon of Escherichia coli shows the existence of a gene (galR) coding for the repressor and two non-tandem operator loci needed for gal repression. One operator (O_E) is located immediately upstream to the two overlapping gal promoters and the other (O_I) is inside the first structural gene. We have investigated the ability of the wild type and mutant O_E and O_I DNA sequences to bind to purified gal repressor. Based on the difference between the electrophoretic mobilities of free and protein bound DNA fragments, we have shown specific binding of gal repressor to each of the two operator DNA segments. The repressor binding protects the two operator DNA fragments from DNase I digestion. Each of the protected regions is about 24 bp long and covers the 16 bp homologous operator sequence. An operator constitutive mutation (O^c) at one of the two sites prevents repressor interaction with that operator.

O_E and O_I are separated by 100 bp and encompass the two gal promoters, P_1 and P_2. We have studied the relationship of the two operators with the intervening DNA. We have found that the addition of a 15 bp DNA sequence, which do not affect the promoter activities, between O_E and O_I derepresses the operon. The implication of these and other results on the mechanism of gal repressor action will be discussed.

0957 HELIX SWAPS; GENE ACTIVATION IN BACTERIA AND YEAST,

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An alpha helix (the "recognition helix") can determine DNA binding specificity of a repressor. Thus, if this (presumed) helix of the 434 phage repressor is replaced (by gene manipulation) with the (presumed) analogous region of 434 cro protein or of P22 repressor, the DNA binding specificity of the hybrid protein is in both cases that of the parent that donated the alpha helix.

Using mutagenesis _in vitro_ we have created a mutant of the CAP protein of _E.coli_ that binds to DNA but is defective in stimulation of transcription of _lac_, _gal_, and _mal_ genes.

A 17 base pair synthetic oligonucleotide has been synthesized and shown to mediate _GAL4_ stimulation of transcription in yeast. Footprinting _in vivo_ indicates that, as expected, _GAL4_ interacts with this sequence and that there are four such _GAL4_ binding sites in the _GAL_ UAS. A bacterial repressor (lex) synthesized in yeast blocks _GAL_ mediated stimulation of transcription if the lex operator had been inserted between UAS and TATA. A yeast putative transcriptional terminator similarly placed also blocks this _GAL4_-mediated stimulation of transcription.

0958 MUTATIONS THAT INCREASE THE AFFINITY AND SPECIFICITY OF LAMBDA REPRESSOR-OPERATOR BINDING.

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We have used reversion and direct selection techniques to obtain mutations that increase the affinity of the bacteriophage lambda repressor for its operator sites. The mutant proteins have been purified and their interactions with operator and non-operator DNA have been characterized. We will discuss the presumed molecular basis for the increased affinity and specificity of these mutant repressors with respect to the interaction proposed for the wild type repressor-operator interaction (1,2).

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Sequence Specificity in Transcription and Control

0959 A GENETIC INVESTIGATION OF THE SPECIFICITY OF AN ACTIVATOR PROTEIN OF PHAGE LAMBDA FOR ITS DNA BINDING SITE, Daniel L. Wulff, Department of Biological Sciences, State University of New York at Albany, Albany NY 12222

The λ CII protein, which activates transcription from the pRE promoter of phage lambda, binds to the four base repeat sequence 5'-TTGCN$_6$TTGC-3', where N$_6$ denotes six intervening bases between the TTGC repeats (1). This repeat sequence flanks the -35 region of the promoter. Promoter minus (pRE$^-$) mutations which alter the four base repeat sequence result in decreased binding of CII protein to pRE, while pRE$^-$ mutations in the -10 and -35 regions of the promoter affect RNA polymerase contacts (1). However no pRE$^-$ mutation has been found in the final C of the repeat sequence, even though methylation experiments indicate that the CII protein makes close contact with this G·C pair. The λ pRE promoter and the NH$_2$-terminal region of the λ cII gene structurally overlap, and mutations within the region of overlap may be examined for their effects on both functions. The ctr-1 mutation, which was identified as an up-translation mutation of the cII gene, lies within the region of overlap and alters the final C of the repeat sequence to yield the sequence 5'-TTGCN$_6$TTGT-3' (2). Since λctr-1 is pRE$^+$, and since a mutant promoter of sequence 5'-TTGTN$_6$TTGC-3' is pRE$^-$, the two TTGC sequences must not be recognized in identical fashion by the CII protein.

Bacteriophage λ is one of a family of bacteriophages with CII-like proteins and pRE-like promoters. The pRE promoter of phage 21 has the same four base TTGC sequences as phage λ, and the pRE promoter of phage P22 has the sequence 5'-TTGCN$_6$TTGT-3' (like λctr-1). The intervening sequences for these three promoters are all quite different from one another. The pRE promoters of all three phages overlap their cII-like genes at precisely the same positions. A CII-like protein of one phage usually does not efficiently activate the pRE promoter of another, although cross-reactions are observed (3). For example, λ pRE is about one fifth as active as P22 pRE when assayed in vivo with the CII-like protein of phage P22. The λ ctr-1 pRE promoter is about half as active as the λ pRE$^+$ promoter when assayed in vivo with either the λ CII protein or the CII-like protein of P22. However the λ dya-5 pRE promoter (dya-5 is a C to T change four bases to the left of the 5'-end of the 5'-TTGCN$_6$TTGC-3' sequence) is five-fold more active than λ pRE$^+$ when assayed with the P22 CII-like protein, and is as fully active as P22 pRE.

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Ribosome Binding Sites

0960 **CORRELATING RIBOSOME BINDING SITE SEQUENCES WITH FUNCTION,** John D. Childs[1], Keith Villanueba[2], Doug Barrick[2], Thomas D. Schneider[2], Gary D. Stormo[2], Larry Gold[2], Moshik Leitner[3] and Marvin Caruthers[2], [1]Atomic Energy of Canada Limited, Chalk River, Ontario, [2]University of Colorado, Boulder, Colorado, [3]Israel Institute for Biological Research, Nessciona.

A typical ribosome binding site in E. coli contains a Shine and Delgarno region and an initiator codon that is normally AUG; the two domains are separated by 5 to 9 bases. Although both elements appear to play a significant role in ribosome binding, other bases surrounding the initiator codon are probably important. For example, between bases -20 and +13 four additional regions have a non-random distribution of bases (1). Since this is the region protected by ribosomes in bind and chew experiments, the non-randomness may be related to ribosome binding. To determine the contribution of each base to ribosome binding, we have constructed a plasmid for cloning random synthetic single stranded DNA into the 5' end of a lacZ gene that has a promoter, but lacks both the first eight non-essential codons and a ribosome binding site. When this vehicle is cut between lacZ and the promoter with BglII and PstI, 5' and 3' overhangs are created; we visualize this arrangement as a notch. We have successfully cloned single strand DNA with ends complimentary to those of BglII and PstI into this vector, a procedure we term "notch cloning". Because the DNA has twelve "ambiguous" bases in the region prior to the initiation codon, we have obtained hundreds of different ribosome binding sites in an isogenic background. Many sequences and associated β-galactosidase values have been determined. Correlation of these data should tell us how each base effects ribosome binding.

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0961 I. TRANSLATIONAL EFFICIENCY: A MUTATIONAL ANALYSIS OF A RIBOSOME BINDING SITE AND OF ITS COMPLEMENT ON THE 16S rRNA. II. THE ROLE OF THE BOX A REGION IN THE RIBOSOMAL RNA ANTITERMINATION SYSTEM,

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In the past two years, we have examined the sequences around the start codon with regard to their role in the efficiency of protein initiation in E. coli. Using synthetic oligonucleotide primers, mutagenized in specific regions by simultaneous couplings of all nucleotides, we obtained two large collections of mutants that differ only at: (1) the three bases preceding the start codon of the β-galactosidase messenger and (2) the three bases following the start codon (i.e., the second triplet) of this messenger. In both of these groups, we found mutants that differ dramatically in the β-gal levels.

In another approach to study the sequence requirements of ribosome binding sites, we developed a portable Shine-Dalgarno region (PSDR). In this system, the Shine-Dalgarno (SD) area of a given sequence was replaced by several other sequences. We have found that an increase in the length of complementarity with the 3'-end of the 16S rRNA (i.e., the anti-Shine-Dalgarno sequence) results in a decrease in translational efficiency. Using the same system, we also found that the sequence in the spacer region; i.e., the region between the SD-sequence and the start codon, affects the mRNA translatability; A's and T's favor the expression, C's lower the expression, and G's essentially abolish translatability. (Note that G residues are almost never found in natural messengers in the spacer region.)

Finally, using the same PSDR system, we exchanged the SD sequence on the messenger with the anti-SD sequence (as found on the 16S rRNA) and the anti-SD-sequence on the 16S rRNA with the SD-sequence. The messenger in which the sequence 5' GGAGG as the SD-region was replaced by 5' CCUCC was completely inactive. We mutated (by site-directed M13-mutagenesis procedures) the anti-SD-region in a 16S rRNA gene of the plasmid pKK3535 from 5' CCUCC (its natural sequence) to 5' GGAGG. Upon reconstruction of the plasmid, transformants were obtained that contained large deletions in the plasmid borne 16S rRNA gene, showing that such a mutation is lethal. (Similar observations were made by A. Dahlberg's group at Brown University, personal communication.) Currently, we are replacing the authentic ribosomal RNA promoters of pKK3535 by an inducible promoter in an attempt to prevent lethality.

Expression of ribosomal RNA genes by other than its own promoters may lead to premature transcriptional termination. For this purpose, we identified the precursor region of 16S rRNA as the region that gives RNA polymerase transcribing rrn-operons antitermination properties. This region contains a "so-called" box A sequence. Using M13 mutagenesis techniques, we made several mutations in and around this region and have demonstrated that the box A sequence is crucial for antitermination.

0962 SELECTION OF TRANSLATIONAL START SITES IN EUKARYOTIC mRNAs,

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Many of the peculiarities of translation in eukaryotes can be explained by postulating that ribosomes scan the 5'-end of messenger RNA. Our working hypothesis is that a 40S ribosomal subunit binds initially at the 5'-end of mRNA and then advances linearly until it reaches the first AUG triplet: if the first AUG codon occurs in an optimal sequence context[1,2,3] all 40S subunits stop there and that AUG serves as the unique site of initiation. But if the first AUG triplet occurs in a suboptimal context, only some 40S subunits stop and initiate there; some bypass that site and initiate at another AUG codon that lies farther downstream. This "modified scanning model" makes several predictions which I have evaluated by introducing mutations around, and upstream from, the AUG initiator codon in a cloned preproinsulin gene. To monitor the effects of sequence variations near the initiator codon, SV40-based plasmids that carry point mutations or small insertions were introduced into monkey (COS) cells and the yield of (pre)proinsulin was measured 48 hours later.

A number of parameters have been studied using this system:

(a) Single nucleotide changes in position -3 (i.e., 3 nucleotides upstream from the AUG codon) or position +4 were found to modulate the yield of proinsulin over a 15-fold range. The optimal nucleotides are A in position -3 and G in position +4; the contributions of those two positions are not simply additive. Single nucleotide changes in positions -1, -2, -4 or -5 have much smaller effects on translational efficiency.

(b) An oligonucleotide has been inserted upstream from the AUG initiator codon that is complementary to a block of 13 nucleotides, including the AUG codon. The formation of a stable hairpin structure in which the AUG triplet is completely base paired does not preclude translation, implying that ribosomes can melt secondary structure barriers in mRNA.

(c) The ability of eukaryotic ribosomes to reinitiate translation following a terminator codon[4] has been confirmed. The efficiency of reinitiation has been shown to depend on the position of the terminator codon relative to the second (downstream) initiator codon.

(d) The possibility that triplets other than AUG can serve as initiator codons in higher eukaryotes was tested by introducing point mutations that changed the AUG codon to AUA, AUU, GUG or UUG. The nonstandard initiator codons that work, at least to some extent, in prokaryotes do not function to a detectable extent in higher eukaryotes.

References

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Sequence Specificity in Transcription and Control

0963 RULES OF TRANSLATION, Fred Sherman, Steven B. Baim, Michael Labhard, Linda R. Friedman, D. Michael Hampsey, Charles T. Goodhue and John I. Stiles*, Departments of Radiation Biology & Biophysics and Biochemistry, University of Rochester School of Medicine, Rochester, NY 14642 and *Department of Botany, University of Hawaii Honolulu, HI 96844

A systematic analysis of mutational alterations within the transcribed region of the *CYC1* gene has revealed certain essential features of the translational process in yeast. Altered DNA sequences were obtained by a variety of techniques, including the selection of mutations, revertants and recombinant *in vivo* and the alteration of cloned DNA segments *in vitro*. The following alterations have been extensively characterized: point mutations of the AUG initiator codon; small deletions and insertion in the 5' untranslated region; bp substitutions adjacent to the AUG initiator codon; relocation of the AUG initiator codon; and formation of sequences with two AUG codons. The levels of the *CYC1* mRNA and of the gene product, iso-1-cytochrome *c*, were determined in strains containing single copies of the altered *CYC1* genes at the normal chromosomal position.

The following major conclusions concerning translation have been reached. (i) An AUG codon is required for effective initiation of translation; however, certain other codons can be used at below 1% of the normal efficiency, and these levels can be enhanced by suppressors unlinked to the *CYC1* locus. (ii) Deletions and AUG relocation indicate there is no requirement for specific sequences or a ribosome binding site adjacent to the AUG initiator codon. (iii) Insertions and deletions indicate that the distance between the CAP sites and the AUG initiator codon can be varied without appreciably affecting the efficiency of translation. (iv) Hairpin and possibly other secondary structures in the mRNA can greatly diminish translation; the degree of inhibition is dependent on the strength of the hairpin structure and on the position of the AUG codon relative to the hairpin structure. (v) The translational efficiency is influenced by nucleotides at position -1 and -3; however, this *context* effect varies with the position of the AUG initiator codon along the mRNA. (vi) Protein synthesis initiates at the 5' proximal AUG codon in most sequences. Insertion of an upstream AUG codon can prevent or diminish initiation from the normal downstream start site, and the efficiency is a function of the sequence context. This result implies that protein synthesis can initiate concomitantly at more than one AUG codon with special contexts.

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Translational Controls; Regulatory Loops

0964 POST-TRANSCRIPTIONAL REGULATION OF COLLAGEN GENE EXPRESSION. Sherrill L. Adams, Richard J. Focht and Eileen S. Allebach, Department of Human Genetics, University of Pennsylvania, Philadelphia, PA 19104

We have examined collagen gene expression in several types of differentiated cells from chick embryos, and in viral transformants of these cells. Our studies indicate that the type I collagen genes may be regulated not only at the level of transcription, but also at the levels of RNA processing and translation. Tendon and skin fibroblasts and smooth muscle cells synthesize type I collagen at widely differing rates, although they display identical amounts of hybridizable type I collagen RNAs. Chondrocytes synthesize no detectable type I collagen, although they display substantial amounts of hybridizable type I collagen RNAs which display altered electrophoretic mobilities. These results indicate that there may be alternative processing pathways which give rise to type I collagen RNAs of differing translational efficiencies. Transformation of chondrocytes results in a dramatic increase in type I collagen RNAs. These RNAs have electrophoretic mobilities comparable to those found in fibroblasts and can be translated very efficiently in vitro, but not in vivo, indicating that, regardless of the form and quantity of the type I collagen RNAs, chondrocytes are unable to utilize them efficiently. We suggest that the type I collagen genes are transcribed constitutively, and that post-transcriptional mechanisms involving RNA processing and translational control have been elaborated to compensate for this.

Sequence Specificity in Transcription and Control

0965 DIRECT MAPPING OF ADENO-ASSOCIATED VIRUS CAPSID PROTEINS B AND C: A POSSIBLE ACG INITIATION CODON. S. Patricia Becerra, James A. Rose, Medora Hardy* and Carl W. Anderson*, NIAID, NIH, Bethesda, MD 20205; *Biology Department, Brookhaven National Laboratory, Upton, NY 11973

Adeno-associated virus (AAV) virions are composed of three major capsid polypeptides, A, B and C, with molecular weights of 90,000, 72,000 and 60,000. Their molar ratio in the virion is similar to that found in infected cells (A:B:C=1:1:10), suggesting that their production is highly regulated. For AAV type 2, these proteins are specified by the entire right half of the viral genome which is 4675 bp in length. In addition, A, B and C share amino acid sequences equivalent to all of C, an overlap which produces a common carboxy terminal segment. The longest open reading frame in the right half of the genome, however, is only sufficient to code for a 503 amino acid polypeptide (i.e., a protein about the size of C). In our current studies, using an amino acid sequencing technique, we have confirmed that protein C is encoded by this open reading frame. As expected, the initiating AUG lies within a favorable sequence context for a functional initiator codon. The start site for protein B maps 195 bases upstream from the initiation site for C. Interestingly, the putative initiator codon for B is a Thr codon, ACG, which also lies within a preferred sequence context for a functional initiator codon. Additional evidence supports the probability that this ACG triplet is the initiator codon for protein B. Initiation by an ACG codon would be novel in eukaryotes. Utilization of an ACG codon as a functional initiator and its role in regulating viral protein synthesis will be discussed.

0966 Human α Interferon Genes: Diversity and Pseudogene Expression, Arthur P. Bollon, Richard Torczynski, and Cheryl Hendrix, Wadley Institutes of Molecular Medicine, Dallas, Texas 75235

Human α Interferons represent a family of related proteins containing anti-viral and anti-proliferative activities. Considerable effort has been made on exploring α Interferon anti-cancer activities against select carcinomas, as well as inhibitory actions against retroviral proliferation and expression. We have developed a method which has permitted the isolation of several α interferon genes from a human genomic library, using 17 base oligomeric probes. The method required several technical refinements, and resulted in the isolation of novel α Interferon genes, which had not previously been isolated from either cDNA or genomic libraries. These results may indicate that short probes are useful for identifying subsets of genes of gene families, for which cDNA probes are less efficient, in a relative sense, in elliciting strong signals. One of the novel α Interferon genes, IFN-αWA, has been sequenced and compared to other α Interferons which previously have been described. The IFN-αWA contains approximately a 10% difference in DNA sequence, compared to human α Interferons A-L. IFN-αWA is most homologous with IFN-αC (2.6% difference) at the 5' end which then diverges to about a 7% difference at the 3' end, whereas the 3' end of IFN-αWA is most homologous with IFN-αH (2.4% difference), which diverges as one moves to the 5' end (16.6% difference). Such comparisions were done at 150 base intervals. IFN-αWA differs with IFN-αA-L at 5 conserved amino acid positions, two of which result in changes from hydrophobic residues to acidic residues. The IFN-αWA has been expressed in *E. coli* using an M13 lac fusion system. A second novel human α Interferon gene as well as properties of these interferons are under analysis. A third isolated human α Interferon gene human is IFN-αL, which is considered a pseudogene since it contains a stop codon in the signal sequence. In an effort to analyze whether the whole IFN-αL gene product was inactive, the stop codon in the signal sequence was corrected. The modified IFN-αL was engineered in *E. coli* for expression, using a M13 lac fusion system. Upon induction with IPTG, an active Interferon protein was generated (10^6-10^7u/l). These results indicate that the mature interferon coding sequence of IFN-αL has been conserved for active interferon protein. These results may bear on the significance and function of so called pseudogenes. One can speculate that cells are capable of utilizing the mature sequence information of IFN-αL, either by bypassing the stop codon via some form of suppression or by DNA or RNA organization. Alternatively, there may be polymorphisms for IFN-αL. Examination of the sequence of IFN-αL gene uncovers some potential internal expression sequences. One can further speculate that multiple forms of α Interferon may be synthesized, secreted and non-secreted forms. It is also interesting to note that several internal procaryotic expression signals have been recognized in not only IFN-αL, but in all the α Interferon genes, which may bear on the nature of interferon protein products generated by genetically engineered bacteria.

0967 TRANSLATIONINITIATION OF THE ECORV ENDONUCLEASE IS MODULATED BY A mRNA TERTIARY STRUCTURAL SIGNAL. J.H. Botterman° & M. Zabeau*, °State University of Ghent, *Plant Genetic Systems N.V. Ghent, Belgium.

The EcorV restriction-modification system has recently been characterized. Analysis of the mRNA secondary structure revealed a very stable stem and loop structure in the region immediately downstream of the endonuclease coding sequence, containing a bulge loop of 7 bases, which is perfectly complementary to the sequence flanking the ATG initiation codon. The possibility that a tertiary structure interaction between these two sequences might inhibit translation, has been tested by site directed mutagenesis. Prior studies showed that this region cannot be removed. Consequently we fused the gene with its ribosome binding site(rbs) to a regulatable lambda P_L promotor, but no appreciable overproduction of the gene product was obtained. Evidence will be presented that subsequent mutation of the putative regulatory signal leads to increased expression. The expression of the endonuclease might thus be regulated at the level of translation initiation by a tertiary structure interaction between the rbs and the signal. When the RNA chain is completed, the rbs becomes masked and hence only nascent mRNA's can be translated. This ensures a stringent control of endonuclease expression by limiting the number of enzyme molecules synthesized from each mRNA.

0968 TRANSCRIPTIONAL AND TRANSLATIONAL STUDIES ON THE MuMTV UNIT II PROVIRUS, Richard W. Connors and Akhil B. Vaidya, Hahnemann University, Philadelphia, PA 19102-1192

The etiologic agent of adenocarcinoma of the breast in the mouse model system is the Mouse Mammary Tumor Virus (MuMTV). Southern blot analysis of Eco RI restricted DNA from BALB/c and C57BL reveal two common MuMTV proviruses, designated unit II and unit III. High levels of functional MuMTV transcripts are found in C57BL lactating mammary glands (LMG), whereas no MuMTV mRNA can be detected in BALB/c LMG. In neither strain are MuMTV proteins detected. To examine this differential regulation of endogenous MuMTV expression, we have isolated molecular clones carrying the MuMTV unit II provirus from BALB/c and C57BL/6 genomic libraries. Restriction endonuclease analysis of the cloned DNA has shown that the unit II provirus is integrated at the same chromosomal locus in both C57BL and BALB/c mice. In addition, there are no restriction site polymorphisms between these clones over a 35 kb region. To examine nucleotide sequence differences that may account for the differential regulation of expression in C57BL and BALB/c LMG, we are sequencing a 500 bp Pst I fragment. This fragment contains the glucocorticoid receptor binding site as well as transcriptional start signals for MuMTV expression. To investigate the regulation of MuMTV unit II expression *in vivo*, we have transfected both the BALB/c and the C57BL/6 clones into NIH3T3 cells and a mammary epithelial cell line of C57BL origin. Preliminary results indicate both BALB/c and C57BL unit II proviruses are transcribed; further studies are being conducted to determine their translational state.

0969 IN VITRO EXPRESSION OF CLONED GENES, R. Contreras and W. Fiers, Laboratory of Molecular Biology, State University of Ghent, B-9000 Ghent, Belgium

Non-manipulated eukaryotic genes are very poorly expressed in prokaryotic systems because they lack a proper ribosomal binding site. In vitro transcription of cDNAs, cloned after a prokaryotic promoter, is simple, specific and rather efficient. Such transcriptions, however, cannot be translated in eukaryotic systems because they lack a 5'-terminal cap structure. Addition of chemically synthesized caps to the transcription mix allows efficient capping of the RNA. A cloning procedure is described which allows the expression of the cloned cDNA through a combination of prokaryotic transcription and eukaryotic translation.

0970 SEQUENCE OF THE MOUSE ALCOHOL DEHYDROGENASE cDNA: TRANSLATION STARTS AT THE SECOND AUG, Howard J. Edenberg and Ke Zhang, Department of Biochemistry, Indiana Univ. School of Medicine, Indianapolis IN 46223

The cDNA encoding the mouse-liver alcohol dehydrogenase (ADH-A subunit) has been cloned from a liver cDNA library, and its sequence has been determined. The cDNA contains 100 nucleotides of 5' non-translated sequence, the entire coding sequence of the ADH-A subunit, and 133 nucleotides of 3' non-translated sequence followed by a short poly(A). This represents the first complete sequence of a cDNA for a mammalian alcohol dehydrogenase. The amino acid sequence deduced from this cDNA closely resembles that of the horse-liver ADH-E subunit: 316 of 374 residues are identical, and 29 of the differences are conservative substitutions. The 5' end of this cDNA is interesting: the second AUG initiates synthesis of the ADH polypeptide. It is preceded by an AUG that could direct synthesis of a tri-peptide before reaching a termination codon. The sequences surrounding both AUGs are similar. Kozak's hypothesis that ribosomes initiate synthesis at the first AUG and, after termination of the peptide, continue traveling along the RNA to reinitiate at the second AUG might explain the initiation of the ADH polypeptide. Such a mechanism might influence the efficiency of translation of this mRNA.

0971 THE RIBOSOMAL RNA INTERVENING SEQUENCE OF TETRAHYMENA; NUCLEOTIDE SEQUENCE DATA FROM SIX DIFFERENT SPECIES, Jan Engberg and Henrik Nielsen, Biochemical Institute B University of Copenhagen, Denmark

We have determined the nucleotide sequence of the rDNA intron region of six different Tetrahymena species in order to test model secondary structures which may be involved in the in vitro and in vivo splicing reaction. The IVS of T. hyperangularis and T. cosmopolitanis are identical in size (407 nucleotides) and sequence and differ from the IVS of T. pigmentosa and T. sonneborni by single base substitutions at two positions only. The IVS of T. thermophila and T. malaccensis are 413 nucleotides and 403 nucleotides long, respectively, and contain several insertions, deletions and single base substitutions relative to the IVS of the other Tetrahymena species. When the differences are positioned on the IVS secondary structure map of T. thermophila (cf. Cech et al. (1983) PNAS 80, 3903) the insertions, deletions and most of the single base substitutions map in single stranded, looped-out regions. In three cases we found that single base substitutions in duplex stem regions have been compensated by secondary base substitutions. One of the single base deletions in T. malaccensis map within the so-called Internal guide sequence which has been implicated in the alignment process during the splicing reaction (Waring et al. (1983) JMB 167, 595). This deletion becomes of special interest since it has been shown - in T. thermophila - that the nucleotide in this position is directly involved in the circulation process (Zaug et al. (1983) Nature 301, 578). The effect of this single base deletion on the splicing and circularization reactions is not known at the molecular level, yet.

0972 DIRECT MEASUREMENT OF THE INTERACTION OF eIF-4A WITH mRNA AND NUCLEOTIDES. D. J. Goss[1], C. L. Woodley[2], and A. J. Wahba[2], [1]Dept. of Chemistry, Hunter College of CUNY, New York, NY 10021 and [2]Dept. of Biochemistry, U. of Miss. Med. Ctr., Jackson, MS 39216.

We have previously used fluorescence techniques to monitor directly protein-protein and protein-nucleic acid interactions. Fluorescent labeling of eucaryotic initiation factor 2 (eIF-2) allowed us to describe a mechanism for the interaction of eIF-2 with the guanosine nucleotide exchange factor (GEF), GDP and GTP (J. Biol. Chem., 259, 7347 (1984)). To explore further the roles of various factors in polypeptide chain initiation, we have fluorescently labeled eucaryotic initiation factor 4A. By monitoring changes in fluorescence intensity and fluorescence anisotropy, direct interaction of eIF-4A, with ATP, ADP, eIF-4B, and various mRNAs has been detected. Analysis of fluorescence quenching data allowed us to determine the K_D for ATP and ADP binding to eIF-4A. The equilibrium constants (K_D) obtained were approximately the same (80 μM) for both nucleotides. The presence of Mg^{2+} decreased K_D more than 2-fold. Messenger RNAs with and without the 5' terminal cap and having different levels of secondary structure will be assayed for binding to eIF-4A and other initiation factors.

Grant Support: NIH GM 25451(AJW)

0973 REGULATION OF HISTONE mRNA DEGRADATION IN MOUSE CELLS. Reed A. Graves, Nunta Chodchoy and William Marzluff, Dept.Chemistry,Florida State University,Tallahassee,Fl.32306

The levels of histone mRNAs encoding the replication-dependent histone variants are regulated coordinately with DNA synthesis. When mouse cells are treated with inhibitors of DNA synthesis which interfere with deoxynucleotide metabolism, both the half-life of the histone mRNA and the rate of transcription are altered. We have investigated the sequences required for regulating the half-life of histone mRNA by constructing altered mouse histone genes and reintroducing these genes into mouse cells. Deletion of the hairpin loop at the 3' end of a histone H3 gene results in the formation of longer transcripts which are polyadenylated. These longer RNAs are found in the cytoplasm and are not rapidly degraded when DNA synthesis is inhibited. Deletion of 200 nucleotides from the center of the coding region of the H3 or H2a gene and replacement of this sequence with a portion of the pBR322 tetracycline gene resulted in formation of cytoplasmic histone mRNAs which were initiated and terminated correctly; These hybrid mRNAs are stable when DNA synthesis is inhibited. We conclude that while the 3' end of the histone mRNA is required for mRNA degradation, the coding regions sequences in the middle of the gene are also required for altered degradation of histone mRNA. The extreme conservation of mouse histone coding region sequences among different genes coding for the same histone protein may reflect the role of these sequences in regulating histone mRNA degradation. Supported by grant GM29832 from NIH.

0974 GLYCOSYLATION OF THE CELL-MEMBRANE ALTERS THE BIOFUNCTION OF THE mRNA TRANSLATION PRODUCTS. Anwar A. Hakim, Charles M. Siraki and Charles E. Joseph*. loyola University Medical Center. Maywood, Illinois 60135 and* Univ. Southern Calif. Los Angeles, Calif. 90024.

Several studies have been conducted to identify the target cell membrane determinants that binds to the effector cells. The present studies were aimed at the chemical nature of the receptor. Human Natural Killer (NK) cells and peripheral blood lymphocytes (PBL) from hepatitis B virus surface antigen vaccine (HBsAg) are cytotoxic (CTL) to human hepatocellular carcinoma (HHCC).When incubated with NK or CTL, neuraminidase (VCN) increased the cytotoxicity of these cells by 10-14 folds. Whereas, if the target cells were treated with VCN, they became resistant to the killing effects. When HHCC are cultured in presence of non-toxic levels (0.3 to 1.0 ug/ml) of tunicamycin (Tn), a potent inhibitor of glycosylation, the cells resist the killing effects of NK and CTL cells. Both HHCC and glycosylation-deficient HHCC were labelled with ^{3}H-N-Acetylglucosamine. Using Ficoll-Hypaque gradient centrifugation, both types of target cells bound to the effector cells, but only HHCC were lysed. mRNA was isolated from HHCC and glycosylation-deficient-HHCC, and were used in an in vitro mRNA-protein synthesis system. On Oligo(dT) columns the two types of mRNA differed in elution patterns. The protein coded by mRNA from HHCC enhanced, whereas the protein coded by mRNA from glycosylation-deficient HHCC had no effect on NK or CTL cytotoxic activities. When applied on SDS-polyacrylamide gel electrophoresis, the protein coded by mRNA from HHCC resembled, whereas that coded by mRNA from glycosylation-deficient cells differed from HBsAg. Therefore, glycosylation of the cell membrane alters mRNA translation products.

0975 EXPRESSION OF HUMAN PROSOMATOSTATIN IN BACTERIAL CELLS, Peter M. Hobart and Philip J. Pawlowski, Pfizer Central Research, Groton, CT 06340

Several bacterial plasmid vectors (pBR322 derivatives) have been constructed in an effort to express human prosomatostatin in bacterial cells. These expression vectors were designed to place the human prosomatostatin cDNA sequence under the regulation of the bacterial tryptophan promoter. Probing of cellular fractions for the prosomatostatin peptide (11,500 MW) and mRNA from cells transformed with initial plasmid constructs indicated very high levels of somatostatin specific mRNA but no prosomatostatin peptide. Northern blots revealed the somatostatin specific transcripts (300-400 bases) were of sufficient length to encode the complete precursor peptide although apparently terminating in the 3′ untranslated region of the eukaryotic sequence (present in all constructs). A computer analysis revealed terminator-like sequences in this 3′ untranslated region similar to those known to terminate beta-lactamase and tetracycline-resistance genes also present on pBR322. Deletion of the 3′ untranslated region and reintroduction into bacterial cells of this otherwise unmodified vector resulted in significant levels of prosomatostatin peptide expression. Analysis of RNA from these cells indicated the presence of specific transcripts two to three times the size necessary to code for the 92 amino acid precursor peptide. The results suggest that bacterial expression of this relatively small eukaryotic peptide requires a minimum size mRNA transcript in order to be efficiently translated by the bacterial ribosome.

0976 VARIATION IN THE NUCLEOTIDE SEQUENCE OF THE β-LACTAMSE S-D AND LEADER REGION IN TWO STRAINS OF B. CEREUS. Erik Hornes and Lars Korsnes, A/S Apothekernes Laboratorium for Specialpræparater. Oslo Norway

The β-lactamase I gene from B. cereus Ni10R, a strain isolated from stream mud was found to be located on a 8.3 kb EcoRI fragment, as opposed to a previously reported 4.3 kb fragment in strain 569/H. Restriction analysis indicates that the flanking regions are different in these two strains. Sequence data from the β-lactamase genes show differences both in the Shine-Delgarno region, leader sequence and structural gene. A series of secretion vectors was constructed using the leader sequence of β-lactamase genes. We are now comparing their function according to the sequence variation in S-D and leader region.

0977 USE OF VACCINIA VIRUS AS AN EUKARYOTIC CLONING AND EXPRESSION VECTOR,

Dennis E. Hruby, and Christine A. Franke, Center for Gene Research, Department of Microbiology, Oregon State University, Corvallis, OR 97331

The use of cloning and expression vectors to study and experimentally manipulate individual genes, independent of their normal resident environment, is a central and vital theme in modern molecular genetic experimentation. Due to a number of unique biological attributes, vaccinia virus (VV) would seem to offer an ideal system for such studies. A number of laboratories have demonstrated the feasibility of this approach by constructing recombinant VV strains which contain and express heterologous viral antigens. Such hybrid vaccine strains may prove useful against a variety of human and animal diseases. Unfortunately, the current methodologies employed to construct recombinant VV are slow, time-consuming, expensive, and do not facilitate genetic engineering of the foreign insert. These drawbacks have thus far retarded the development of VV as a generalized eukaryotic expression vector.

Experiments currently in progress have three objectives: 1) To streamline the methods employed to construct chimeric genes and to assay their biological activities; 2) To construct insertion plasmids containing dominant selectable markers which will allow direct, one-step, selection of VV recombinants containing foreign inserts; and 3) To use VV as a research tool with which to study gene systems that are not readily amenable to more conventional approaches - RNA viruses (animal and plant), histocompatibility antigens, and immunoglobulin genes.

0978 EFFECTS OF CODON BIAS ON IFN-γ EXPRESSION IN YEAST.

M. Jones, R. Koski, K. Egan, and G. A. Bitter. Amgen, Thousand Oaks, CA 91320.

Two synthetic IFN-γ genes which differ in codon usage have been constructed. One gene contains optimal yeast codons, the other has codon usage optimized for *E. coli*. Both genes were expressed in the yeast *S. cerevisiae* from multi-copy plasmids, using the PGK promoter, or a modified G3PDH promoter, and the PGK transcription terminator. Side-by-side comparisons have been performed on yeast transformants containing either the IFN-γ YC or the IFN-γ EC gene. Results indicate use of yeast optimal codons significantly increases translation efficiency of IFN-γ mRNA. Comparison of codon bias effects on IFN-γ expression, plasmid stability, mRNA levels and polyribosome sedimentation will be discussed.

0979 MOUSE APOLIPOPROTEIN E cDNA SEQUENCE: ANALYSIS OF INTERNAL HOMOLOGY,

John S. Kaptein, Tripathi Rajavashisth, Karen L. Reue, Stuart Rich and Aldons J. Lusis, University of California, Los Angeles, CA 90024

Apolipoprotein E (apo E) is responsible for the binding of VLDL and chylomicron remnants to cellular receptors thereby effecting their removal from the circulation. We have isolated and determined the sequence of a nearly full-length cDNA clone of C57Bl/6J mouse apo E. The clone encodes 284 amino acids and the entire 3' untranslated region of 112 nucleotides. Comparison with the sequences of human and rat apo E reveals a high degree of conservation. There are two regions, however, which in each species is characterized by unique insertions and deletions. The resulting reading frame shift is most extensive in mouse and could alter an entire potential amphipathic α-helix. Analysis of the sequence homologies within apo E reveals that the entire sequence is made up of repetitive units despite an intron interruption. The most primitive unit appears to be an 11-nucleotide repeat within higher order repeats of 22 or 33 nucleotides. These repeats are read in different reading frames in various portions of the molecule; hence, at the amino acid level, the homologies are not always apparent. We postulate that apo E and those other apolipoproteins related to it have arisen by duplication and subsequent modifications of an 11-nucleotide unit or multiples thereof. Furthermore, insertions and deletions in one particular region have been tolerated because the alternate amino acid sequence yields comparable structural features.

0980 IDENTIFICATION OF THE COMPONENTS NECESSARY FOR ADENOVIRUS TRANSLATIONAL CONTROL AND THEIR UTILIZATION IN cDNA EXPRESSION VECTORS,

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A transient expression system was used to study the role of the adenovirus late and SV40 early mRNA leader sequences and adenovirus virus-associated (VA) RNAs in mRNA translation. Hybrid transcription units containing the adenovirus late and SV40 early promoters fused to various coding regions were introduced into monkey COS cells on plasmids containing an SV40 origin of replication. The translational efficiencies of the mRNAs produced from these plasmids were determined after alterations in the viral leader sequences or in the presence of VA RNAs provided by adenovirus infection of the transfected cells or by cotransfection with plasmids containing the VA genes. Efficient translation of mRNA with either adenovirus or SV40 leader sequences is dependent upon the presence of VA RNA. Translational stimulation by VA RNA of mRNAs containing the adenovirus tripartite leader sequences is dramatically reduced if leader exons 2 and 3 are removed or if their orientation is altered. Sequence analysis has indicated a homology between the nontranslated 5' end of SV40 early mRNA and sequences at the border of the 2nd and 3rd tripartite leader exons which may be responsible for the increased translation of these mRNAs in the presence of VA RNA.

0981 HOST tRNA CLEAVAGE AND REUNION IN T4-INFECTED *E. COLI* STRAINS RESTRICTING POLYNUCLEOTIDE KINASE AND RNA LIGASE MUTANTS.

G. Kaufmann, Biochemistry Dept., Tel Aviv University, Ramat-aviv, Israel 69978.

Anticodon loop cleavages of two host tRNAs occur in T4-infected *E. coli* CTr5x, a strain restricting phage mutants deficient in polynucleotide kinase (*pnk*) or RNA ligase (*rli*). The cleavage products accumulate with the mutant infections but are religated with wild type. Some revertants of *pnk*$^-$ or *rli*$^-$ phage, with a second mutation in *stp*, lack the anticodon nuclease. Other *pnk*$^-$ revertants lack in addition an endonuclease that cleaves the host leucine tRNA$_1$, suggesting the existence of a phage-coded factor common to both enzymes. In *E. coli* BJMn10, a transduced host strain carrying the restrictive *E. coli* CTr5x locus *prr* (Abdul Jabbar and Snyder (1984), J. Virol 51, 522), the anticodon nuclease reaction products appear transiently during *wt* infection, accumulate with *pnk*$^-$ and *rli*$^-$ mutants and are absent with *stp*$^-$ mutants. The genomes of the restrictive donor and transductant bacteria, but not of the permissive recipient, contain a common DNA restriction fragment that hybridizes to a CTr5x-specific tRNA fragment probe, suggesting that *prr* encodes tRNA species vulnerable to the anticodon nuclease. Regarding the restriction mechanism, it is proposed that the anticodon nuclease reaction products inhibit late phage gene expression, unless further processed by polynucleotide kinase and RNA ligase.

0982 HYBRID-ARREST OF INJECTED mRNA IN XENOPUS OOCYTES USING COMPLEMENTARY OLIGONUCLEOTIDES,

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Several oligonucleotides, complementary to the Interleukin 2 and Interleukin 3 coding sequences, were tested for their ability to hybrid-arrest the translation of their respective mRNAs in Xenopus oocytes. The oligonucleotides, which ranged in size from 18 to 23 bases, were all found to be very effective in blocking the biological activities of IL-2 and IL-3 mRNA, and in some cases more than 99% of the activity could be eliminated. Several parameters were tested which included: (1) Whether the oligomer was derived from the 5', middle or 3' portion of the coding sequence. (2) The molarity of NaCl used in the hybridization buffer. (3) The concentration of oligomer used. In summary, we found that the position of the oligomer in the coding sequence was not too significant in its ability to block mRNA translation. Oligomers were able to hybridize to mRNA AFTER injection into the oocyte since nearly quantitative inhibition could occur even when no NaCl was used in the hybridization buffer. Under optimal conditions, as little as 5 pmoles of oligomer was effective in eliminating greater than 90% of the mRNA activity.

0983 MECHANISM OF TRANSLATIONAL ACTIVATION OF GENE 14 OF BACTERIOPHAGE T7,

Peter C. Keck, John J. Dunn, Barbara Lade and F. William Studier,
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Genes 13 and 14 of T7 are transcribed as a single mRNA in which the stop codon of gene 13 is seven bases ahead of the start codon of gene 14. The gene 14 message has a good Shine-Dalgarno sequence, but nevertheless, translation of the gene 14 message is strongly coupled to translation of the gene 13 message. To examine the mechanism of this coupling we have analyzed the rate of synthesis of gene 14 protein in E. coli, using mRNAs transcribed by T7 RNA polymerase from cloned fragments carrying gene 14 and various upstream sequences. High rates of synthesis of gene 14 protein are observed when both gene 13 and 14 are translated, but a very low rate is found when the mRNA contains 155 bases of untranslated upstream gene 13 sequence. An in-frame fusion between gene 10 and 13 restores translation of the upstream gene 13 sequence, and also restores the normal rate of synthesis of gene 14 protein. Removing all but the last 60 bases or so of the gene 13 coding sequence eliminates the requirement for translation of the gene 13 message, and increases the rate of synthesis of gene 14 protein above that found in coupled synthesis. Replacement of the sequence ahead of the start codon for gene 14 by the sequence ahead of the start codon for gene 10 produces a still higher rate of synthesis. In the natural gene 13-14 mRNA, potential secondary structures in which the gene 14 initiation region is base-paired to sequences located more than 70 bases upstream have been identified. Presumably, translation of the gene 13 message melts out these structures, thereby making the gene 14 initiation site accessible.

0984 PAIRING BETWEEN IS10'S pIN AND pOUT TRANSCRIPTS IN VITRO, J.D. Kittle Jr. and N. Kleckner, Dept. of Biochemistry and Molecular Biology, Harvard U.,Cambridge MA.02138

At high copy numbers of transposon Tn10 and IS10, expression of IS10 transposition function is negatively regulated at a post-transcriptional level by a small trans-acting IS10 encoded transcript, RNA-OUT. RNA-OUT and the transposase mRNA, RNA-IN, share a 36 base region of complementarity at their 5' ends. This overlap region includes the translational start signals of the transposase gene. Genetic analysis suggests that RNA-OUT pairs with RNA-IN at this region, thereby preventing the translation of the transposase message. We have now developed an in vitro assay that measures the kinetics of pairing between RNA-IN and RNA-OUT. A mixture of RNA-IN and RNA-OUT transcripts from one or more templates is obtained by transcription of restriction fragments with purified E. coli RNA polymerase. Pairing between RNA-IN and RNA-OUT occurs after the completion of transcription, and the paired species's detected by non-denaturing polyacrylamide gel electrophoresis. The second order rate constant for in vitro pairing is 3×10^5, which is very similar to the pairing rate of RNAI with primer RNA in the ColE1 system. (Tomizawa, J. (1984). Cell 38, 861-870.) The calculated secondary structure of free RNA-OUT predicts that of the region complementary to RNA-IN is involved in large, stable intrastrand stem-loop structure, with the 3' portion of this region residing in the loop. Our working model proposes that interstrand basepairing initiates when the 5' end of RNA-IN contacts the RNA-OUT loop. Mutations that act to close up this loop or prevent the propagation of pairing from beyond the inital contact point abolish transcript pairing in vitro, and negative regulation in vivo. These and other experiments support our working model.

0985 ANALYSIS OF mRNA DEGRADATION IN ESCHERICHIA COLI, Sidney R. Kushner, Cecilia M. Arraiano, Stephanie D. Yancey, and William P. Donovan, Univ. of Georgia, Athens, GA 30602

E. coli mRNAs are rapidly degraded to mononucleotides with an average half life of 60-90 seconds. Although previous work has suggested the involvement of a number of ribonucleases such as ribonuclease II and polynucleotide phosphorylase in mRNA turnover, until recently it has not been possible to demonstrate any clear change in the degradation pattern of mRNAs. The finding that pnp (polynucleotide phosphorylase) rnb (RNase II) double mutants could not be constructed by P1 transduction suggested a means of examining RNA metabolism in E. coli. Using in vitro mutagenesis of the cloned rnb structural gene, it has been possible to isolate temperature sensitive rnb mutations. Double mutants carrying one such allele (rnb-500) and an absolute mutation in the structural gene for polynucleotide phosphorylase (pnp-7) are conditionally lethal for growth and accumulate partially degraded mRNA species at the nonpermissive temperature. These partially degraded mRNA species range in size from 20-500 nucleotides. In viability is directly correlated with the inability to totally degrade mRNA species. The turnover of rRNA species does not appear to be affected. Experiments are currently in progress to determine the fate of individual mRNAs such as lac and uvrD in order to examine the nature of the initial degradative steps in the overall process of mRNA turnover. (This work was support by grants from NIH, GM28760 and GM27997)

0986 AUTOREGULATION OF TUBULIN SYNTHESIS, Joseph T. Y. Lau and Don W. Cleveland, Johns Hopkins University School of Medicine, Baltimore, Md

An apparently autoregulatory pathway determines the level of new tubulin synthesis in virtually all animal cells. Increase in the intracellular pool of free tubulin subunits results in dramatically lowered levels of tubulin mRNAs. To understand the molecular basis for this regulation, we have transiently introduced a cloned tubulin gene into cultured mouse fibroblasts and demonstrated that the heterologous gene is transcribed and correctly processed into stable mRNA. Moreover, using colchicine to induce depolymerization of endogenous microtubules and a corresponding elevation of the pool of free tubulin subunits, we have shown that the expression of the heterologous tubulin gene is suppressed concomitantly with that of the endogenous mouse tubulin genes. However, such down-regulation is not observed following transfection of a hybrid actin gene which is transcribed under the control of a tubulin promotor. This observation, together with our previous findings that tubulin gene transcription in nuclei from control and colchicine-treated cells is not subject to down-regulation, strongly suggests that the autoregulation of tubulin synthesis is not modulated on a transcriptional level. Results with other recombinant gene constructs are consistent with this hypothesis and serve to define more closely the genetic region(s) which contains the regulatory signal.

0987 ANALYSIS OF mRNA SECONDARY STRUCTURE BY CROSSLINKING
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The UV photoreaction of the hen oviduct mRNA for ovalbumin with the nucleic acid crosslinking reagent 4'-aminomethyl-4,5',8-trimethyltrioxsalen (AMT) has been investigated as a method of stabilizing intramolecular secondary structure for further analysis. Fluoresence emission spectroscopy revealed that this reaction occurs in two stages - monoadduction followed by crosslinking. Measurement of ^{3}H-AMT incorporation into ovalbumin mRNA indicated the maximum level of monoadduction but not crosslinking depended on the reaction conditions. In all cases,ovalbumin mRNA crosslinked with AMT exhibited a characteristic positive electrophoretic mobility shift on agarose gels, which could be reversed by breaking the crosslinks. Partial T1 RNase digestion of crosslinked ovalbumin mRNA followed by "northern blot" hybridization with an 86 bp cDNA restriction fragment containing the ovalbumin mRNA region used to initate protein synthesis indicates that the RNA fragment containing this initiation region also undergoes a postive electrophoretic mobility shift relative to the corresponding native RNA fragment. This result is consistant with the previous prediction of a hairpin structure in the initiation region of ovalbumin mRNA (Kuebbing, D. and Liarakos, C. D. (1978) Nucleic Acids Res.5,2253-2266) and suggests that crosslinking with AMT can be developed as a method for mapping intramolecular secondary structure in RNA.

0988 TRANSLATIONALLY ASSOCIATED HELIX-DESTABILIZING ACTIVITY IN RABBIT RETICULOCYTE LYSATE, Stephen A. Liebhaber, Faith E. Cash and Susan H. Shakin, University of Pennsylvania, Philadelphia, Pa 19104

The problem addressed in this work is to examine how the ribosome reads through regions of secondary structure in mRNA during peptide elongation. We have approached this problem by testing the ability of the rabbit reticulocyte lysate system to translate human globin mRNA which is hybridized to specifically positioned cDNA fragments. These cDNA-mRNA hybrids are used as an experimental model of intra-strand mRNA duplexes. Hybridization of globin mRNA to cDNA fragments which extent 5' of position +10 to +15 (10-15 nucleotides 3' to the initiation AUG) fully block mRNA translation while cDNA fragments beginning 3' to this position have no adverse affect upon translation. Using two independent approaches we demonstrate that cDNAs in the latter group remain attached to the mRNA during translation. These results imply that the ribosomal complex, once fully assembled at the AUG initiation codon can locally destabilize secondary structures as it moves along the mRNA. This activity may be critical for the translation elongation reaction.

0989 TRANSCRIPTION AND TRANSLATION IN GENOME-LIMITED ORGANISMS, Jack Maniloff and Todd L. Sladek, Dept. of Microbiology, Univ. of Rochester, Rochester, NY 14642

Mycoplasmas are genome-limited organisms: their 500 or 1000 Mdalton genomes limit the genetic complexity of these cells. The low % G+C of mycoplasma DNAs (in general, 23-34% G+C) puts additional constraints on their information storage and codon usage. We have shown that mycoplasmas arose by degenerative evolution from Gram-positive eubacteria: the initial phylogenetic branch produced mycoplasmas with 1000 Mdalton genomes (hence, cell wall loss and genome reduction were probably coupled), and subsequent branchings gave rise to mycoplasmas with 500 Mdalton genomes. Recent DNA sequence studies show that mycoplasma ribosomal protein cistrons make maximal use of codons rich in A and T, and 16S-23S rRNA spacer DNA is noncoding and rich in A and T. Mycoplasma rRNAs have slightly lower amounts of G+C than eubacterial rRNAs, but are still close to 50% G+C. We have identified Shine-Delgarno sequences near the 3'-terminus of mycoplasma 16S rRNA, but no data are available on promoter or Shine-Delgarno sequences upstream from DNA coding regions. To investigate gene structure and regulation in mycoplasmas, we are studying mycoplasma virus L2: a temperate noncytocidal phage, containing 11.8 kb circular DS DNA. We used L2 DNA as template in *in vitro* *E. coli* and *B. subtilis* coupled transciption-translation systems. Both systems produce most virion proteins, plus several other different proteins. These data will be discussed and the two *in vitro* systems compared, both for L2 and L51 (a mycoplasma virus containing 4.5 kb SS DNA). In addition, mycoplasma transcription and translation regulatory sequences are being described by sequencing the L2 genome, and will be discussed.

0990

USE OF A PORTABLE RIBOSOME BINDING SITE FOR STUDYING THE EFFICIENT EXPRESSION OF A EUKARYOTIC GENE UNDER VARIOUS PROKARYOTIC PROMOTERS. David Marquis, Jo Marie Smolec and David H. Katz. QUIDEL, 11077 North Torrey Pines Road, La Jolla, CA 92037.

The cloning of eukaryotic genes, for potentially therapeutic proteins, has prompted studies on maximizing expression of eukaryotic genes in prokaryotic cells. It is known that for high level expression, a strong prokaryotic promoter and an efficient ribosome binding site are required. Contained within the ribosome binding site is a Shine-Dalgarno sequence, followed by the initiation codon. The distance between the Shine-Dalgarno sequence and the ATG has been shown to have some effect on the efficiency of translation. In order to maximize expression of a eukaryotic gene in *E. coli*, we constructed a portable cassette containing a synthetic ribosome binding site in which the Shine-Dalgarno sequence was intermixed with stop codons in all three reading frames. Following the Shine-Dargarno, at a distance varying from 5-9 bases, was an initiation codon and our model eukaryotic gene, human T cell growth factor (TCGF). The cassette was inserted into seven different expression systems that varied with regards to promoter and the presence or absence of necessary translational signals. The level of protein produced varied considerably and was dependent upon the promoter and the distance between the Shine-Dalgarno sequence and the initiator codon.

0991 ADENOVIRUS VA RNA REGULATES THE INITIATION OF PROTEIN SYNTHESIS VIA eIF-2 PHOSPHORYLATION, Michael B. Mathews[1], Patricia Reichel[1], William C. Merrick[2] and John Siekierka[3]. [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724; [2]Case Western Reserve University, Cleveland, OH 44106; [3]The Roche Institute of Molecular Biology, Nutley, NJ 07110.

Large amounts of two small (160 nucleotide) RNAs, the VA RNAs, accumulate in the cytoplasm of adenovirus-infected cells. A mutant virus unable to produce the major species, VA RNA$_{I}$, is deficient in the translation of both viral and cellular mRNAs. The defect occurs at the level of polypeptide chain initiation and is corrected *in vitro* by addition of the initiation factor eIF-2 or its recycling factor GEF: none of the other initiation and elongation factors tested are effective. Furthermore, assays for GEF show that the activity of this factor is severely depressed in mutant-infected cells. Two lines of evidence suggest that the role of VA RNA is an indirect one: addition of this RNA fails to rescue initiation activity, and mixing experiments reveal the presence of a translational inhibitor in mutant-infected cells. This inhibitor appears to be a protein kinase capable of phosphorylating the α subunit of eIF-2, thereby trapping GEF and preventing the recycling of eIF-2 in its catalytic role. Identification is underway of the VA RNA$_{I}$ sequences important for its function.

0992 PROPERTIES OF THE mRNA DISCRIMINATING PROTEINS eIF-4A and eIF-4F, William C. Merrick, Richard D. Abramson, Angela Caliendo and Jamie A. Grifo, Case Western Reserve University, Cleveland, Ohio 44106

Recent studies have indicated that two of the initiation factors required for the binding of mRNA to ribosomes are RNA-dependent ATPases (Grifo et al. J. Biol. Chem. 259, 8648, 1984). Both of these factors, eIF-4A and eIF-4F, seem to share a common peptide of 46,000 daltons, although identity has not been proven yet. However, the specificity of these two factors is quite different. eIF-4A is an active ATPase in the presence of all four ribohomopolymers although poly(G) works poorly. eIF-4F is quite dependent on the presence of eIF-4B for ATPase activity and then displays an m^7GDP sensitive preference for globin mRNA. Neither protein is an effective ATPase in the presence of poly(dA), poly(dT) or oligo(dT).

Current studies are intended to extend the biochemical studies into the area of protein chemistry to identify regions of similar sequence such as the peptide site responsible for reacting with the affinity label flourosulfonylbenzoyl adenosine (FSBA) and by double label peptide mapping of the 46,000 dalton peptides of eIF-4A and eIF-4F. At present, very preliminary data indicate a high degree of similarity, but non-identity of these two peptides. Supported in part by NIH Grant GM 26796.

0993 TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS - ACTIVATION OF TRANSLATIONALLY REPRESSED HSP70 mRNA AT THE LEVEL OF ELONGATION. Richard I. Morimoto, Nicholas G. Theodorakis and Sunandita S. Banerji, Dept. of Biochemistry, Molecular and Cell Biology Northwestern University, Evanston, Illinois 60201

Chicken reticulocytes respond to elevated temperatures (43C) by the increased synthesis of one heat shock protein, HSP70, and the repression of normal cellular proteins such as α and β globin. The globin mRNA in heat shocked cells is neither degraded nor structurally modified and is apparently blocked in vivo at the level of initiation. The preferential synthesis of HSP70 following heat shock is not due to an increase in HSP70 mRNA as the level of messenger RNA increases only two-fold following heat shock while the level of HSP70 synthesis increases over twenty-fold. We find that HSP70 mRNA is maintained in the cytoplasm of normal red cells in a translationally repressed state that can be activated following heat shock. Over 70% of the HSP70 mRNA is associated with polysomes in its translationally repressed state, and can be released from ribosomes by treatment with EDTA. Furthermore, we find that HSP70 mRNA is associated with RNP particles with the density expected of polysomes on metrizamide gradients. Our data suggests that the control of HSP70 synthesis in avian reticulocytes is regulated at the level of elongation of protein synthesis.

0994 ISOLATION OF cDNA CLONES ENCODING INITIATION FACTOR eIF4A, Peter J. Nielsen, Gary K. McMaster and Hans Trachsel,Univ. of Basel,Switzerland

In most cases, regulation of eukaryotic gene expression at the translational level probably occurs by regulation of mRNA association with ribosomes. Several protein factors have been identified as being necessary for the binding of mRNA to 40S ribosomal subunits. However, very little is known about how these proteins act during messenger binding and how their activities might be regulated. One of the initiation factors required for mRNA binding is eIF4A, a single polypeptide of 43kd. Interestingly, it is required as a single polypeptide for mRNA binding, and is present, along with several other proteins, in a high molecular weight complex involved in cap recognition. It also has the ability to discriminate between different mRNAs, that is, it stimulates the translation of different messengers to different extents. In order to better understand the function and possible regulation of eIF4A, we have isolated mouse cDNA clones coding for this factor. We describe the isolation and characterization (including the nucleotide sequence) of these clones. We have used these clones to examine the expression of mRNA coding for eIF4A and present evidence suggesting the existence of two different messengers coding for eIF4A.

0995 HIGH-LEVEL EXPRESSION OF CLONED EUKARYOTIC GENES IN E. COLI PLASMID VECTORS

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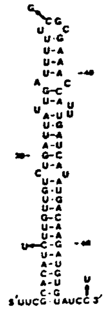
The level of expression of eukaryotic genes in E. coli using established vectors is highly variable and unpredictable. Various features of the mRNA structure were examined and altered in order to give both increased mRNA stability and improved efficiency of translation initiation. A scheme was devised which may make these improvements generally applicable to the expression of other eukaryotic genes in E. coli.

0996 RNA PRODUCT OF THE POUT PROMOTER OF IS10,

Francis J. Schmidt and Younghoon Lee, University of Missouri-Columbia, Columbia, MO 65212

RNA species encoded by outward promoters of the IS10 elements have been proposed to translationally inhibit tansposition of Tn10 by sequestering the mRNA encoding transposase functions (Simons, R.W. and Kleckner, N., Cell, 34, 683-691). In the present work, RNA species hybridizing to the outer 400 bp of the IS20 sequences flanking transposon Tn10 were analyzed by hybrid selection, nuclease S1 mapping and RNAase T_1 fingerprinting. These experiments detected a single RNA transcript (RNAout1) which was 70 nucleotides in length. RNAout1 was synthesized from pOUT of IS10 (right) but not pOUT of IS10 (left) even though these promoter sequences are identical. A sequence alternation in a potential stem and loop structure of the IS10 (left) transcript apparently decreases its stability in vivo, leading to a reduced steady-state RNA concentration and a concomitant inability to inhibit Tn10 transposition.



0997 INITIATION OF TRANSLATION AT INTERNAL ATG CODONS IN MAMMALIAN CELLS,

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We have studied the consequences of inserting ATG triplets in all three reading frames upstream of the translational initiation codon of the Hepatitis B virus surface antigen (HBsAg) gene. As expected, these additional ATG codons can severely depress the initiation of translation at the authentic start codon. Such inhibition, however, can be totally suppressed by the presence of in-frame translational stop codons following the upstream ATG codon. We have positioned translational stop codons at various points after the upstream translational start codon in order to ask whether the rate of translational initiation at the authentic HBsAg start codon is affected by the distance between the translational stop codon of the upstream reading frame and the initiation codon of the HBsAg gene. Immunoprecipitation of S^{35}-labeled intracellular extracts prepared from cells transfected with vectors having the upstream AUG in the same translational reading frame as the HBsAg gene demonstrated that the upstream ATG codons are efficiently recognized. These results show that mammalian ribosomes can reinitiate translation at internal ATG codons after having terminated translation from an upstream reading frame. This suggests that polycistronic mRNAs may indeed function in mammalian cells.

0998 POST-TRANSCRIPTIONAL CONTROL OF ORNITHINE DECARBOXYLASE AND THYMIDINE KINASE ACTIVITIES IN SYNCHRONIZED MAMMALIAN CELLS,

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In the course of characterizing several temperature sensitive Chinese hamster cell mutants we focused our attention on the enzymes ornithine decarboxylase (ODC) and thymidine kinase (TK). These enzymes are regulated in a cell cycle dependent manner in synchronized cells. ODC reaches a sharp maximum at 6 hours after stimulation of quiescent cells with serum, while TK activity rises abruptly with the onset of DNA synthesis (S-phase) at about 10 hours.

cDNA probes for ODC and TK were used to monitor the levels of ODC mRNA and TK mRNA in stimulated cells following a 48 hour serum starvation. While we observed the dramatic changes in enzyme activities previously reported by ourselves and others, we found no corresponding changes in the levels of the specific mRNAs as measured by both slot blots and Northern blots. The levels of both mRNAs remained almost constant during progression through the cell cycle. These observations suggest that post-transcriptional regulatory mechanisms operate to control a number of enzyme activities during the cell cycle.

0999 REGULATION OF PHAGE T4 GENE 32 TRANSLATION BY OLIGONUCLEOTIDES COVALENTLY LINKED TO INTERCALATING AGENTS,

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We have synthesized oligonucleotides covalently linked to an acridine derivative (Acr) through their 3'phosphate group via a pentamethylene arm. We have used such oligonucleotides as regulators of phage T4 gene 32 expression. The region [-80 + 1] upstream of the AUG codon on the gene 32 mRNA is crucial for gene 32 translational self-regulation. It contains a sequence $^{3'}$[UUAAA] repeated three times in tandem. The effect of modified oligonucleotides complementary to one (r_1 Acr) two (r_2 Acr) or three (r_3 Acr) repeated sequences, on gene 32 expression has been investigated in a coupled *in vitro* transcription-translation system using a plasmid DNA containing an *amber* gene 32 as a template. These oligonucleotides inhibit gene 32 expression with a increasing efficiency in the order r_1 Acr < r_2 Acr < r_3 Acr. We have shown that the inhibition is due to both an unspecific effect on transcription and to a specific one on translation. The first effect is most likely due to the binding of the modified oligonucleotides to RNA polymerase. The second is ascribed to their specific binding to gene 32 mRNA. Unmodified homologous oligonucleotides have a much smaller effect. This must be ascribed to the stabilization of oligonucleotide-mRNA hybrids following intercalation of the acridine derivative.

1000 AN UNUSUAL 5.8 kb TANDEMLY REPEATED SEQUENCE THAT IS TRANSCRIBED AND CONSERVED AMONG MALARIAL PARASITES,

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Malarial parasites with their complex life cycle, and the ability for growth at two different temperatures, as well as intracellular parasitism, offer many challenges to molecular geneticists. These organisms have a genome size of about $3x10^7$ bp and an A/T content of about 80%. During screening of a genomic library of the rodent parasite Plasmodium yoelii with nick-translated total parasite DNA, we have identified a repetitive sequence with unusual properties. This 5.8 kb sequence is tandemly and directly repeated in the parasite genome, making up about 2.8% of the P. yoelii genome. A sequence of the same complexity is also detected as tandem repeats in the DNA from each of the three other species of malarial parasites tested: P. berghei, P. chabaudi, and P. falciparum. The 5.8 kb element does not encode histones or ribosomal RNA. Poly A^+ RNA transcripts of P. yoelii ranging in size from less than 300 nucleotides to 1.6 kb are recognized by this sequence in the Northern blot analysis. We do not know the biological function of this highly conserved and expressed tandemly repeated sequence. However, an organism that has only 4 or 5 dispersed copies of ribosomal RNA genes must have some compelling reasons to have such a large number of copies of a highly conserved sequence.

1001 Translational control mechanisms at the replicase cistron of RNA bacteriophage MS2.

Jan van Duin and Ben Berkhout. Dept. of Biochem. University of Leiden.

The start region of the replicase gene of MS2 RNA fulfills at least 4 different functions.
a. it serves as an efficient ribosome binding site,
b. it can fold into an imperfect hairpin that can bind the MS2 coat protein resulting in repression of replicase synthesis.
c. it can assume an alternative basepaired structure with a part of the coat gene, thought to be responsible for polarity of coat amber mutants (1).
d. it is part of the coding sequence of the overlapping lysis gene,

Using recombinant DNA techniques we have:
a. determined the 5' boundary of the replicase ribosomal binding site .
b. measured repression by the coat protein on shortened variants of the replicase region. One variant can no longer bind the coat protein, but is fully active in ribosome binding (2)
c. confirmed the Min Jou model (1): deleting the relevant coat gene sequence uncouples replicase synthesis from coat protein cistron translation.
d. asked whether coat protein bound to the replicase start interferes with the progress of ribosomes synthesizing the lysis protein. Coat protein concentrations sufficient to repress replicase starts do not affect the amount of lysis protein synthesized.

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1002 ANALYSIS OF THE *SUF12* GENE AND GENE PRODUCT, Patricia G. Wilson and Michael Culbertson, Univerisity of Wisconsin, Madison, WI. 53706

Non-triplet reading of the genetic code has been proposed as an explanation of such phenomena as leaky expression of genes containing certain frameshift mutations and expression of out-of-phase overlapping genes. Our lab has examined non-triplet translocation by genetic and molecular analysis of extragenic revertants of +1 frameshift mutations. The majority of these suppressor mutations are located in tRNA genes. In contrast to such codon-specific suppression, one group of suppressors, *suf12*, *suf13* and *suf14*, suppresses frameshift mutations in both glycine and proline codons. This indicates a non-codon specific pattern of suppression.

Two classes of suppressors can be identified within this group. One class is comprised of *suf13* and *suf14*. Suppression by this class is apparently limited to frameshift mutations. The second class is comprised of different alleles of *suf12*. Unlike *suf13* and *suf14*, *suf12* suppresses UAA and UAG nonsense mutations as well as different types of frameshift mutations.

We have utilized the recessive phenotype of *suf12* to isolate a clone encoding the wildtype *SUF12* gene. Genetic evidenc suggests that the *suf12* locus encodes a protein gene product, possibly a ribosomal protein. We are currently investigating the nature of the *suf12* gene product by hybrid selection and *in vitro* translation of the *suf12* transcript.

1003 CONTROL OF VITAMIN B_{12} BIOSYNTHESIS IN AN AEROBIC BACILLUS, Julie B. Wolf and Robert N. Brey, Genex Corporation, Gaithersburg, Maryland 20877

The goal of our research is to develop a strain of *Bacillus megaterium* that produces large quantities of vitamin B_{12}. The biosynthesis of this complex molecule is very tightly controlled and probably coordinately regulated with porphyrin and heme synthesis as well as with nitrogen and sulfur metabolism. To understand the regulation of synthesis of the vitamin, we first isolated a series of auxotrophs blocked in different steps in the vitamin B_{12} pathway. Some of these mutations have been mapped by generalized transduction and appear to fall into two linkage groups. Several of the wild-type genes have been cloned by complementation onto multicopy plasmids. We are examining the effect of these cloned vitamin B_{12} biosynthetic genes on the production of vitamin B_{12}.

1004 CHARACTERIZATION OF A TRANSLATIONAL CONTROL MECHANISM BY COMPARATIVE DNA SEQUENCE ANALYSIS, Richard E. Wolf, Jr. and Gerard J. Barcak, University of Maryland Baltimore County, Catonsville, MD 21228.

Previous studies have suggested that the growth rate-dependence of 6-phosphogluconate dehydrogenase (6PGD) level in *Escherichia coli* K12 requires a site for negative control that lies deep within the structural gene, *gnd*. As an approach to characterizing this unusual genetic regulatory mechanism we have taken advantage of naturally occurring *Escherichia coli* that encode 6PGDs of differing electrophoretic mobilities. A set of isogenic strains carrying 6 different *gnd* alleles was prepared. The pattern of growth rate-dependence of 6PGD level in the strains was similar but quantitative differences in the amount of enzyme activity were observed. In contrast, expression of a *Salmonella typhimurium gnd* gene was growth-rate independent. The respective *gnd* genes were cloned. Restriction enzyme polymorphisms distinguishing each gene were readily found. Initial comparative DNA sequence analysis revealed regions of sequence divergence and conservation. The use of these data in identifying specific regulatory sequences will be described.

1005 THREE DIMENSIONAL ORGANIZATION OF THE E. COLI 16S RIBOSOMAL RNA.

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A model for the organization of the E. coli 16S ribosomal RNA in the 30S subunit is being constructed. This model is based on the 16S rRNA secondary structure, RNA-protein interactions (which are useful because the positions of the proteins in the subunit are known) and photochemical and chemical intramolecular RNA crosslinks that indicate the higher order structure of the RNA. These considerations allow the assignment of most of the RNA helices to approximate positions within the 30S subunit. The long distance secondary structure interactions that are in the center of the secondary structure remain in the center of the three dimensional form; several of the helix end loops that are on the periphery of the secondary structure are also organized so that they are at the center of the three dimensional form. The three major secondary structure domains are preserved for the most part as autonomous folded regions. In addition, many close contacts occur between the 5' domain and the middle domain in the body of the subunit. The overall asymmetric Y shape is similar to the description made for the molecule from direct visualization.

Regulation of Translation

1006 micRNA AS A REPRESSOR OF GENE EXPRESSION

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In Escherichia coli, the expression of the ompF gene, a gene for a major outer membrane protein, is inhibited by a small RNA transcript (micRNA; mRNA-interfering complementary RNA) produced under certain growth conditions (1,2). The micRNA is complementary to part of the ompF mRNA and presumably hybridizes to it to block the synthesis of OmpF protein. This observation prompted the construction of several recombinant genes, each capable of inducibly synthesizing, from the lac promoter/operator, a micRNA complementary to a specific E. coli mRNA when introduced into bacteria. In the presence of lac inducers, these genes blocked the synthesis of the proteins they were targeted to affect (3). In this manner, the artificial mic system facilitated the inducible repression of two genes normally expressed constitutively, ompA and lpp, and over-powered the normal osmoregulation of another gene, ompC. The micRNAs directed towards the 5'-end of the ompA mRNA were found to repress gene expression less effectively than mic(ompC)RNAs or mic(lpp)RNAs. To better understand the nature of this response, micRNAs complementary to two different regions of the ompA mRNA have been introduced into the same E. coli cells. In addition, micRNAs complementary to the entire ompA mRNA have also been constructed. From the analysis of the repressor activities of these micRNAs, it was concluded that (1) the induction of micRNA production blocks the expression of the specific target gene very rapidly; (2) the micRNA also reduces the amount of target mRNA in the cell; (3) there is a clear effect of mic gene dosage; (4) the level of repression elicited by two different micRNAs present in the same cell is greater than when either is present alone; (5) micRNAs complementary to regions of the RNA known to interact with ribosomes are the most effective.

We will also report the construction and analysis of micRNAs directed against other E. coli genes as well as viral and yeast genes.

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1007 MECHANISM OF IS10-MEDIATED MULTICOPY INHIBITION

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The transposition rate of IS10 decreases as the number of IS10 copies per cell increases. This phenomenon, known as multicopy inhibition, results from decreased expression of IS10 transposase at high transposon copy numbers. Genetic experiments (Simons and Kleckner, Cell *34*: 673, 1983) have suggested that multicopy inhibition is mediated by direct RNA/RNA pairing between the transposase messenger RNA and a small IS10-encoded regulatory RNA which is complementary to the 5' end of that message. By hypothesis, the extent of inhibition depends upon the level of RNA-OUT, which should increase with increasing IS10 copy number.

We have now shown that RNA-OUT and the transposase mRNA (called RNA-IN) can pair *in vitro* with a second-order rate constant similar to that observed for pairing between RNAI and primer RNA in the ColE1 system of Tomizawa (1984, Cell *38*, 861). Analysis of RNA-OUT secondary structure and of mutations encoded by RNA-IN and/or RNA-OUT has suggested a model for how this pairing occurs. We propose that the rate-limiting step in this reaction is pairing between the 5' end or RNA-IN and an unpaired loop in an extended step-loop structure of RNA-OUT. This initial interaction is followed by full pairing between the two RNA's along their entire 36 basepairs of complementarity.

We have isolated and are presently characterizing several types of mutations that alter multicopy inhibition *in vivo*. Thus far we can draw the following conclusions: (1) Mutations that abolish transcription initiation from the promoter for RNA-OUT (pOUT) also abolish multicopy inhibition. (2) pOUT$^-$ mutations can be suppressed by insertion upstream of any other strong promoter. Apparently the sequence at the 5' end of RNA-OUT is not important for multicopy inhibition. (3) All four mutations that abolish pairing between (mutant) RNA-OUT and (wild type) RNA-IN *in vitro* also abolish the ability of the mutant element to multicopy inhibition wild type IS10. (4) A mutation that moves the start of RNA-IN transcription several basepairs upstream renders the mutant element insensitive to multicopy inhibition by wild type IS10 and eliminates pairing between that RNA-IN and wild type RNA-OUT *in vitro*. These results support our hypothesis for the mechanism of multicopy inhibition and our working model for the mechanism of multicopy inhibition and our working model for the mechanism of pairing between RNA-OUT and RNA-IN. Several mutations that inhibit multicopy inhibition *in vivo* do not affect RNA pairing *in vitro*. Experiments to distinguish among the several possible explanations for these mutants are underway.

1008 RIBOSOMAL GENES OF YEAST AND THEIR REGULATION,

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The construction of ribosomes requires the simultaneous availability of nearly 100 macromolecules, which are utilized in equimolar amounts. The provision of these molecules in a timely and efficient manner calls for substantial coordination between the transcriptional and translational facilities of the cell. In practice this coordination is very effective, for under normal circumstances we see less than 10% overproduction of any component. We have studied this coordination by analyzing the effects of introducing extra copies of ribosomal protein genes into the cells of the yeast, *Saccharomyces cerevisiae*. For most non-ribosomal genes studied in this way the amount of gene product is nearly proportional to the copy number. This is generally not true for ribosomal protein genes.

We have found that the synthesis of several ribosomal proteins is regulated largely at the level of translation. Although excess copies of the gene leads to several-fold excess mRNA in the polyribosomes, only the normal amount of protein is synthesized. Two lines of evidence suggest that the regulation is at the level of initiation of translation, and involves the 5' sequences of the mRNA.

In yeast, introns are very rare. Of the 20 or so genes in which introns are present, all but two are ribosomal protein genes. We were intrigued, therefore, to find that when certain of the ribosomal protein genes are present in excess, there is an accumulation of unspliced pre-mRNA, but only for the transcript of the gene in excess. Other transcripts are spliced normally, and all ribosomal proteins are synthesized normally. We propose a model in which a ribosomal protein can regulate the splicing of the transcript of its own gene, and are carrying out experiments designed to test this model.

Finally, when certain genes are present in excess, their products are made in excess; the excess ribosomal proteins are rapidly degraded.

Two generalizations result from our studies:

1) A variety of mechanisms control the accumulation of ribosomal proteins.

2) The synthesis of each ribosomal protein is regulated independently. Excess copies of the gene for one ribosomal protein do not alter the synthesis of any other ribosomal protein.

For complementary studies on the mechanisms of ribosomal RNA synthesis and its regulation we have constructed an artificial rRNA gene in which most of the rDNA transcription unit has been replaced with a small fragment of T7 DNA which can be specifically detected. Correct transcription is observed. By manipulation of the 5' flanking sequences we have demonstrated that the major promoter element of rRNA transcription lies more than 2 kb upstream from the site of initiation.

Sequence Specificity in Transcription and Control

1009 POST-TRANSCRIPTIONAL ENHANCEMENT OF GENE EXPRESSION IN BACTERIA BY A RETROREGULATORY ELEMENT, Hing C. Wong and Shing Chang, Department of Microbial Genetics, Cetus Corporation, 1400 Fifty-Third Street, Emeryville, CA 94608

A locus that positively regulates the expression of the upstream gene(s) has been identified within a ~400-bp restriction fragment containing the transcriptional terminator of the crystal protein (*cry*) gene from *Bacillus thuringiensis* vs. *Kurstaki* HD-1. This fragment was fused to the distal ends of either the penicillinase (*penP*) gene from *B. licheniformis* or the interleukin-2 (IL-2) cDNA from the human Jurkat cell line. Synthesis of the corresponding polypeptides in *Escherichia coli* and *B. subtilis* strains carrying the fusion plasmids was increased to 2.7 - 7 times the level of synthesis in the cells harboring the parental plasmids. The enhancement of expression of upstream genes was observed to be independent of the orientation of the distal transcriptional terminator fragment. Deletion analysis revealed that the locus conferring the enhancing activity is located within a 78-bp fragment that includes the inverted-repeat, the 22-bp upstream-, and the 13-bp downstream- flanking sequences. We propose that the observed enhancing effect is a result of the transcription of this sequence which generates a mRNA with the corresponding stem-and-loop structure at its 3'-end; this stem-and-loop structure increases the stability of the mRNA and thus enhances the expression of the target gene(s).

RNA Processing

1010 **RNA-RNA INTERACTIONS DETERMINE THE SPECIFICITY OF PRE-rRNA SELF-SPLICING**

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Intervening sequence (IVS) excision and exon splicing of the pre-rRNA from *Tetrahymena* occur in the absence of protein, indicating that the catalytic activity required for the splicing reaction resides in the structure of the pre-rRNA molecule. Two transesterification reactions are involved in splicing. A free guanosine attacks at the 5' splice site, is covalently bound to the IVS and generates a 5' exon with a free 3' hydroxyl which then attacks at the 3' splice site, resulting in ligated exons and excised linear IVS. In a third transesterification reaction the guanosine at the 3' end of the linear IVS attacks at a site near the 5' end of the IVS, releasing an oligonucleotide and producing a circular IVS. Three pyrimidines precede the 5' splice junction (UCU) and the primary (UUU) and secondary (CCU) sites of circularization.

The sequence requirement at the circularization site has been studied by two different approaches. Addition of certain short oligonucleotides, containing free 3' hydroxyl groups, will reopen the circular form of the IVS, yielding a linear IVS with the oligonucleotide covalently attached to its 5' end. The product of this reaction will recircularize with release of the added oligonucleotide, indicating two nucleotides 5' to the site of circularization are sufficient for circularization. By varying the sequence of the added oligonucleotides in the reaction, it was found that certain combinations of pyrimidine dimers and trimers work best for reopening the IVS. Relative reactivity of oligonucleotides is sequence dependent: CU works better than UU, which works approximately as well as CC; UUU works better than UU. These data support the existence of an oligopyrimidine binding site within the IVS.

In a second approach, the nucleotide sequence at the site of circularization was altered using recombinant DNA techniques. RNA was transcribed *in vitro* from a plasmid containing a single *BamHI* linker (CGGATCCG) inserted in the IVS at the primary circularization site. The IVS was found to excise and to circularize to the UCC sequence within the linker. Deletions at this site, generated by S1 nuclease treatment of *BamHI*-cut plasmid, shifted the preferred sites of circularization either to the secondary site or to cryptic sites. The presence of three *BamHI* linkers at this site displaced the major site of circularization out of the linker sequences to the secondary site, presumably because the repeated linker sequences form a stem-loop structure. The major site of circularization in each of six constructs was immediately preceeded by three pyrimidines, suggesting the existence of a binding site for a tripyrimidine sequence, but position and RNA secondary structure also appear to be important in the selection of circularization sites.

1011 THE BACILLUS SUBTILIS RNASE P, Norman R. Pace, Terry L. Marsh, Katheleen Gardiner and Claudia I. Reich, Department of Biology, Indiana University, Bloomington, Indiana 47405.

The RNase P of Bacillus subtilis consists of protein (17K mol. wt.) and RNA (395 nucleotides) elements, both required for cleavage of 5' precursor segments from tRNAs under "physiological" conditions. In the presence of high cation concentrations (NH_4^+>1M, Mg^{+2}>0.2M), the RNase P RNA alone carries out the reaction with perfect fidelity. The high cation concentrations probably in part provide counterion shielding to overcome anionic repulsion between the two interacting polynucleotides, the RNase P RNA and the precursor tRNA. However, the character of the ion dependence, inhibition of the reaction by high SO_4^{-2} concentration and potentiation of the reaction by solvents (ethanol, DMSO) suggest that RNA conformational transition is involved in the reaction. It may be that the reason for catalysis by RNA rather than protein in the RNase P reaction is a requirement for fluidity in the structure of the catalyst, so that it can accomodate many tRNA substrates, which vary in their structural details.

The RNase P and Tetrahymena rRNA self-splicing reactions both involve phosphodiester bond scissions, but they seem mechanistically distinct. Whereas the nucleolytic self-splicing reactions require a 3'-OH group, destruction of the RNase P RNA and substrate tRNA 3'-OH groups by periodate does not result in diminution of the reaction.

Some understanding of the RNase P higher order structure is imperative for future progress on the problem. This best will drive from phylogenetic comparisons, seeking common foldings among homologous RNase P RNAs with different sequences. The sequences of the RNase P RNAs of B. subtilis (395 NT) and E. coli (377 NT) are known. Even though the RNAs utilize the proteins from the other species, the RNA sequence homologies are remarkably low, ca. 40 percent in the "best" alignment. The two sequences may be credibly aligned for secondary structure comparisons over only about 25 percent of their lengths, however. In those regions, common folding is evident. The two compared sequences seem to vary by addition or deletion of short helical domains. RNase P RNAs from other organisms currently are under sequence analysis.

1012 MECHANISM OF MESSENGER RNA SPLICING IN HELA CELL EXTRACTS, Richard A. Padgett, Maria M. Konarska, Paula J. Grabowski, and Phillip A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

The splicing of mRNA precursors in HeLa cell extracts proceeds in two steps (1). In the first step, a cleavage occurs at the 5' splice site and the 5' end of the intervening sequence is joined to the 2' position of an adenosine residue near the 3' splice site to produce a lariat structure. The two RNAs produced by this reaction are held together in a specific complex. In the second step of the reaction, the lariat RNA containing the intervening sequence and the 3' exon is cleaved at the 3' splice site and the 5' and 3' exons are joined by a 3'-5' phosphodiester bond. The intervening sequence is released as a lariat RNA. These reactions conserve the number of phosphate bonds in the precursor RNA and are consistent with a coupled transesterification mechanism. Although the reaction requires ATP, both the newly formed 3'-5' and 2'-5' phosphodiester bonds involve phosphate groups from the precursor RNA.

In addition to the complementarity observed between the sequences at the 5' splice site and sequences at the 5' end of U1 RNA, there is a striking complementarity between the same sequences at the 5' splice site and sequences around the site of branching. This complementarity may help determine the specificity of the splicing reaction by bringing the 5' splice site into close proximity with the branch site.

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Sequence Specificity in Transcription and Control

Prokaryotic Promoters, Repressors and Activators

1013 THE P2 OGR PROTEIN - A POSITIVE REGULATOR OF P2 LATE GENE TRANSCRIPTION. Gail E. Christie[1], Elisabeth Ljungquist[2], Robert Feiwell[3] and Richard Calendar[3], [1]Virginia Commonwealth University, Richmond, VA, [2]Karolinska Institute, Stockholm, Sweden, and [3]University of California, Berkeley, CA.

The late genes of temperate coliphage P2 are organized into 4 transcription units. Expression of these genes during P2 infection depends on P2 DNA replication, *E. coli* RNA polymerase, and a positive regulatory protein encoded by the P2 *ogr* gene. The 4 promoters from which P2 late mRNAs are transcribed share a consensus sequence at -10 and -35 which is different from that normally recognized by the host RNA polymerase. These promoters do not possess obvious repeated or symmetrical sequences that would suggest a specific binding site for a positive regulatory protein. In order to gain insight into the mechanism of activation of P2 late transcription by the *ogr* gene product, we have determined the nucleotide sequence of the *ogr* gene and undertaken analysis of *ogr* protein. The *ogr* gene is defined by a class of mutations which overcome the block to P2 late gene expression imposed by a point mutation, *rpoA109*, in the α subunit of *E. coli* RNA polymerase. The *ogr* gene product is a protein of 72 amino acids. The pI predicted from the amino acid sequence is 12.0, and there is some clustering of the basic residues at the amino terminus. In this respect, *ogr* resembles other known transcriptional effectors which bind to DNA. We have cloned the *ogr* and *ogr52* alleles into an overexpression vector under the control of the λ P_L promoter, and have demonstrated synthesis of a small protein consistent with the predicted molecular weight of the *ogr* gene product. The plasmid carrying the *ogr52* allele allows the growth of P2 in an *E. coli* rpoA109 host, confirming the production of functional protein.

1014 ELEMENTS OF TRANSCRIPTION ANTITERMINATION: A GENE FUSION ANALYSIS. Asis Das, Sailen Barik, Balaram Ghosh, Fred Warren, William Whalen and Krystyna Wolska. Univ. of Connecticut, Farmington, CT 06032.

Employing specifically engineered plasmids in which the expression of E. coli *galK* cistron is regulated by transcription termination, we have analyzed transcription antitermination mediated by phage lambda N protein *in vivo* and *in vitro*. Antitermination requires the presence of a *nut* site, the N-recognition element, between the promoter and the terminator. It also requires the direct participation of three cellular proteins NusA, NusB and S10 in roughly stoichiometric amounts. S10 is a normal component of the ribosome. In addition to S10, ribosomes contain active N, NusA and NusB proteins. It seems likely that N-action in the cell involves the ribosome. However, N-action need not involve the translational coupling phenomenon since translation upstream, across and downstream of the *nut* locus does not play a necessary role in antitermination. We propose that a specific interaction of the N-ribosome complex with RNA polymerase-*nut* RNA complex leads to the formation of the antitermination apparatus.

1015 ANALYSIS OF MUTATIONS IN THE *CRP* GENE OF *ESCHERICHIA COLI*, Susan Garges and Sankar Adhya, National Cancer Institute, NIH, Bethesda, MD 20205.

The cyclic AMP receptor protein (CRP), working in conjunction with cyclic AMP (cAMP), is involved in either the activation or the repression of a number of genes in *Escherichia coli*. CRP, when cAMP is bound to it, binds to a specific DNA site in the promoters of these genes. To study how CRP acts, we have cloned, into a bacteriophage lambda vector, the gene (*crp*) that encodes the protein. The resulting λcrp^+ phage can transduce crp^-, but not cya^- (defective in cAMP synthesis), mutant hosts and complement their pleiotropic defects. Mutagenizing λcrp^+, a class of *crp* mutants have been isolated which have acquired the ability to complement cya^- hosts. The mutant *crp* genes (called *crp**) have been crossed into the chromosome, and the physiology of the mutants studied. *crp** mutants can express *lac*, *srl*, and *ara*, but not *mal*, independently of cAMP; this expression is stimulated by exogenous cGMP. To determine what the changes in the mutant proteins are, *crp** mutations have been cloned into a multicopy plasmid for DNA sequence analysis.

Sequence Specificity in Transcription and Control

1016 REPRESSION OF A MUTANT λ P_{RE} PROMOTER BY cII PROTEIN, Gary Gussin, University of Iowa, Iowa City, IA 52242.

The -35 region of wild-type P_{RE} contains the sequence shown below. The *TTGC* sequences,

5'-TTGCGTTTGTTTGC-3'

which are sites at which cII protein binds to activate wild-type P_{RE}, flank the wild-type -35 sequence, **GTTTGT**. We have investigated transcription initiation from a derivative of P_{RE} constructed by D. Court and M. Zuber; the derivative contains a 2-base pair (bp) insertion at -17. This shifts the P_{RE} sequence 2 bp to the left so that the underlined **TTG** corresponds to the initial three nucleotides in the -35 consensus sequence, **TTGACA**. We find that, in contrast to wild-type P_{RE}, the altered promoter is active in the absence of cII protein. Furthermore, cII protein substantially represses transcription, presumably because the 2-bp insertion positions the *TTGC* sequences in such a way that cII-binding prevents recognition of the -35 region by RNA polymerase. The concentrations of cII protein required for maximal repression of the mutant promoter and maximal activation of the wild-type promoter are the same. Thus, insertion of 2 bp provides a way of assaying the effects of mutations on cII-binding independent of their possible effects on transcription initiation once cII protein has bound. To this end, we are constructing derivatives of the promoter having both the 2-bp insertion and a previously-isolated cy (P_{RE}^{-}) mutation. Of particular interest are mutations that appear to affect both cII-binding and subsequent initiation by RNA polymerase.

1017 REGULATION AND EXPRESSION OF THE PHAGE MU mom GENE, Stanley Hattman[1], Janet Ives[1], and Martha Howe[2], University of Rochester[1], Rochester, NY 14627, University of Wisconsin[2], Madison, WI 53706.

The bacteriophage Mu mom gene specifies an unusual DNA modification function. Expression of the mom gene is under a novel set of regulatory controls. For example, transcription of mom requires the host Dam methylase activity (methylates A in GATC sequences) and a trans-acting phage gene function (designated Dad). The Dam requirement can be circumvented by deleting certain regions 5' to the structural gene. The mom promoter has been localized by S1 nuclease mapping and shown to be downstream from the Dam target region. To facilitate studies on the signals mediating mom regulation, we have constructed a mom lacZ fusion plasmid which synthesizes β-galactosidase only when the Mu Dad transactivating function is provided in cells which are dam^+. This plasmid has been useful for a variety of studies. For example, mutants arise at low frequency that are capable of constitutively producing β-galactosidase activity. These plasmid mutations, therefore, result in Dad independent expression. They have not yet been characterized by DNA sequencing. Preliminary complementation analyses to localize the gene(s) responsible for transactivation function have been carried out. The results of these studies suggest that the Mu C gene encodes this activity; the C gene function is known to be required for turn on of all late Mu structural genes.

Eukaryotic Promoters and Enhancers

1018 ANALYSIS OF AN ACTIVATABLE PROMOTER: SEQUENCES IN THE SV40 LATE PROMOTER REQUIRED FOR T ANTIGEN MEDIATED TRANS-ACTIVATION. James C. Alwine and Janis M. Keller, Department of Microbiology/G2, University of Pennsylvania, Philadelphia, PA 19104.

Expression of the simian virus 40 late genes is activated by the viral early gene product, T antigen. This trans-activation is independent of viral DNA replication. We have used the chloramphenicol acetyltransferase (CAT) transient expression system to determine the sequences of the SV40 late promoter region necessary for trans-activation. We find that the T antigen binding sites at the origin of replication can be removed without loss of trans-activation. Deletion of the GC-rich 21 bp repeat regions causes a 5-fold decrease in T antigen stimulated gene expression in plasmids where the 72 bp repeat regions are not present; however, when the 72 bp repeat regions are included, the deletion of the 21 bp repeats has no effect on activated gene expression. This implies that the 21 bp repeats may contain a weak promoter element which is superseded in the presence of promoter elements in 72 bp repeat region. The major promoter elements needed for trans-activation are concurrent with the 72 bp repeat region. We have evidence of at least two elements in this region; one is within a 33 bp fragment between nucleotides 167 and 200, overlapping the junction of the 72 bp repeats. This element alone will allow T antigen trans-activation when inserted in the late orientation. These data indicate that trans-activation requires specific sequence elements and that they may function in a unidirectional manner. We have also examined the trans-activation ability of T antigen, adenovirus E1A protein and the herpes immediate early (IE) protein on a variety of promoters. While T antigen and herpes IE were promiscuous, activating all promoters tested (including the promoter of the Rous sarcoma virus long terminal repeat), the E1A protein activated only its homologous adenovirus promoter.
The promiscuity of T antigen and IE protein may indicate the mechanism by which these proteins contribute to transformation. The data with E1A indicated that trans-activation can also be specific. The finding that many promoters can be trans-activated implies that many genes may be controlled by specific trans-activation mechanisms.

Sequence Specificity in Transcription and Control

1019 TISSUE AND SPECIES SPECIFIC EXPRESSION IS CONFERRED BY A SEQUENCE FROM THE 5' END OF THE RAT ALBUMIN GENE, Marie-Odile Ott*, Linda Sperling*, Mary C. Weiss*, Philippe Herbomel@, Jean-Michel Heard@, and Moshe Yaniv@, *Centre de Genetique Moleculaire, C.N.R.S., Gif-sur-Yvette 91190 and @Department of Molecular Biology, Institut Pasteur, 75724 Paris Cedex 15, France.

To test the mechanisms involved in the control of the expression of hepatic functions, we made use of transient expression assays after transfection of a variety of cell lines. A DNA fragment preceding the 5' end of the rat albumin mRNA was cloned upstream of the CAT (chloramphenicol acetyl transferase) gene in a promoter-less derivative of pSV CAT. Comparing the activity of this promoter with those of pSVCAT or pRSVCAT constructions showed that the albumin promoter is inactive in rat or mouse fibroblasts, whereas it is as active as the SV 40 early promoter in rat hepatoma cell lines. Surprisingly the rat albumin promoter is inactive in mouse hepatocytes. Furthermore, dedifferentiated derivatives of rat hepatoma cells or hepatoma X fibroblasts hybrids that do not express the endogenous albumin gene are unable to sustain CAT synthesis with the pAlb CAT construction. Bal 31 deletion analysis was undertaken to map precisely the sequences involved in the tissue recognition of this promoter element. The implications of these findings the possible mechanisms of gene control during terminal differentiation will be discussed.

1020 TISSUE-SPECIFIC tRNA GENE EXPRESSION IN <u>BOMBYX MORI</u>, Lisa S. Young and Karen U. Sprague, University of Oregon, Eugene, OR 97403

The alanine tRNAs from silkworms (<u>Bombyx mori</u>) accumulate in a tissue-specific fashion. One species ($tRNA^{Ala}_{C}$) is produced constitutively in all silkworm tissues, while a second major species ($tRNA^{Ala}_{SG}$) is limited to the silkgland. We wish to learn whether differences in the transcriptional properties of the genes encoding these RNAs can explain the tissue distribution of alanine tRNA. To that end, we have examined the transcriptional activities of representatives of these two classes of genes <u>in vitro</u>. The two genes behave very differently in homologous transcription extracts prepared from <u>Bombyx mori</u> silkglands. To identify sequences responsible for the observed functional differences, we have constructed $tRNA^{Ala}_{C}/tRNA^{Ala}_{SG}$ hybrid genes. Remarkably, sequences upstream from the transcription initiation site determine whether the gene behaves like a constitutive type or silkgland-specific type gene. We are currently using partially purified components of the silkworm transcription apparatus to learn which component(s) interact differentially with the two kinds of genes.

Transcripton Termination and RNA Processing

1021 ANALYSIS OF PROCESSING/POLYADENYLATION SIGNALS BY USING DERIVATIVES OF THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE, Chuck Cole, Department of Biochemistry, Dartmouth Medical School, Hanover, N.H. 03756

Most gene transcribed by RNA polymerase II contain the hexanucleotide, 5'-AAUAAA-3', just upstream from the polyadenylation site. We have examined the signal requirements for processing and polyadenylation by using derivatives of the herpes simplex virus thymidine kinase gene (HSV-tk). Three lines of evidence suggest that the hexanucleotide alone is sufficient to signal processing and polyadenylation: (i) The HSV-tk gene was resected from its 3' end. All derivatives which retained the AAUAAA were able to produce poly A+ tk mRNA in transfected Cos-1 cells. The HSV-tk gene contains two copies of the AAUAAA. Resection with Bal 31 nuclease was used to generate deletion derivatives of the tk gene. Derivatives retaining at least 50 bp of information distal to these AAUAAAs produced near wildtype levels of tk mRNA. Retention of fewer than 40 nucleotides distal to the AAUAAAs resulted in a reduced level of poly A+ tk mRNA. (ii) Poly A+ tk mRNA was produced when the HSV-tk polyadenylation signal was replaced with an 88 bp fragment of SV40 DNA, containing an AAUAAA that is never used as a polyadenylation signal during SV40 infection. (iii) Random fragments of either simian (4.0-15 kb) or prokaryotic (0.2-1.5 kb) DNA were inserted into an HSV-tk derivative lacking a polyadenylation signal. Poly A+ tk mRNA production was restored by 83% of the simian and 92% of the prokaryotic DNA fragments. Together, these results suggest that the minimum signal necessary for processing/polyadenylation is a hexanucleotide (AAUAAA or similar) and that additional sequences in the vicinity of the AAUAAA affect the efficiency of the reaction, probably by affecting the secondary structure of the precursor RNA.

Sequence Specificity in Transcription and Control

1022 **FUNCTION OF *E. COLI nusA* PROTEIN: TS MUTANT AND RNA BINDING,** Yoshikazu Nakamura, Akiko Tsugawa and Saeko Mizusawa, Inst. of Medical Science, Univ. of Tokyo, Tokyo. Previous *in vitro* studies have attributed apparently antagonistic activities to the NusA protein of *E. coli*; namely both termination and stimulation of transcription. To determine which activity is functional *in vivo*, we isolated a temperature-sensitive mutant of the *nusA* gene (*nusA(tsl)*). The mutant cells produce a thermolabile NusA protein and grow at 32°C but not at 42°C. The effect of the mutation on the termination of transcription at several terminators was studied using terminator-assay vectors *in vivo*. The efficiency of termination at the *E. coli trp* and *nusA* attenuators as well as at the λt_{R1} and λt_{L1} terminators appeared to decrease and read-through transcription increased. However, the overall synthesis of β-galactosidase and the ββ' subunits of RNA polymerase was reduced by the mutation. These results suggested that the NusA protein plays both negative and positive modulator roles, and that it is involved in both ρ-dependent and ρ-independent terminations *in vivo*. Furthermore, we studied the interaction of *nusA*$^+$ and *nusA(tsl)* proteins with *boxA* RNA *in vitro*, as the previous genetic data have suggested that the NusA protein interacts with the *boxA* sequence of λ. Several mRNAs were synthesized by *in vitro* transcription of truncated λ DNA templates and their binding capacities to NusA were examined by a filter-trapping assay. Thus, we conclude that the NusA protein bound to mRNAs containing *boxA*. tRNA competition experiments revealed that this binding occurs specifically at the *boxA* site or its close vicinity. The mutant protein exhibited reduced and thermolabile RNA-binding activity, whereas that of the wild-type protein was heat-resistant. Studies on the RNA-binding domain of NusA and its role in transcription termination are currently in progress.

1023 SPECIFIC INTERACTIONS OF RHO FACTOR WITH RNA TRANSCRIPTS, John P. Richardson, Marion A.F. Ceruzzi and Susan L. Bektesh, Department of Chemistry and Institute for Molecular and Cellular Biology, Indiana University, Bloomington, IN 47405

The specificity and strength of binding of *Escherichia coli* rho factor to various coliphage and *E. coli* gene transcripts is compared with the function of rho in termination of transcription of those genes. Rho binds tightly ($K_a \sim 10^9\ M^{-1}$) to a number of specific transcripts that were terminated within or downstream from a rho-dependent site but has very little affinity for transcripts that were synthesized without being affected by rho. Rho protein is also unable to bind tightly to incomplete transcripts of the λ *cro* gene that have not attained a minimum length of 290 nucleotides, which is the size of the shortest rho-terminated transcripts of that gene. In contrast, rho binds to incomplete transcripts of the early region of T7 D111 DNA that are as much as 180 nucleotides shorter than the smallest transcripts of that gene region. These binding studies support the concept that the binding of rho protein to an RNA is a necessary prelude to the termination of transcription of that RNA by an action of rho. The results also indicate that the interval between the point where a nascent transcript is large enough to interact with rho and the point where it is large enough to be terminated by rho action varies with different genes.

Translation and Its Regulation

1024 SITE-DIRECTED MUTAGENESIS OF RIBOSOMAL RNA. A. Dahlberg, R. Gourse, M. Stark, C. Zwieb, D. Jemiolo, R. Skinner, W. Jacob and M. Santer*, Brown University, Providence, RI 02912 and *Haverford College, Haverford, PA 19041

Structural and functional aspects of *E. coli* ribosomal RNA are being studied by a genetic approach. Using a recombinant plasmid containing the *rrnB* cistron and site-directed mutagenesis techniques we have produced a series of small deletions and base substitutions at specific sites in the rRNA genes. The use of M13 and synthetic oligonucleotides has permitted us to introduce single base changes in the Shine-Dalgarno region of 16S rRNA, at G_{41} in 5S rRNA, and at the site in 23S rRNA which binds to the antibiotic thiostrepton. The altered plasmids have been transformed into "maxi cells" to determine the effects of the deletions on rRNA processing, subunit assembly and ribosome function.

1025 CODOMINANT ANTIBIOTIC RESISTANCE MUTATIONS IN RIBOSOMAL RNA GENES OF ESCHERICHIA COLI. Curt Sigmund, Mohamed Ettayebi, and Edward A. Morgan. Roswell Park Memorial Institute, Buffalo, New York 14263.

Ribosomal RNA operons of E. coli have not been extensively analyzed by genetics because of a lack of dominant selectable mutations in these operons. Mutations in rRNA operons have probably been difficult to isolate because there are seven rRNA operons in E. coli. Therefore, mutations in rRNA genes might escape detection because the mutations are recessive or alter only a small fraction of the ribosomes. An rRNA operon (rrnH) cloned on a multicopy plasmid increases the contribution of a single rRNA operon to ribosomes and allows isolation of codominant mutations in 16S and 23S ribosomal RNA genes. One mutation at position 2058 of a 23S RNA gene confers resistance to macrolide, lincosamide, and streptogramin type B antibiotics. Two other mutations which are located nearby confer resistance to a subset of the macrolide antibiotics and to chloramphenicol. A mutation at position 1192 of a 16S RNA gene confers resistance to spectinomycin. The alterations in 23S RNA are in a region involved in peptidyl transfer. The alterations in 16S RNA are in a region which may be involved in translocation. These mutations enable genetic methods to be applied to studies of ribosomal RNA struture, function, and regulation.

The Heat Shock Response

1026 EXPRESSION OF MEMBERS OF THE HSP70 GENE FAMILY OF YEAST, Elizabeth A. Craig, Michael R. Slater and Kurt Jacobsen, Department of Physiological Chemistry, University of Wisconsin-Madison, Madison, Wisconsin 53706.

The genome of S. cerevisiae contains a family of genes related to the heat inducible 70K gene of higher eucaryotes and to the dnaK gene of E. coli. Eight genes have been isolated and designated YG100-YG107. There are two pairs of highly homologous genes. YG100 and YG102 are 97% identical in the protein coding region, while YG101 and YG103 are ~94% identical. Homologies outside the protein coding regions are negligible. The homology amongst the less similar members is ~60-80%.

The expression of YG100-YG103 has been analyzed most extensively. Although the four genes are expressed at the optimal growth temperature of 30°C, their regulation differs. The abundance of both YG101 and YG103 transcripts decreases upon an increase in temperature from 23°C to 37°C. However, the abundance of YG100 transcripts increases after a temperature shift, while that of YG102 changes only slightly. Using the method of gene replacement, strains containing mutations in the four genes have been constructed. Although no phenotype of any of the single mutants as detected, haploid strains containing both the YG100 and YG102 mutations were not able to form colonies at 37°C. As the temperature is lowered, the effect on growth rate diminishes. These results indicate that YG100 or YG102 protein product is needed to obtain normal growth rates at all temperatures, but is essential at higher temperatures. Strains containing both the YG101 and YG103 mutations display altered growth properties at all temperatures. The cells grow fastest at 37°C; the severity of the effect increases with decreasing temperature, although colony forming ability is retained even at low temperatures. At 37°C the YG101 YG103 double mutant grows 22% slower than wild-type; at 23°C it grows 116% slower. These results suggest that the Hsp70 multigene family of yeast contains genes which have specialized but similar functions. Some of the gene products (YG100 and YG102) are required for sustained growth at high temperature and others (YG101 and YG103) at low temperatures.

Within the Hsp70 family, we have analyzed the heat inducible promoter of YG100 in most detail. A protein fusion to E. coli β-galactosidase was constructed through the tenth amino acid of YG100. This construction shows heat inducible β-galactosidase activity in yeast whether present as single-copy integrants or present in multicopy plasmid vectors. A 5' deletion series was constructed using Bal31. Sequences upstream of -340, where +1 is the first base of the initiation codon, are necessary for constitutive expression of YG100. A deletion at -246 is heat inducible whereas a deletion at -212 is not. A deletion at -236 has an intermediate behavior. Therefore, sequences between -246 and -212 are necessary for heat shock induction. A YG100 fragment from -320 to -190 was used to replace the upstream activating sequence of the yeast CYC1 promoter. This construction demonstrated that sequences from -320 to -190 contain a heat shock specific upstream activating sequence and those sequences are sufficient to elicit the heat shock response.

1027 Regulation of the Heat Shock Response in *E. coli*. Alan D. Grossman, Deborah W. Cowing, James W. Erickson, David B. Straus, and Carol A. Gross, Dept. of Bacteriology, Univ. of Wisconsin, Madison, WI 53706.

When *E. coli* are shifted from 30°C to 42°C, the rates of synthesis of heat shock proteins (*hsps*) increase between 5 and 20 fold, depending on the particular protein. The increased rates of synthesis peak 5-10 min after the upshift and then decline to new steady state rates of synthesis somewhat greater than those at 30°C. The increased rate of synthesis of hsps reflects increased synthesis of their mRNAs and increased transcription initiation at heat shock promoters.

The transcriptional regulator of heat shock gene expression is the *rpoH* (*htpR*) gene product. *rpoH* encodes a 32 kd sigma factor(σ^{32}) which promotes transcription initiation at heat shock promoters. Mutations in *rpoH* result in a defect in the heat shock response (and a defect in degradation of some unstable proteins).

Five *E. coli* promoters have been identified that are heat-inducible *in vivo* and transcribed *in vitro* by RNA polymerase holoenzyme containing σ^{32}. We have compared these promoters and proposed a consensus sequence for heat shock promoters. The consensus sequence has T-tC-cCTTGAA in the -35 region and CCCCATtT in the -10 region.

In vivo, induction of hsp synthesis after a temperature upshift could be due to an increased *amount* of σ^{32} and/or increased *activity* of σ^{32}. We have fused *rpoH* to the inducible lactose promoter in order to regulate σ^{32} synthesis. At 30° and 42° induction of σ^{32} synthesis by IPTG causes a transient increase in hsp synthesis. Thus, increased synthesis of σ^{32} can increase the synthesis of hsps.

In addition, σ^{32} is unstable *in vivo*, and has a physical half-life of about 5 min. Because it is rapidly turned over, changes in the rate of synthesis of σ^{32} will rapidly lead to changes in its intracellular concentration. Consistent with the notion that the amount of σ^{32} might be increased at high temperature, we have found that in wild type strains the amount of σ^{32} mRNA increases after a temperature upshift. We do not know if the increase is sufficient to cause the rapid increase in synthesis of hsps.

Overproduced σ^{32} migrates as 2 spots in the isoelectric dimension of 2D gels while purified σ^{32} migrates as only one spot. σ^{32} was purified based on its activity. Perhaps only one isoelectric form of σ^{32} is active. Reversible or irreversible inactivation of σ^{32} could provide a direct mechanism for both induction and shut-off of the heat shock response.

1028 INDUCTION OF THE BACTERIAL HEAT SHOCK RESPONSE, Frederick C. Neidhardt and Philip M. Kelley, Department of Microbiology and Immunology, University of Michigan Medical School, Ann Arbor, MI 48109.

Sudden heating of a bacterial culture to growth-restricting temperatures suppresses the synthesis of most cellular proteins and induces high rates of synthesis of 17 polypeptides. The induced proteins resemble the heat-shock proteins of other organisms in their pattern of regulation and, in some cases, their molecular structure. In *Escherichia coli* these heat-shock proteins are products of unlinked operons forming a regulon under the control of the regulatory gene, *htpR* (1). This gene has been cloned and sequenced (2). Its protein product has been identified on two-D gels as cellular protein F33.4. Its predicted structure possesses 43% identity or conservative replacement of amino acid residues with the carboxy-terminal half of RNA polymerase sigma factor (3). Protein F33.4 purifies with RNA polymerase, and purified preparations direct the initiation of transcription from heat-shock promoters without requiring normal sigma factor. On this evidence F33.4 has been designated sigma-32 (the normal sigma, sigma-70) (4).

At temperatures above 45 C part of the differential induction of heat-shock proteins is the result of suppression of expression of most other genes, but the sudden, high rates of heat-shock protein synthesis upon shifts to the 37 - 45 degree range must involve a pronounced absolute activation of heat-shock transcription (1). How transcription of heat-shock promoters by sigma-32 is activated at high temperature is not known. Our recent work is directed at this process. We have examined the effect on *E. coli* of a large number of agents known, or claimed, to induce the heat-shock response in other organisms - including various oxidants, DNA replication inhibitors, and damagers of DNA or membrane integrity. Also, we have examined the synthesis of various dinucleotides during treatment with heat and these other agents. Our results indicate that ethanol is the only agent that closely mimics high temperature in inducing the heat-shock regulon in *E. coli*, and that there appear to be several, perhaps related, stress regulons inducible in this organism by various toxic substances.

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1029 EXPRESSION AND FUNCTION OF HSP70, Hugh Pelham, Sean Munro and Michael Lewis, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England.

Most cells synthesize the major heat shock protein (hsp70) when exposed to stress, but the precise function of this protein is obscure. We have studied the properties of intact Drosophila hsp70 and of a variety of deletion mutants expressed from cloned genes in monkey COS cells. In order to detect the mutants, the genes were tagged with a synthetic oligonucleotide which encodes a short peptide recognized by a monoclonal antibody.

Hsp70 is mostly concentrated in the nucleus of unstressed COS cells; on heat shock a large fraction of it transiently associates with nucleoli. Deletion analysis defines two domains of the protein, a large N terminal one which has been strongly conserved during evolution and a smaller C terminal one. Both are able to accumulate in the nucleus, but only the N terminal one is able to enter nucleoli.

The latter interaction appears to be a functional one. Nucleoli are visibly damaged by heat shock, but subsequently recover. Overproduction of Drosophila hsp70 by fusion of the gene to a strong constitutive promoter greatly accelerates their recovery. These and other results suggest that hsp70 may catalyze the reassembly of heat-disrupted RNPs. We are currently studying the properties of purified hsp70, and attempting to devise more direct assays for its function both in vivo and in vitro.

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Pelham, H.R.B. (1984) EMBO J., in press.

Enhancers; Heat Shock

1030 DOUBLE-STRANDED DNA INDUCES THE PHOSPHORYLATION OF SEVERAL PROTEINS INCLUDING THE 90,000 M_r HEAT-SHOCK PROTEIN IN ANIMAL CELL EXTRACTS, Carl W. Anderson, Anthony I. Walker, Richard J. Jackson, and Tim Hunt, Biochemistry Department, Cambridge University, Cambridge CB2 1QW, England

Double-stranded DNA (dsDNA) has been found to induce the transfer of phosphate from ATP to several proteins in extracts of widely divergent eukaryotic cells. Rabbit reticulocyte lysates, extracts of HeLa cells, Xenopus (frog) egg and oocyte extracts, Arbacia (sea urchin) egg and oocyte extracts, and Spisula (surf clam) oocyte extracts all show dsDNA-dependent protein phosphorylations. The mechanism is specific for dsDNA and will not respond to either RNA or single-stranded DNA. The minimum sized dsDNA fragment capable of inducing phosphorylation in HeLa extracts is about 14 base-pairs. One of the proteins phosphorylated in reticulocyte lysate, HeLa, and Xenopus egg extracts has a molecular weight of 90,000 and has been identified as a heat shock protein (hsp90). HeLa extracts and reticulocyte lysates possess kinase activities that bind to DNA-cellulose; DNA-cellulose depleted extracts no longer phosphorylated hsp90 in response to DNA. The pattern of dsDNA-independent phosphorylations is not significantly altered by DNA-cellulose depletion of extracts.

1031 A NOVEL ENHANCER SEQUENCE IS LOCATED WITHIN THE GAG GENE OF AVIAN SARCOMA VIRUSES, Karen L. Beemon, Mary Yun and Jeanne Lindquist, The Johns Hopkins University, Baltimore, MD 21218

The hypothesis that transcriptional regulatory sequences are located within the coding regions of avian sarcoma viruses (ASVs), as well as in their long terminal repeats, is being investigated. A GTGGTTTG sequence, matching the consensus core sequence observed in many enhancers, is present in the gag-coding region of many ASVs, approximately 900 nucleotides from the 5' end of the RNA genome. An internal portion of the gag gene of Fujinami sarcoma virus (532 to 1185 nucleotides) was inserted into the pSV1cat plasmid at a position downstream of the chloramphenicol acetyltransferase (CAT) gene. This plasmid contains an SV40 promoter but most of the SV40 tandem 72 bp enhancer sequences have been deleted. After DEAE-dextran mediated transfection into mouse Ltk$^-$ cells, the level of CAT activity assayed with the gag-pSV1cat construct was higher than that obtained with pSV2cat, containing intact SV40 regulatory sequences. Strong enhancement with the gag-containing plasmid was also observed in chick embryo fibroblasts. We are currently studying the mechanism of this observed enhancement of CAT activity.

1032 SUPERCOILING, CHROMATIN STRUCTURE, AND THE REGULATION OF ACID PHOSPHATASE GENE EXPRESSION, Lawrence W. Bergman, Martin C. Stranathan, Connie S. Agnor and Lisa Preis, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD 21228.

To investigate the mechanism of transcriptional activation, we have developed a high copy plasmid system containing the entire structural and regulatory sequences of the acid phosphatase (PHO5) gene and the TRP1/ARS1 sequences of the yeast *Saccharomyces cerevisiae*. The resulting 3.4kb plasmid has been utilized to transform either wild type or a number of acid phosphatase regulatory mutant strains. Analysis of mRNA levels under repressed and derepressed growth conditions reveals that normal transcriptional regulation of the gene persists, although gene copy number has been increased approximately 50-fold. Analysis of changes in the linking number of the plasmids isolated under repressed and derepressed growth conditions has revealed that the transcriptionally active plasmid contains 2-3 less negative supercoils than the inactive plasmid. This difference in linking number is similarly seen in a plasmid containing a second sequence-related phosphate-repressible acid phosphatase gene, however, is not seen in plasmids isolated from either fully constitutive or non-derepressible regulatory mutants suggesting that the change in linking number is associated with the mechanism of regulation of the acid phosphatase gene. Finally, analysis of the chromatin structure of these plasmids has revealed a localized disruption of nucleosome positioning associated with transcriptional activity.

1033 CLONING OF SOMATOMEDIN-C DOUBLE-STRANDED DNA, Gary N. Buell, Biogen SA, Geneva 1201, Switzerland

Double-stranded DNA encoding the human hormone, somatomedin-C (SMC) has been synthesized. This synthetic gene was expressed in *E. coli* from a plasmid bearing the strong leftward promoter (p_L) of bacteriophage lambda. Optimal codons for the N-terminal amino acids of SMC were chosen in an SMC/β-galactosidase fusion assay using a 256x degenerate DNA linker. The levels of SMC accumulated by heat induction of p_L were influenced by two *E. coli*, *lon*, an intracellular protease and *htpR*, a pleiotropic positive regulator of heat shock.

1034 HOST SPECIFICITY OF POLYOMAVIRUS DNA IS NOT ALTERED BY THE SV40 72 BASE PAIR REPEATS, Bruce A. Campbell and Luis P. Villarreal, University of Colorado, Denver, Colorado 80262

Mouse 3T6 cells and monkey CV-1 cells were transfected with polyomavirus DNAs containing; (i) a deletion of sequences known to possess enhancer activity; (ii) the SV40 72 base pair (bp) repeats in place of the deleted enhancer; and, (iii) the SV40 72 bp repeats, 21 bp repeats, and origin of replication substituted for the enhancer. The levels of replication and transcription were measured following transfection. Deletion of the polyomavirus enhancer resulted in a genome that was defective for replication and transcription. The SV40 72 bp repeats readily substituted for the polyomavirus enhancer allowing replication to nearly wild type levels in 3T6 cells. Polyomavirus early gene transcripts were present among the cytoplasmic RNAs of 3T6 cells, however, no replication or transcription was observed in CV-1 cells. The polyomavirus genome containing the SV40 72 bp repeats, 21 bp repeats, and origin of replication was found to replicate at very low levels in 3T6 cells and CV1 cells. Small amounts of early gene transcripts were found in the cytoplasmic RNAs isolated from 3T6 cells, but no polyomavirus specific transcripts were detected in CV-1 cells. Whereas the polyomavirus and SV40 enhancers are thought to promote expression in a cell type specific manner, our results suggest that the SV40 72 bp repeats will substitute to promote polyomavirus DNA replication and early gene expression in 3T6 cells. The SV40 enhancer is unable to alter the host specificity of the polyomavirus genome.

1035 THE EFFECT OF HEAT SHOCK ON PLANT PATHOGEN PROMOTERS, Nathan M. Chu, Arthur G. Hunt, Nam-Hai Chua, The Rockefeller University, New York, NY 10021-6399.

We have observed the stimulation of gene expression by elevated temperature in plant cells harboring genes contributed by plant pathogens. Genes encoding nopaline (nos) and octopine (ocs) synthetase activities are transferred into plant chromosomes upon oncogenic transformation by the soil bacterium Agrobacterium tumefaciens. These enzymatic activities are constitutively expressed in plant cells. Cauliflower mosaic virus (CaMV) is a double strand DNA plant virus which infects members of the Brassica family. CaMV promoter sequences are functional in other plant species. Gene activities under CaMV promoter control are also constitutively expressed in plant cells.

We are interested in studying stress effects on the regulation of expression of genes under the control of plant pathogen promoters. Here we present evidence for stimulation of expression of nos, ocs, and chimeric genes containing the CaMV 35S promoter by elevated temperature.

1036 THE BOVINE LEUKEMIA VIRUS LTR IS A CELL-SPECIFIC PROMOTER UNIT, David D. Derse and James W. Casey, Section of Genetics, NCI, Frederick, MD 21701

Bovine leukemia virus expression is highly restricted in vivo and in vitro. To examine the molecular basis for the control of BLV expression, we excised BLV LTRs from cloned proviruses and fused them to the bacterial chloramphenicol acetyltransferase (CAT) gene. Plasmids carrying the CAT gene controlled by the BLV LTR or LTR fragments were introduced into a variety of cells. The BLV LTR was an inactive promoter in all cell lines tested except those previously established as BLV producers. In FLK-BLV or BLV-bat2 cells, the BLV LTR directed high levels of CAT expression. Deletion mapping experiments revealed that removal of LTR sequences located between 100bp and 170bp upstream of the RNA start site reduced CAT expression by 90%. A 75bp DNA fragment encompassing this region was cloned into pSV Ecat (a pSV2cat derivative lacking SV40 72bp repeats and requiring the insertion of "enhancers" for CAT expression). The resulting plasmids, p E75cat, directed CAT expression only in the BLV producer cell lines, indicating that the 75bp fragment contains the BLV cell type-specific enhancer element. Surprisingly, deletion of LTR sequences on the 3'-side of the RNA start site caused an 87% reduction in CAT expression. Whether this relfects an effect on transcription or post-transcriptional stages of gene expression is currently being investigated. Thus, the cell type-specific promoter activity of the BLV LTR probably results from an interaction of unique transcriptional factor(s), present in BLV producer cells, with the BLV enhancer element(s).

1037 SEQUENCE, STRUCTURAL AND SPATIAL REQUIREMENTS FOR THE cis AND trans ACTIVATION OF TRANSCRIPTION ENHANCERS OF DNA AND RNA TUMOR VIRUSES; Thomas Firak, Ramesh Kumar, Chris Schroll and Kiranur Subramanian.

We have dissected the transcriptional enhancer elements of Adenovirus 5 (Ad5), Rous Sarcoma Virus (RSV) and Simian Virus 40 (SV40) in an attempt to locate the essential domains involved in transcriptional enhancement, transformation in culture, and activation in trans by viral oncogene products. We have utilized transient and long term assays using chloramphenicol acetyl transferase (CAT), thymidine kinase (TK) and neomycin phosphotransferase (NEO) as marker genes in human, monkey and mouse cells in culture. Our studies indicate that each enhancer comprises of several interactive domains which have critical spatial constraints. All three transcriptional control elements are activated by adenoviral E1A protein in transient assays. There appears to be no rigid sequence specificity in the E1A mediated modulation; However both the structure and spatial configuration of the enhancer region contribute to the trans induction by SV40 T antigen. Marker gene constructs with Ad5 and RSV enhancers when introduced into the genome of human and mouse cells in culture are responsive to the trans induction by the E1A protein. We have also critically examined the importance of spacing between the various domains and Z DNA elements of the enhancers and the consequences of varying the distance separating the TATA box and the enhancer on the transcriptional enhancement activity in the presence or absence of trans acting inducers.

1038 IDENTIFICATION OF THE FUNCTIONAL MODIFICATION OF A POLYOMA EC HOST RANGE MUTANT, K.H. Hales, J.G. McMahon and J.M. Lehman, Department of Pathology, University of Colorado Health Sciences Center, Denver, CO 80262

Polyoma mutants capable of producing lytic infections in embryonal carcinoma (EC) stem cells contain mutations in the transcriptional enhancer region. This laboratory has isolated a polyoma mutant which appears to differ from those previously isolated. The mutant, Py F9340, contains a 2500 bp insert at or near the Bgl I site on the early side of the origin. This insert appears to consist of five 500 bp repeats which map to a region from -100 bp from the origin of replication and extending 400 bp into the early region. Removal of the inserts renders the virus incapable of expressing in EC cells but does not prevent expression in mouse embryo cells. Insertion of the repeats into wild-type polyoma confers upon it the ability to express in EC cells. We are presently addressing the question of how many inserts are required for expression in EC cells and which, if any, subfragment of the 500 bp repeat is sufficent for expression. This will allow us to determine if this mutant has acquired a modified enhancer or if it has overcome the EC host restriction by some other mechanism. (Supported by CA-16030, CA-09157 and CA-15823 from the National Institutes of Health, and by a gift to the department from R.J. Reynolds Industries, Inc.)

1039 ACQUIRED THERMOTOLERANCE IN *TETRAHYMENA* IS THE RESULT OF A CHANGE IN THE CELL'S TRANSLATIONAL MACHINERY, Richard L. Hallberg and Kevin W. Kraus, Iowa State University, Ames, Iowa 50011.

When *T. thermophila* cells growing at 30°C were shifted either to 40°C or 43°C, the former lived (100% survival for 24 hr) and the latter died (<0.01% survival after 1 hr). The mRNAs for hsp 73 and hsp80 were induced by both the 40°C and 43°C treatments. However, only in the 40°C treated cells was the induced synthesis of hsp73 and hsp80 detected. Cells shifted from 30°C to 40°C for 15 min and then to 43°C survived 1 hr at that temperature (ca. 70% survival). Similarly, cells treated with 1 ug/ml cycloheximide at 30°C for 3 hr, when shifted to 43°C, also survived for 1 hr (ca. 60% survival). That is, in both cases, cells had an acquired thermotolerance.

We examined the protein synthetic activity of cells treated continuously at 30°C with cycloheximide. Fowllowing an initial >95% inhibition, protein synthesis began to recover by 1 hr and by 2.5-3 hr was completely restored to the pre-drug-treatment level. The kinetics of this recovery was coincidental with the induction of acquired thermotolerance. An examination of the array of proteins synthesized during this time showed no synthesis of any heat shock proteins. Furthermore, no detectable induction of mRNAs for hsp73 or hsp80 was found. Throughout the 3 hr of cycloheximide treatment, hsp mRNAs were equally inducible by either a 40°C or 43°C heat shock, but their translation could not be detected until after 2.5 hr of cycloheximide treatment. These and other data suggest that a) hsp synthesis is not necessary to induce the acquired thermotolerant state and, b) acquired thermotolerance involves a change in the translational properties of the cell.

1040 DUPLICATIONS OF A MUTATED SV40 ENHANCER RESTORE ITS ACTIVITY, Winship Herr and Yakov Gluzman, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

The SV40 enhancer contains two 8 bp stretches of alternating purines and pyrimidines that have been implicated in transcriptional activation (Nordheim & Rich, *Nature* *303*, 674). We investigated the functional role of these sequences within a SV40 enhancer that contained only one copy of the 72 bp element; the pattern of alternating purines and pyrimidines was destroyed by making two transversion point mutations in each 8 bp segment. This mutant enhancer is four- to six-fold less active than the wild type enhancer in a transient Hela cell expression assay when linked to the human B globin gene. SV40 virus carrying these four point mutations also grows very poorly in CV-1 cells; revertants with improved growth potential could be obtained, however, after passage of mutant virus stocks in CV-1 cells. Nucleotide sequence analysis of 18 plaque-purified revertants showed that each revertant carried a tandem duplication of the mutated enhancer region, but the mutations were always preserved even when duplicated. The revertant phenotype and improved enhancer function were both shown to result from these duplications. Although the duplicated regions range from 45 to 135 bp in length and the sequences contained in each duplication vary, there is a 15 bp sequence, TGTGGAAAGTCCCCA, that is consistently duplicated in each reactivated enhancer. This region contains the 'core' sequence, GTGGAAAG, that was initially suggested (Laimins *et al.*, *PNAS* *79*, 6453) to be functionally significant because related sequences were identified in a variety of viral enhancers. Our results suggest that the 'core' and surrounding sequences are a *cis*-acting element that can function independently of and compensate for the alternating purine and pyrimidine sequences.

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1041 ENHANCER SEQUENCES IN VISNA VIRUS: A RETROVIRUS CAUSING A SLOW DISEASE IN SHEEP,

Jay L. Hess and Janice E. Clements, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

Visna virus is a non-oncogenic retrovirus which causes a chronic demyelinating disease in sheep beginning months to years after the initial infection. The major target cell for visna in infected animals are monocytes, which express virus upon differentiation into macrophages in lung and brain. For unknown reasons, other tissues remain free from virus. As a step toward understanding the tissue tropism of visna virus, we sequenced the U3 region of the visna LTR and have identified sequences which resemble enhancer elements - a 43 base pair repeat 77 base pairs upstream from a TATAA box. Although the repeats in visna virus lack the consensus seuqence GXTGTGG-TTT or AAA found in most viral and eukaryotic enhancer elements, they act as strong enhancers when inserted into pSV1CAT, a plasmid containing the chloramphenicol acetyltransferase (CAT) gene under the transcriptional control of an SV40 promoter. In both L-cells and sheep choroid plexus cells, plasmids containing visna repeats inserted either 3' or 5' to the CAT gene gave a 29-fold enhancement of CAT activity in a 5'—→3' orientation, while a plasmid with repeats inserted 3' to the CAT gene in the opposite orientation had a 6 to 7 fold enhancement as compared to the parent plasmid. In sheep macrophages, plasmids containing an SV40 promoter and either SV40 or visna enhancer sequences were poorly expressed. pVIS2CAT, a plasmid containing the CAT gene with visna promoter and enhancer sequences, gave high levels of CAT activity in all three cell types examined.

1042 LOCALIZATION OF HEAT SHOCK INDUCED TRANSCRIPTION ON DROSOPHILA DNA,

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After Heat shock a massive redistribution of RNP-proteins is observed by immunolocalisation of antigens on polytene chromosomes of Drosophila and by velocity gradient analysis of extracts of Drosophila tissue culture cells. One of the largest heat shock puffs, 93D , accumulates giant RNP particles after temperature shift from 25°C to 35°C.

We have isolated a series of DNA fragments from the region 93D-6-7 as shown by in situ hybridisation to polytene chromosomes. In vitro labeled nuclear RNA prepared from heat shocked third instar larvae hybridized specifically to one fragment within 85 kb of cloned DNA. The region which is specifically transcribed after heat shock could be defined to a cluster of internally repetitive DNA and its neighbouring proximal sequences. Over a sequence of 12 Kb the DNA is cut into repeat units of 280 nucleotides by the restriction endonuclease Taq I.

A comparison beween heat shock locus 93D and the corresponding locus in Drosophila hydei revealed a similar arrangement of a repetitive transcript in close vicinity to a unique one.

1043 NEW GENE EXPRESSION ACCOMPANYING ACTIVATION OF MOUSE LIGHT CHAIN GENE TRANSCRIPTION,

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Transcription of the immunoglobulin heavy chain and light chain genes is mediated through enhancer elements present in their respective J-C introns. These enhancers are very cell type specific, since cloned functionally rearranged Ig alleles are transcribed correctly and efficiently only when introduced into lymphoid cells. This suggests the presence of cell type specific gene products which can activate an enhancer from a silent to a functional transcriptional control element. We are interested in isolating cDNA sequences from mRNA coding for factors which can activate the mouse kappa light chain enhancer. We assume that these sequences are expressed in B-cell populations in which the kappa gene is transcribed but not in kappa mRNA negative B-cells. Using a subtractive hybridization technique which allows a substantial enrichment for cell type specific sequences, we have measured gene expression differences between the mouse pre-B cell line 70Z/3, in which the rearranged kappa allele is not transcribed and lipopolysaccharide (LPS) treated 70Z/3 cells which produce kappa mRNA and the B-cell line WEHI231 which produces kappa mRNA constituvely. Our results show that the cDNA sequences specific for LPS induced 70Z/3 cells are less than 1% of the total mass of cytoplasmic mRNA. We will attempt to isolate clones representing these specific cDNAs and test them for the ability to induce kappa mRNA in 70Z/3 cells in the absence of LPS.

1044 STABLE ACTIVATION OF AN INTEGRATED HUMAN BETA GLOBIN PROMOTER IN MONKEY CELLS

Thomas Kadesch and Paul Berg, University of Pennsylvania, Philadelphia, PA 19104 and Stanford University, Stanford, CA 94305

The human beta globin promoter has been linked to the *gpt* gene of *E. coli* to form a hybrid transcription unit, BG-gpt. This gene is normally inactive when introduced into CV1 monkey cells. It can be activated, however, when co-transfected with the SV40 enhancer acting *in cis* or with either the pseudorabies virus IE gene or adenovirus Ela gene acting *in trans*. CV1 cell lines have been isolated that contain an integrated copy of the hybrid gene in an inactive form. A subset of these lines can be transiently induced for expression of the BG-gpt gene (up to 100-fold) either by fusing them with 293 cells or by infecting the cells with adenovirus. Cells that stably express a gpt-positive phenotype can be directly selected after these inducible lines are transfected with plasmid DNA that carries the pseudorabies virus IE gene. These inducible cell lines are currently being used to identify other genes that may activate the human beta globin promoter.

1045 IDENTIFICATION OF DNA SEQUENCES AND CHROMATIN STRUCTURES INVOLVED IN HEAT SHOCK GENE INDUCTION,

Robert Kay, Roland Russnak and E. Peter M. Candido, University of British Columbia, Vancouver, B.C., Canada

A pair of divergently transcribed C. elegans small heat shock protein genes have been transfered to mouse C127 cells using a BPV/G418^r vector. They are stably maintained in an episomal form with copy numbers ranging from 10 to 1000. Transcription of the heat shock genes is repressed under normal conditions but strongly induced by heat shock or arsenite. Measurements of nuclease sensitivity indicate that all copies can be simultaneously induced. Sequence elements involved in modulating heat shock promoter induction and transcription are being identified with a series of deletions, rearrangements and site directed mutations. A heat shock-induced enhancer-like element has been found downstream from the promoter. Sequences required for efficient scission of the transcript 3' to the AATAAA site have been found immediately upstream from the promoter. The mechanism by which these sequences affect distal processing events will be examined with a further series of reconstructions. Essential protein-DNA interactions are being identified by chromatin mapping of intact and reconstructed heat shock genes and with nuclease footprinting assays.

Minimal vectors containing less than a kilobase of BPV DNA are being developed to facilitate isolation and direct analysis of transfected heat shock genes.

1046 REGULATION OF MOUSE-GOAT HYBRID β-GLOBIN GENES IN MOUSE ERYTHROLEUKEMIA CELLS.

Carol Kherlakian, Eileen D. Kuempel and Donal Luse. University of Cincinnati, Cincinnati, OH 45267-0522

We are investigating the regulation of hybrid globin genes during the differentiation of murine erythroleukemia (MEL) cells. We have therefore constructed three hybrid globin genes consisting of 1.4 kb of 5' flanking sequences from the mouse β-major gene along with its first and most of its second exon, linked to 3' sequences from one of three goat β-like genes: β^c (early adult), γ (fetal) or E^{II} (embryonic). Following the insertion of these constructs via transformation into a tk^- MEL cell line we induced the transformants with DMSO. Three out of the eight transformants containing the hybrid β^c gene showed induced mRNA levels of 30 to 200 copies per cell with no constitutive mRNA expression. One showed down-regulation upon induction and the rest showed no signal. Four out of nine transformants containing the hybrid γ gene showed induced mRNA levels of 500 to 3000 copies per cell. One had constitutive expression and the rest showed no signal. We are currently analyzing the hybrid E^{II} transformants; preliminary experiments with 15 different transformants, all of which induce their endogenous β-globin genes, showed no expression of the E^{II} hybrid before or after induction. We are currently determining (i) the copy number of the E^{II} hybrid genes and (ii) the expression of a control mouse-human hybrid gene in these transformants. The fact that the β^c and γ hybrids do induce, but the E^{II} hybrid apparently does not, provides further evidence for the control of β-globin expression by internal sequences. (Recall that all three genes have the mouse β major promoter). it is worth noting that the goat γ gene is much more homologous with goat adult β genes than with goat embryonic β-like genes. Thus, the induction of the mouse-goat γ hybrid is not necessarily surprising, even though MEL cells do not induce their endogenous fetal β-like genes.

1047 REGULATION OF HEAT SHOCK PROTEIN 70 GENE EXPRESSION BY VIRAL AND CELLULAR ONCOGENES.

R. E. Kingston, A. S. Baldwin and P. A. Sharp, Center for Cancer Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts.

In order to investigate regulation of heat shock protein 70 (hsp70) expression, the promoter region of a Drosophila hsp70 gene has been linked to either dihydrofolate reductase (DHFR) or chloramphenicol acetyl transferase (CAT). Cotransfection of the hsp70-DHFR vector into DHFR deficient CHO cells with vectors expressing either the adenovirus EIa region, the polyoma virus early region, or the mouse c-myc gene results in an increase in the number of colonies that can form under selective growth conditions. Similar results are obtained when expression is assayed 48 hours after transfection through use of hsp70-CAT vectors. Small deletion mutants were used to demonstrate that the protein products of the c-myc gene, the EIa region and the polyoma early region were responsible for stimulation of expression. The sequences necessary for regulation of expression of the hsp70 chimeric gene by these gene products lie more than 200 bases upstream of the normal hsp70 transcription start site. Vectors containing the hsp70 promoter but deleted for this regulatory sequence are still responsive to heat shock regulation, implying that hsp70 expression is regulated by the viral and cellular gene products through a specific mechanism that is different than that of the heat shock response.

1048 TRANSLATIONAL CONTROL OF THE HEAT SHOCK RESPONSE IN *DROSOPHILA MELANOGASTER*,

Roman Klemenz, Dan Hultmark and Walter Gehring, University of Basel, CH-4056 Basel, Switzerland

One of the effects of temperature elevation above 35^{0}C is the selective translation of heat shock mRNA. We tested whether the transcript of a non heat shock gene is being translated at high temperature provided it is synthesized during the heat shock. A fusion construct between the alcohol dehydrogenase (adh) gene and the promoter of a heat shock gene was introduced into adh^- flies. Such transformed flies synthesize adh mRNA during heat shock in massive amounts but translation of this mRNA is restricted to non heat shock temperatures. Thus, the information that allows the translation machinery to discriminate between heat shock and non heat shock mRNA must reside in the primary sequence of the heat shock mRNA.

Internal deletions of different parts of the non translated leader region of a heat shock gene were performed in order to identify such a sequence element. Over 80% of the leader sequence are dispensable for efficient translation. Deletion to within 25 bases from the cap results in a drastic drop in expression both at high and low temperature.

1049 Reconstitution of an *in vitro* transcription system to search for regulatory factor,

Takashi Matsui, Univ. of Occup. and Environ. Health, Japan

My current interest is to know how the expression of genes coding for proteins are regulated in eukaryotic cells. There is relatively much information on the DNA regions which may control the transcription levels, while little is known about protein factor which might interact to the DNA regulatory region to regulate their expression. There are few works to search such regulatory proteins using *in vitro* transcription system. However, the regulatory mode of expression *in vitro* has not been shown in the case of most of genes studied so far. This kind of *in vitro* study is totally based on an observation of the transcription enhanced effect by the extracts from the cells in which a given gene is actively transcribed. It is not clear whether the enhancement of transcription levels observed *in vitro*, if any, mimics truely regulatory mechanism of gene expression occurred *in vivo*. Moreover, it may not be assured if the *in vitro* transcription of all genes can be stimulated in same manner with the homologous cell extracts. Therefore, it seems to be essential to establish an improved *in vitro* transcription system in which any regulatory proteins could be identified. There is much evidence showing that 5'-upstream region of the transcriptionally active gene is free of nucleosome and that a certain cell-specific protein(s) interacts specifically with this region of gene. The binding of the regulatory protein(s) to the DNA must be essential for gene to be actively transcribed through conformational change of the regulatory region. My approach along this line is to use nucleosome assembled *in vitro* and search protein(s) required for an activation of the *in vitro* transcription initiation of a given gene.

1050 TRANSLATIONAL CONTROL PROCESSES WHICH GOVERN THE SYNTHESIS OF HEAT SHOCK PROTEINS,

Thomas McGarry and Susan Lindquist, Department of Biology, The University of Chicago, Chicago, IL 60637.

When *Drosophila* cells are exposed to high temperatures, the synthesis of most proteins stops, but there is a dramatic, coordinate induction of a small group of proteins, the heat shock proteins (hsp's). This response is brought about by both an activation of heat shock gene transcription and a preferential translation of heat shock mRNAs; other cellular messages are stably retained in heat shocked cells even though they are not translated. When cells are returned to normal temperatures after a heat shock, the synthesis of hsp's continues until a certain amount of the major heat shock protein, hsp70, has accumulated. At this point, heat shock transcription shuts down and heat shock messages are destabilized and degraded.

We have been trying to identify specific sequences on heat shock messages which confer their characteristic translational regulation. Our attention has focused on the unusually long (242 bp) leader sequence on the hsp70 message, which contains two sequence elements that have been conserved in several other heat shock mRNAs. A number of linker insertion mutations and small deletions in this leader have been constructed *in vitro*. We are generating stably transformed *Drosophila* cell lines which harbor these constructions in order to compare their regulation to that of the resident wild-type genes.

1051 A NON TISSUE SPECIFIC ENHANCER FLANKING THE HUMAN GROWTH HORMONE GENE PROMOTER,

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Comparison of sequences of promoter regions from several mammalian growth hormone genes reveals an area of strong conservation. This 70 base pair segment stretches from approximately position -145 to -75 relative to the start of transcription, and is more strongly conserved than the protein coding portions of the genes. Fragments containing this sequence show enhancer activity when fused to the herpes simplex virus thymidine kinase promoter in a transient expression vector. In contrast to previously described cellular gene enhancers which show tissue or cell type specific activity, the growth hormone sequence shows enhancer activity in a variety of cell lines. The presence of this positive element is particularly remarkable since transient expression from the intact growth hormone promoter is very weak in all cell types examined.

1052 CONTROL DNA SEQUENCES FROM A *DROSOPHILA* HSP 70 AND ACTIN GENE ARE HEAT SHOCK REGULATED AFTER TRANSFECTION INTO CULTURED *DROSOPHILA* CELLS,

W.C. Nierman, T.V. Feldblyum, and K.K. Watanabe, American Type Culture Collection, Rockville, MD 20852.

A recombinant plasmid has been constructed in which the promoter from a *Drosophila* 70K dalton heat shock protein gene (hsp 70) has been fused to a bacterial chloramphenicol acetyltransferase (CAT) structural gene. Introduction of the plasmid into *Drosophila* cells in culture results in the expression of functional CAT enzyme. At some levels of transfected DNA, CAT expression is inducible by exposure of the transfected cells to a brief heat shock. When larger amounts of plasmid DNA are transfected, CAT activity is expressed at a very high level and heat shock control of CAT expression is lost. This DNA induction of CAT activity is, in fact, brought about by plasmid DNA. The DNA induction is demonstrated by cotransfection of a small amount of the hsp 70-CAT plasmid with a large amount of plasmid pBR322. A similar plasmid in which a CAT gene was placed under the control of a *Drosophila* 88F actin promoter was constructed. Expression of CAT is dependent on the actin promoter when the plasmid is introduced into *Drosophila* cells. CAT activity is repressed in the transfected cells after a brief exposure to a temperature elevation.

1053 SPECIFIC EXPRESSION OF AN ELASTASE-HUMAN GROWTH HORMONE FUSION GENE IN PANCREATIC ACINAR CELLS OF TRANSGENIC MICE, David M. Ornitz[1], Richard D. Palmiter[1], Robert E. Hammer[2], Ralph L. Brinster[2], Galvin H. Swift[3] and Raymond J. MacDonald[3], [1]University of Washington, Seattle, Washington 98195, [2]University of Pennsylvania, Philadelphia, Pennsylvania 19104, [3]University of Texas Health Science Center, Dallas, Texas 75235. To identify the DNA elements required for pancreas specific expression of the rat elastase I gene, we joined the 5' flanking region of the elastase I gene to the human growth hormone (hGH) structural gene. Three elastase-hGH fusion genes with 4.2, 0.5 and 0.2 kb of elastase 5' flanking sequence were introduced into mice. Of 18 transgenic mice with the fusion genes, 5 had no detectable hGH mRNA, one mouse had a very low level of hGH mRNA in the pancreas, while the remaining 12 mice had between 1,100 and 39,000 molecules of hGH mRNA per pancreatic cell. Most of these transgenic mice had undetectable levels of hGH mRNA in nonpancreatic tissues. The data indicate that 213 bp of elastase promoter region are sufficient to direct pancreas-specific expression. To ascertain whether expression was specific to the exocrine cells of the pancreas, we immunologically stained sections of the pancreas for the presence of hGH. The acinar cells and lumen of pancreatic ducts of mice expressing the elastase-hGH gene fluoresced brightly, while no fluorescence was detectable in the Islets of Langerhans, lymph nodes, connective tissue or blood vessels.

1054 CHANGES IN MRNA EXPRESSION FOLLOWING HEAT SHOCK IN DEVELOPING DROSOPHILA WINGS, Nancy S. Petersen and Herschel K. Mitchell, Univ. of Wyo. Laramie WY, 82071 and Caltech, Pasadena CA 91125.

Brief heat shocks (40 min at 40.8°C) during pupal development in Drosophila result in developmental abnormalities (phenocopies) in adult flies. These abnormalities resemble mutant defects and in some cases can only be induced in the heterozygote of the corresponding recessive mutant. Our hypothesis is that these phenocopies are due to failure of a gene to be expressed at the correct time in development. During the pupal differentiation period there are very rapid changes in wing messenger RNA concentration which are reflected in changing patterns of protein synthesis. A heat shock during this period can change the program of messenger RNA synthesis and decay. When flies are heated at 38 hrs of pupal development several wing messages which would normally disappear within 4-6 hours at 25° continue to be present for more than 20 hours following the heat shock. The disappearance of these messages follows the recovery of normal protein synthesis and is coincedent with the appearance of new messages in the developmental program. During the period when these developmental messages appear to be stabilized, message coding for heat shock proteins is synthesized and then decays. A 35° treatment for 30 min before the 40.8°C shock can prevent phenocopies and also allows a much more rapid recovery of the normal program of protein synthesis and mRNA synthesis and decay. These results suggest that both the normal pattern of mRNA synthesis and the expression of heat shock genes are tightly regulated. We are currently looking at changes in expression of specific mRNA's which may result in phenocopies.

1055 TRANSCRIPTIONAL REGULATION OF THE MURINE IAβ GENE, William D. Roeder and Richard Maki, La Jolla Cancer Research Foundation, La Jolla, California 92037

The initial events that trigger the immune response depend upon the interaction of cells bearing Ia-antigen complexes and T-cells bearing Antigen/Ia-specific receptors. A number of accessory cells, expressing Ia-antigens on their surface, are able to participate in this reaction. Some of these cells, notably resting B-cells, express Ia glycoproteins constitutively while recent evidence indicates that the expression of Ia by macrophages, is positively regulated by interferon-γ (INF-γ). We have subcloned various portions of the 5'-flanking sequence of the murine IAβ gene into expression vectors so that the IAβ sequences control the expression of a bacterial gene encoding the enzyme chloramphenicol acetyl transferase (CAT). Consistent with observations of Ia expression by FACS analysis we find that CAT activity is low when plasmid clones containing approx. 1800 bp of 5'-flanking region are introduced into the macrophage-like cell line, P388D$_1$; the level of expression increases about three-fold when this plasmid is introduced into P388D$_1$ treated with INF-γ. Deletion of this clone so that 930 bp of promoter proximal DNA sequence is present results in plasmids that are expressed in P388D$_1$, irrespective of the presence or absence of INF-γ. The deleted sequences must contain signals that "repress" expression in the absence of inducing signals given by INF-γ. Included in the deleted segment is a highly conserved sequence found in four other class II genes: CTAGCAACAGAAGN$_{20}$CTGATTGG. Preliminary evidence indicates that this sequence may be the binding site for a protein under INF-γ inducing conditions.

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1056 INTRODUCTION OF EXOGENOUS HUMAN PLACENTAL LACTOGEN AND GROWTH HORMONE GENES INTO CULTURED HUMAN CHORIOCARCINOMA CELLS, Bruce L. Rogers, Mark G. Sobnosky and Grady F. Saunders, The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

Human placental lactogen (hPL) and human growth hormone (hGH) are members of a multigene family which share amino acid sequence homology and similarity in gene structure and nucleotide sequence, but differ in both function and expression. hGH is involved in the regulation of growth and is expressed in the pituitary, whereas hPL stimulates fetal and mammary gland development and is produced in the placenta. The hPL/hGH gene cluster provides an excellent model system to examine tissue-specific differential expression of specific genes. The action of tissue specific enhancers may account for the differential expression of the highly conserved members of this gene family. To probe for the presence of transcriptional enhancer sequences, members of the hPL/hGH multigene complex have been subcloned into the EcoRI site of the pSV2gpt vector. Analogous clones have also been constructed using an enhancer-deleted variant of pSV2gpt. These recombinant DNAs have been introduced into JEG-3 cells, a human choriocarcinoma cell line, using the calcium phosphate co-precipitation method. Transformed cell lines, able to grow in the presence of selection media, have been isolated. These stably transformed cell lines are being used to probe for the presence of transcriptional enhancer sequences in the hPL/hGH gene cluster.

1057 Glucocorticoid Regulation and Tissue Specific Expression of MTV Sequences in Transgenic Mice, Susan R. Ross, University of Illinois, Chicago, Ill. 60612.

Transcription of mouse mammary tumor virus (MTV) can be induced by glucocorticoids in infected tissue culture cells or in explants of mammary carcinomas. Although several cell types can be infected in vitro with MTV and can express these sequences, and although there are active and functional receptors in most mouse tissues, transcription of proviral sequences in vivo occurs predominantly in lactating mammary glands and in mammary tumors in the mouse. We were interested in whether the tissue-specific expression of MTV, in addition to the response to glucocorticoids, was due to MTV-encoded sequences. We have introduced a chimeric plasmid, $\mathrm{pLTR_2tk}$ containing the MTV long terminal repeat linked to the HSV I thymidine kinase gene into the mouse germline by microinjection. In these mice, the tk gene is appropriately expressed in the lactating mammary glands of females; it is also expressed in the ovaries of some of these mice. Transcription of these new germline sequences also occurs in the testes of males, unlike the endogenous MTV sequences. This testes-specific transcription is specifically stimulated by glucocorticoids. Thus, the MTV LTR may contain elements which allow its expression both in lactating mammary glands and in testicular tissue. We are also constructing molecules containing the MTV internal glucocorticoid-receptor binding sites, in order to determine their role in the tissue-specific expression of MTV.

1058 THE STRONG ENHANCER ELEMENT IN THE IMMEDIATE EARLY REGION OF THE HUMAN CYTOMEGALOVIRUS GENOME, Rüdiger Rüger[1], Michael Boshart[1], Frank Weber[2], Karoline Dorsch-Häsler[2], Gerhard Jahn[1], Bernhard Fleckenstein[1], and Walter Schaffner[2], [1]Institut für Klinische Virologie der Universität Erlangen Nürnberg, D-8520 Erlangen; [2]Institut für Molekularbiologie II der Universität, CH-8093 Zürich

An enhancer was identified in human cytomegalovirus (HCMV) DNA by co-transfecting cloned DNA from the immediate early region of HCMV with an "enhancer trap" of linear simian virus 40 (SV40) DNA lacking its own enhancer. Two replication competent SV40-like viruses were isolated containing HCMV DNA inserts of 341 and 262 bp. These sequences are located upstream of the major immediate early promoter, between nucleotides -118 and -524. Transient expression assay with a cloned rabbit ß-globin gene indicated that the upstream region of the major immediate early gene of HCMV contains the strongest enhancer element identified so far. Studies with deletion mutants showed that different subsets of this enhancer can substitute for the SV40 enhancer. Studies with more extended deletions and various fusion genes are in progress.

1059 THE REGULATION OF TRANSLATION IN HEAT SHOCK IN *DROSOPHILA*,

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The translational regulation observed in *Drosophila* cells in heat shock conditions requires that: 1) messenger RNAs coding for heat shock polypeptides (hsp's) have a structural element identifying them for selection for translation and 2) the translational apparatus in the cell changes such that mRNAs coding for hsp's are actively translated while normal cellular mRNAs are not. An early event in heat shock in *Drosophila* and other organisms is the dephosphorylation of the ribosomal protein, S6. To determine whether this change plays a role in translational regulation in heat shock we have set up an RNA-dependent *in vitro* translation system from normal and heat shocked *Drosophila* Kc cells which mimics the regulation of translation seen in intact cells in these conditions. We have fractionated the lysates into washed ribosomal and supernatant fractions and used the fractions to determine whether ribosome or supernatant factors control the changes in translation regulation in heat shock. In supplementation experiments where either ribosomes or supernatant factors are added to intact normal or heat shock lysates, we find that supernatant factors from control lysates restore normal patterns of protein synthesis to heat shock lysates. Ribosome fractions have no effect. In reconstitution experiments where ribosomes are mixed with supernates from either normal or heat shock lysates, we find the regulation of translation in heat shock is supernatant dependent and independent of ribosome source. In the lysates, the state of phosphorylation of S6 is the same as that seen in intact cells. At least three other polypeptides in the lysates have changed their state of phosphorylation in heat shock. One of these is ribosomal and the other two are supernatant factors.

1060 INHIBITION OF THE SYNTHESIS OF THE 78-kDALTON POLY(A)-BINDING PROTEIN IN FRIEND ERYTHRO LEUKEMIA CELLS SUBSEQUENT TO HEAT SHOCK.

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When Friend erythroleukemia cells are incubated at 43 degrees there is a rapid and nearly complete inhibition of protein synthesis which can be reversed when cells are returned to their normal growing temperature of 37 degrees. Examination of the recovery of FEC from heat shock indicates that most cellular mRNAs behave as a cohort and return to translation at approximately the same rate. We found a noteable exception to this rule in the case of a 78 kD basic protein (named protein A) whose rate of return to a normal synthetic rate is markedly inhibited subsequent to heat shock. For example, the synthesis of protein A is inhibited by greater than 60 percent compared with a host of other cell proteins when cells heat shocked for 30 minutes at 43 degrees are allowed to incorporate [35S] methionine for one hour at 37 degrees. Longer periods of heat shock increases the disparity between the rate of protein A synthesis and other cell proteins. We found that the inhibition of protein A synthesis is not due to an increase in protein turnover after heat shock nor to a disappearance of protein A mRNA. We show that protein A corresponds to the 78 kD polypeptide commonly found to be associated with the poly(A) tails of mammalian mRNA (PABP). Further analysis of heat-shocked FEC indicates that the composition of messenger ribonucleoprotein (mRNP) particles changes after stress so that PABP comprises a significantly smaller percentage of mRNP polypeptides.

1061 CHROMOSOMAL INTEGRATION-SITE SPECIFIC ACTIVATION OF EXOGENOUS RETROVIRUS PROMOTERS,

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Embryonal carcinoma (EC) cells normally do not support the gene expression of exogenous proviruses. We have infected EC cells with a recombinant retrovirus containing a selectable gene and have found that approximately one in 10,000 integration events leads to the expression of the provirus from the viral promoter (1). The expression is due to an unknown cis-acting mechanism (1). We have now cloned two of these expressed proviruses along with their flanking EC DNA into λ phage. The flanking DNA in each case hybridizes to a single poly A^+ RNA species expressed in undifferentiated, uninfected EC cells. We have isolated cDNA clones for these RNA species and are in the process of: 1) identifying the genes, 2) identifying the gene's promoter/enhancer, and 3) determining the influence that the promoter/enhancer might have on viral gene expression.

1. Sorge, J., Cutting, A.E., Erdman, V.D., and Gautsch, J.W., Proc.Natl.Acad.Sci. 81: November, 1984.

1062 DNA SEQUENCE-POSITIONED CHROMATIN STRUCTURES OF THE YEAST *HSP83* LOCUS, C. Szent-Gyorgyi, D.B. Finkelstein, and W.T. Garrard, The University of Texas Health Science Center at Dallas, Dallas, Tx. 75235

We have investigated at high resolution the chromatin structure of the locus containing a *Saccharomyces cerevisiae* gene that encodes an 81 kd heat shock protein (the homolog of *Drosophila HSP83*). Northern hybridization enables us to determine that within 10 min of heat shock (39°) the steady state level of HSP83 transcript is induced at least tenfold over the basal level (27°). DNase I and micrococcal nuclease cutting sites in nuclei isolated from either heat shock induced or uninduced cells were mapped along the *HSP83* locus by indirect end-labeling. A persistent set of nuclease hypersensitive features were noted. Regions ∿150 bp in breadth that are hypersensitive to DNase I are centered at -1625 bp, -545 bp, -155 bp, +2400 bp, and +3375 bp relative to the transcription start. The DNase I sites (except +2400 bp) each display centrally located protected regions of 20-40 bp, implying the presence of DNA binding proteins. The Pelham heat shock consensus sequence lies within a protected subdomain of the site at -155 bp. Sequences corresponding to poly(A) addition and possibly transcription termination reside within the site at +2400 bp. A clear array of DNA sequence-positioned nucleosomes, detectable by either nuclease, resides 3' to the DNase I hypersensitive region at +3375 bp; a similar array may exist 5' to the site at -1625 bp. These two hypersensitive regions are also each demarcated 5' and 3' by exceptionally strong micrococcal nuclease cleavages ∿ 160 bp apart, a length which coincides with the canonical nucleosomal repeat. (Supported by grants from NIH and The Robert A. Welch Foundation).

1063 EXPRESSION IN TERATOCARCINOMA STEM CELLS OF A RECOMBINANT GENE FLANKED BY MOLONEY MURINE LEUKEMIA VIRUS LTR; Makoto Taketo[1,2], Eli Gilboa[3], Michael I. Sherman[2] and Masafumi Tanaka[1], [1]The Jackson Laboratory, Bar Harbor, ME 04609, [2]Roche Institute of Molecular Biology, Nutley, NJ 07110 and [3]Princeton University, Princeton, NJ 08554

By infecting murine embryonal carcinoma (EC) cell lines with a recombinant transducing virus that contains neomycin resistance gene (*neo*) linked to Moloney murine leukemia virus (M-MuLV) LTR, we have isolated clonal lines that are resistant to the neomycin analogue, G418. These transductant lines consist of undifferentiated EC cells as judged by morphology, tumorigenicity and cell surface antigenic markers. Analysis of the integrated *neo* sequence by Southern blot hybridization revealed that some of the lines have single copies whereas others have multiple copies in multiple sites. Although these transductant lines contained many copies of helper M-MuLV integrated in the cellular genome, expression of their genes was not detected either by reverse transcriptase activity or by XC plaque assay. Two F9tk$^-$ transductant lines were superinfected with a second recombinant transducing virus that contains herpes simplex virus thymidine kinase gene flanked by the M-MuLV LTR. The frequency of transduction to yield clones able to grow in HAT medium was similar to that of the parental cells. These results suggest that the expression of the *neo* gene linked to M-MuLV LTR in the trnasductant EC cell clones is due to a cis-acting mechanism; e.g., rearrangement of the enhancer sequence in M-MuLV LTR or effect by a cellular enhancer element flanking the integrated recombinant gene. To investigate these possibilities, the integrated recombinant gene has been cloned together with its flanking cellular sequences, and their structures are being analyzed.

1064 COMPARTMENTATION OF NORMAL AND STRESS PROTEIN mRNAS IN STRESSED CELLS. G.P. Thomas, Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724

Of ~~the~~ myriad agents which induce stress responses, amino acid analogues prove highly effective in mammalian cells. The general features of the response of HeLa cells to treatment with the proline analogue azetidine-2-carboxylic acid (AzC) have been described (Thomas and Mathews, 1984, Mol. Cell. Biol. **4**, 1063) including transcriptional controls as well as two apparent translational controls, effected at the levels of initiation and elongation. The latter is of interest since it demands specificity among mRNAs which have overcome the initiation defect and has been investigated by monitoring the distributions of newly-synthesized mRNA under conditions where this comprises stress protein mRNAs exclusively. Analysis of AzC-treated HeLa cells by equilibrium density centrifugation reveals a novel RNP complex which (i) contains a good portion of pulse-labeled RNA, (ii) is devoid of normal cellular mRNAs as detected with cloned probes, (iii) contains both 18*s* and 28*s* rRNAs and (iv) bands at densities distinct from those of normal mRNAs. This component appears restricted to cytoplasmic fractions of stressed cells. Based on the synchrony with which both RNA production and protein synthesis become restricted to the fabrication of stress proteins, we have suggested that protein synthesis becomes limited to translation of newly-made mRNAs: This novel RNP fulfills a number of predictions arising from such a scenario and attempts at its further characterization will be presented.

1065 *CELL SPECIFIC EXPRESSION OF THE RAT INSULIN GENE: EVIDENCE FOR ROLE OF TWO DISTINCT* 5' FLANKING SEQUENCES. Michael D. Walker, Thomas Edlund and William J. Rutter, Hormone Research Institute, University of California, San Francisco, Ca 94143
Recombinant plasmids were constructed containing 5' flanking DNA of the rat insulin I gene linked to chloramphenicol acetyltransferase (CAT) coding DNA. Use of a transient transfection procedure results in selective expression of CAT activity (50-200 fold) following introduction to insulin producing tissue culture cells (HIT cells) as compared with other cells. The insulin 5' flanking DNA sequence is able to augment the activity of the thymidine kinase (tk) promoter (20-30 fold) but only in insulin producing cells. We therefore conclude that this region contains a cell specific enhancer-like element. A fragment from -103 to -249 efficiently enhances (about 20 fold) the tk promoter in an orientation-independent manner. To test whether DNA sequences more proximal to the transcription start site also contribute to cell specificity we removed the insulin enhancer from the intact flanking DNA region, replacing it with the murine sarcoma virus (MSV) enhancer and fusing to insulin gene sequences at -114. This hybrid retains substantial cell specificity (about 50 fold) despite the absence of significant enhancer activity in the insulin sequences. Thus the cell specificity associated with the insulin 5' flanking DNA sequence appears to result from the activities of two distinct elements: one located more distal to the transcription start site and exhibiting properties of an enhancer and the second more proximal to the transcription start site.

1066 EFFECTS OF CYCLOHEXIMIDE ON HEAT SHOCK PROTEIN SYNTHESIS, HEAT SHOCK RNA ACCUMULATION AND THERMOTOLERANCE EXPRESSION IN MAMMALIAN CELLS. Randall B. Widelitz and Eugene W. Gerner, University of Arizona, Tucson, AZ 85724
We have investigated the effects of a range of cycloheximide (CHX) concentrations (0.2 to 20 μg/ml) on thermotolerance, protein synthesis, and RNA accumulation in heat treated Rat-1 embryonic fibroblasts in order to determine the function of the heat shock proteins (hsps) and their mode of regulation in mammalian cells. Thermotolerance, as measured by colony formation following two 45°C heat doses, developed equally when CHX, at all concentrations tested, was present throughout the treatment protocol. CHX inhibited general and hsp synthesis in a concentration dependent manner, such that at 20 μg/ml, 95% inhibition was achieved as determined from acid precipitable ^{35}S methionine counts. However, after the heat treatments, the drug was removed, and the cells were able to resume general and heat shock protein synthesis. Since the expression of thermotolerance was not reduced while hsp synthesis was inhibited throughout the heat treatments, our data suggest that the hsps may act in a repair capacity after the thermal damage is done. While heat shock induced the expression of several RNA species which hybridize to probes encoding the 68, 70 and 83 kd hsps, in the presence of CHX, only high molecular weight species of RNA were accumulated. Preliminary data suggest that these species are not polyadenylated nor are they translated by in vitro translation systems.

1067 SECONDARY STRUCTURE OF ENHANCERS, Donna M. Williams, Therese Brendler and Patricia E. Berg, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, Maryland 20205
Our analyses of the primary sequences of various enhancer elements has revealed regions of extensive dyad symmetry suggesting a possible role of secondary structure in enhancer activity. Stable regions of potential stem and loop structures also appear to be essential for the efficient termination of transcription in prokaryotes (Rosenberg et al. (1983) Science 222:734). Based on these observations, we have developed a biological assay which allows us to screen for secondary structure in enhancer elements by determining their ability to terminate transcription in E. coli. A terminator cloning vector (pDR720) has previously been developed which contains the tryptophan (trp) promoter and the galactokinase (gal K) structural gene from E. coli separated by several unique restriction sites (Russell and Bennett (1984) Gene, in press). Insertion of DNA containing a terminator into one of these sites causes decreased levels of gal K activity which directly reflects the amount of transcription of the gal K gene and can be observed as changes in colony morphology. We have found that insertion in either orientation of a 470 bp DNA fragment containing the enhancer element from the Harvey Sarcoma virus (HaSV) or of a 250 bp DNA fragment containing the SV40 enhancer element significantly reduces transcription of the gal K gene. S_1 nuclease analysis of mRNA isolated from cells containing pDR720 with the HaSV enhancer indicates that termination of transcription occurs within the fragment containing the enhancer element. It should be possible to utilize this system to identify mutations affecting the secondary structure of enhancer elements.

Sequence Specificity in Transcription and Control

1068 THE HUMAN HSP70 GENE: CLONING, CHARACTERIZATION AND EXPRESSION

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The synthesis of the 70,000 dalton heat shock protein (HSP70) is induced in human cells in culture following exposure to a variety of agents including incubation at elevated temperatures and infection by adenovirus. In the latter case the induction of HSP70 is dependent on the adenovirus transforming gene E1A (Nevins, 1982). We have cloned the human HSP70 gene, characterized the transcriptional unit, and determined the DNA sequence for the gene and flanking regions. The human HSP70 gene is transcribed as an uninterrupted 2.3kb transcript. The cytoplasmic levels of HSP70 mRNA in HeLa cells increases 10 - 12 fold during two hours of heat shock at 43C. Fusion of sequences flanking the 5' terminus of the HSP70 gene to the bacterial chloramphenicol acetyltransferase (CAT) gene confers heat shock induced expression of the CAT gene. In an adenovirus transformed human embryonic kidney cell line, 293, in which E1A is constitutively expressed, synthesis of HSP70 is elevated as is the cytoplasmic level of HSP70 mRNA relative to Hela cells. The regulation of expression of the chimaeric HSP-CAT gene in 293 cells mimics the endogenous HSP70 gene. Therefore, the 5' flanking sequences of the HSP70 gene contains the regulatory sequences for both heat shock and E1A induction. Accumulation of HSP70 mRNA in HeLa and 293 cells is cell cycle dependent, reaching maximal levels just prior to the peak of DNA synthesis. Because the human HSP70 gene sequence shares 50% homology with an E. coli protein involved in DNA replication (dna K), we suggest that HSP70 may function during the synthetic phase of the cell cycle.

Complex Regulatory Loops

1069 **MOLECULAR AND FUNCTIONAL ORGANIZATION OF THE PHOSPHATASE GENETIC SYSTEM IN S. CEREVISIAE**

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The phosphorus metabolism system in S. cerevisiae is a dispersed gene system consisting of numerous structural and regulatory genes involved in the basic cellular housekeeping functions responsible for balancing intracellular pools of Pi, polyphosphate, phosphate nucleotides and other Pi metabolites and for maintaining metabolic homeostasis. The acid and alkaline phosphatases, members of this multigene family, exist in multiple forms, as constitutive and repressible enzymes. The constitutive and repressible APase, PHO3 and PHO5 respectively, form a cluster on chromosome II, whereas the repressible alkPase (PHO8) is on chromosome IV. Expression of these genes is mediated by numerous regulatory genes scattered throughout the yeast genome: PHO4, PHO81, PHO80, PHO85, PHO2, PHO6 and PHO7. Recessive mutations in PHO4 and PHO81 block the derepression of both repressible APase and alkPase, while PHO2 mutants show a phenotype lacking only the repressible APase. Recessive mutations occurring at the PHO80 or PHO85 loci result in constitutive synthesis of both enzymes. PHO6 and PHO7 are involved in expression of the constitutive APase.

Based on genetic and biochemical data (Toh-e et al. 1981, J. Bacteriol. 145:221; Bostian et al. 1980, PNAS 77:4504), a specific model has been proposed for the control of the yeast phosphatase gene family. This model involves the simultaneous functioning of regulatory proteins by direct molecular interaction. In its simplest form, the regulatory factors are produced constitutively. Under repressed conditions the PHO4 gene product aggregates with a complex of the PHO80 and PHO85 gene products at a protein domain identified by pho82, and is unable to activate PHO5. Under derepressed conditions, the PHO81 product binds to the PHO80, PHO85, PHO4 aggregate, regulated by a co-repressor (Pi metabolite), and the PHO4 product is released. It binds to the "operator" of a structural gene, in combination with a second regulatory factor bearing target gene specificity, to activate transcription.

This model provides a view of how these dispersed structural genes might be regulated in response to transcriptional control signals and serves to facilitate specific experimental questions. It is not yet known, for example, whether the target of the PHO4 gene is a controlling sequence of DNA or a regulatory intermediate such as a DNA binding protein. Recent data from our laboratory establish that the interplay of these positive and negative regulatory genes does occur prior to transcription, and that their functions are all indispensible for normal regulation of mRNA synthesis. Biochemical evidence suggests that the regulatory gene products are expressed constitutively, and sensitive to fine control by regulatory mechanisms reminiscent of bacterial control circuits, involving feedback regulation of transcription and autoregulation. Data on the expression of the "constitutive" APase (PHO3) suggest that it is in fact regulated in trans by PHO5, either through a mechanism similar to autogenous regulation, or by feedback suppression.

1070 TRANSLATIONAL REGULATION OF THE ACTIVATOR OF GENERAL AMINO ACID CONTROL IN YEAST,

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Starvation of yeast for any amino acid leads to increased transcription of many unlinked genes encoding enzymes in diverse amino acid biosynthetic pathways. This regulation is mediated by the sequence -TGACTC- that is repeated in the 5' non-coding regions of the co-regulated genes and which functions as an orientation-independent, *cis*-acting positive regulatory site.

The *GCN4* gene encodes a *trans*-acting positive regulator of amino acid biosynthetic genes in yeast. The expression of this activator is itself controlled by amino acid levels and this regulation occurs at the translational level. Other known trans-acting factors in general amino acid control mediate the translational regulation of *GCN4* mRNA. The *GCN2* and the *GCN3* products act as positive regulators of translation during amino acid starvation, whereas the *GCD1* product appears to act as a translational repressor in non-starved cells. The *GCN4* mRNA 5' leader is nearly 600 nucleotides in length and contains four small open reading frames, each of which is two or three codons in length and complete with initiation and termination codons. A deletion of the small open reading frames leads to constitutive derepression of *GCN4* translation. A mutation in the *GCD1* gene appears to have nearly the same effect, suggesting that *GCD1* mediates the translational repression exerted by the *GCN4* mRNA 5' leader sequences. The *GCN4* leader deletion suppresses the requirement of the *GCN2* and *GCN3* products for derepression of GCN4 translation, suggesting that *GCN2* and *GCN3* antagonize this negative element in response to amino acid starvation.

1071 COUPLED CIRCUITS OF GENE REGULATION,

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A large number of mechanisms for the control of gene expression have been described at the molecular level. This richness of molecular description has become an embarrassment because there is no comparable theory of gene regulation to give meaning to the alternative designs--no theory that allows one to predict the type of mechanism that might be expected to evolve for a given set of genes in a given organism. Although there is no general theory of gene regulation, some of the elements of such a theory are beginning to emerge. I shall give a report of just two such elements that help us to understand coupled circuits of gene regulation. The first is what I have called the "demand theory" of gene regulation . It provides rules for predicting the modality of gene regulation--that is, whether the molecular mechanism will be positive or negative. This theory has implications regarding what can be called the "logical coupling" of regulatory circuits. Examples of these implications will be given for a variety of microbial systems. The second element, which I shall call the "circuit theory" of gene regulation, provides rules for predicting the patterns of regulatory interactions --what can be called the "topological coupling" of regulatory circuits. Even with only two coupled units of transcription, the number of patterns of regulatory interactions is very large and these give rise to a diverse behavioral repertoire. The patterns will be enumerated for an inducible system and those patterns that are physically realizable will be distinguished from those that are not. Predictions for the use of classical, autogenous and coupled circuits will be given based upon the behavior of the intact systems.

1072 MATING TYPE REGULATION IN YEAST, Jeffrey Strathern[1], Margaret Kelly[2], Brenda Shafer[1], and Carolyn McGill[1]. [1]NCI-Frederick Cancer Research Facility, Laboratory of Eukaryotic Gene Expression, P.O. Box B, Frederick, MD 21701; [2]Cold Spring Harbor Laboratory, NY 11724.

The two alleles of the mating type locus, MATa and MATα control the a, α, and a/α cell types of the sexual cycle in *Saccharomyces cerevisiae*. MATα encodes two proteins, a positive regulator (MATα1) of several unlinked α-specific genes and a negative regulator (MATα2) of unlinked a-specific genes. MATα2 together with MATa1 control the genes for the sporulation functions specific to a/α cells. MATa has no known role in a cells. MAT is on chromosome III in yeast. An unexpressed copy of MATα is stored at the HMLα locus on the left arm of chromosome III, and an unexpressed copy of MATa is stored on the right arm of chromosome III at HMRa. HML and HMR are kept silent by trans-acting functions encoded by the MAR/SIR genes. Some yeast strains (homothallic) can change from the α cell type to the a cell type and from a to α. These switches reflect changes from MATα to MATa and MATa to MATα. They occur by substituting copies of HMRa or HMLα into MAT. These specific genome rearrangements occur at high efficiency (over 80%) in particular cells within a clonal pedigree. Thus, this system has many of the features common to development. The switch is heritable but can be reversed, it is not randomly distributed among cells, and a single change activates a set of cell-type-specific genes.

This complex regulatory pathway by which cell type is differentiated and maintained in bakers yeast can be divided into several areas of research.

1) How does the DNA cassette switching mechanism occur?
2) How is homothallic switching regulated in terms of its distribution in the population of cells and its timing in the cell cycle?
3) How are the HML and HMR copies of the MAT sequences kept from being expressed?
4) How do the products of the MATa and MATα genes regulate the expression of the genes specific to the a, α and a/α cell types?
5) What are the cell-type-specific genes?

Research sponsored by the National Cancer Institute, DHHS, under Contract No. N01-CO-23909 with Litton Bionetics, Inc.