MOLECULAR APPROACHES TO EUCARYOTIC GENETIC SYSTEMS

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200 THE EXPRESSION OF EUKARYOTIC DNA SEGMENTS IN ESCHERICHIA COLI, John Carbon and Barry Ratzkin, Department of Biological Sciences, University of California, Santa Barbara, California 93106

Segments of yeast (S. cerevisiae) DNA cloned on a Col E1 plasmid vector in E. coli can be functionally expressed. Specific yeast DNA segments have been isolated that are capable of complementing auxotrophic mutations (deletions) in various E. coli genes, such as leuB, hisB and trpAB. This type of complementation is apparently not a rare phenomenon.

Previous studies (1,2) have shown that the use of the poly(dA·dT) "connector" method to join sheared E. coli DNA with linear Col E1 DNA yields a preparation that will transform E. coli cells to colicin E1 resistance with high efficiency, thereby establishing a collection or "bank" of transformants containing hybrid plasmids representative of the entire E. coli genome. Using these methods, randomly sheared yeast DNA (average MW = 8 x 10^6 daltons) containing poly(dA) tails was annealed to poly(dT)-tailed linear Col E1 DNA (L_{RI}). This DNA preparation was used to transform E. coli cells, selecting colicin E1-resistant clones that contain hybrid Col E1-yeast DNA plasmids. Sufficient transformant clones were obtained to insure that the hybrid plasmid population was representative of the entire yeast genome.

Various hybrid Col E1-yeast DNA plasmids capable of complementing E. coli auxotrophic mutations were selected from this collection. Plasmid pYeleu10 will complement several different point or deletion mutations in the E. coli or S. typhimurium leuB gene (β-isopropylmalate dehydrogenase). Plasmids pYeleu11, pYeleu12, and pYeleu17 are specific suppressors of the leuB6 mutation in E. coli C600 strains. Plasmid pYehis2 will complement a deletion in the E. coli hisB gene (imidazole glycerol phosphate dehydratase). Plasmid pYetrp1 will complement deletions of the E. coli trpAB region (tryptophan synthase).

The above plasmids were shown to contain yeast DNA segments by studying the kinetics of reassociation of labeled single-stranded plasmid DNA in the presence of excess unlabeled single-stranded yeast or E. coli DNAs. In addition, pYehis2 contains a portion of the yeast Eco RI fragment recently shown by Struhl et al. (3) to complement hisB mutations when cloned on a modified lambda phage vector (λgt-Schis). In certain cases, smaller restriction fragments can be recloned which retain the complementation function.

Relatively high levels of the appropriate enzyme activities can be detected in deletion mutant strains bearing the complementing hybrid plasmids. The β-isopropylmalate dehydrogenase activity expressed by pYeleu10 is quite cold-sensitive, a property of the yeast enzyme.

1) L. Clarke and J. Carbon, Proc. Natl. Acad. Sci. U.S.A., 72 (1975) 4361.
2) L. Clarke and J. Carbon, Cell, 9 (1976) 91.
3) K. Struhl, J. R. Cameron and R. W. Davis, Proc. Natl. Acad. Sci. U.S.A., 73 (1976) 1471.

201 SV40 VECTORS FOR MOLECULAR CLONING IN MAMMALIAN CELLS, George C. Fareed, Department of Microbiology and Immunology, Molecular Biology Institute, University of California, Los Angeles, CA 90024

The approach for our obtaining mammalian transducing viruses arose from studies of reiteration mutants of papova viruses (1). These mutants, derived from serial diluted passage of SV40 or polyoma in permissive cells, characteristically have deleted a large part of the WT genome and reiterated in tandem a small part bearing the origin for viral DNA replication. Structural analysis has indicated that this origin is the only required cis function for replication. Thus, in analogy to the cloning of foreign DNA segments in E. coli by enzymatic insertion into a suitable plasmid or phage λ replicon, one could use a segment of SV40 DNA containing its initiation site as the vector for a eukaryotic or prokaryotic DNA segment, of appropriate size for encapsidation. We found in our initial study (2) that the short, monomer segments from SV40 reiteration mutants can serve as vectors for propagating specific segments of λ DNA in monkey kidney cells. In the presence of helper SV40 DNA to supply trans functions, the hybrid genomes are replicated and those whose sizes are near that of WT SV40 DNA (5000 bp) are encapsidated in progeny virions. In these initial experiments, a defective hybrid bearing a specific 520 bp segment from the λ immunity region was propagated but not cloned in monkey cells. In a subsequent report (3) we described the partial cloning in monkey cells of a 2300 bp segment neighboring that 520 bp segment. The cloning of this hybrid in mammalian cells not only showed the efficacy of defective SV40 vectors but also provided an opportunity for examining a well-characterized prokaryotic genetic regulatory site (the leftward operator) in a eukaryotic environment.

An alternative method for molecular cloning with SV40 vectors takes advantage of the ability of the vector to express an early or late gene function and, thereby, complement a ts mutant helper genome. We have used this approach to clone a SV40 genome bearing an E. coli suppressor gene, $tRNA^{Tyr}su^{+}III$. The bacterial DNA segment was inserted in a unique orientation via EcoRI and HpaII termini into a deletion in the late genes of SV40. It was propagated with the aid of a helper tsA mutant of SV40. Transcription of the prokaryotic sequences in monkey cells was demonstrated although no mature, acylatable suppressor tRNA could be found (Hamer, Davoli, Thomas and Fareed, unpublished results). This hybrid genome has also been employed for the transformation of rat embryo cells.

1) Ganem, D., Nussbaum, A.L., Davoli, D. and Fareed, G.C. (1976) J. Mol. Biol. 101, 57.
2) Ganem, D., Nussbaum, A.L., Davoli, D. and Fareed, G.C. (1976) Cell 7, 349.
3) Nussbaum, A.L., Davoli, D., Ganem, D. and Fareed, G.C. (1976) Proc. Nat. Acad. Sci. USA 73, 1068.

202 NIH GUIDELINES FOR RECOMBINANT DNA RESEARCH, William J. Gartland, Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20014

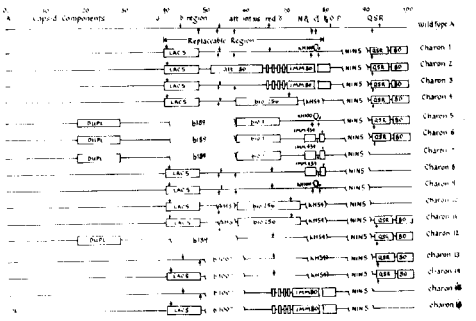
In June 1976 the National Institutes of Health (NIH) issued Guidelines for Research Involving Recombinant DNA Molecules (1). The NIH has also undertaken an environmental impact assessment in accordance with the National Environmental Policy Act of 1969 (2). The guidelines establish carefully controlled conditions for experiments involving the production of recombinant DNA molecules and their insertion into organisms such as bacteria. The NIH guidelines replaced recommendations of the Summary Statement of the Asilomar Conference on Recombinant DNA Molecules.

The chronology of events leading up to the version of the guidelines released in June 1976 was described (3). NIH policies and procedures for implementation of the guidelines by grantee institutions and contractors was discussed (4). Possible options for adoption and implementation of the guidelines by other Federal agencies and by private industry were presented.

1) Federal Register, 41, 27902-27943 (1976).
2) Federal Register, 41, 38426-38433 (1976).
3) Recombinant DNA Research - Volume I, U.S. Government Printing Office, Washington, D.C. 20402.
4) NIH Guide for Grants and Contracts, November 30, 1976.

241 CONSTRUCTION OF PHAGE LAMBDA CLONING VEHICLES, Williams, B.G. and Blattner, F., Genetics, U.W. Madison, Madison, Wisconsin 53706

The following derivatives of phage λ have been constructed for DNA cloning with Eco RI. The Charon series, named after the mythological boatsman of the river Styx, all lack RI sites in the essential regions of the genome. The structures have been confirmed by genetic analysis, by gel electrophoresis of Eco RI and Hind III digest, by density analysis in analytical CsCl ultracentrifugation, and by heteroduplex mapping in the electron microscope. Downward "↓" arrows indicate Eco RI sites, upward "↑" arrows indicate Hind III sites. Parantheses () indicate deletions, and boxes ▭ are substitutions of non-lambda DNA.

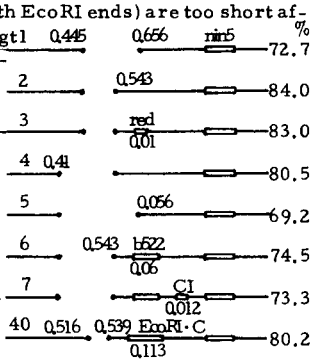


242 BACTERIOPHAGE f1 AS A VECTOR FOR CONSTRUCTING RECOMBINANT DNA MOLECULES, Gerald F. Vovis, Mariko Ohsumi and Norton D. Zinder, The Rockefeller University, New York, N. Y. 10021

Bacteriophage f1 is a filamentous particle consisting of a circular, single-stranded DNA molecule covered with protein. The existence of miniphage, i.e. deletion mutants where as much as 80% of the genome has been deleted, and multi-unit length phage, even though the DNA within is unit length, suggest that large filamentous phage containing DNA molecules much greater in size than the size of the f1 genome should be able to be formed. Experiments to test this hypothesis and the resulting implications for the use of f1 as a vector for the construction of recombinant DNA molecules will be discussed.

243 CLONING OF YEAST DNA WITH λ PHAGE VECTORS, Peter Philippsen, David Benton, John Cameron, Richard Kramer, Tom St. John, Kevin Struhl, Marjorie Thomas and R.W. Davis, Dept. of Biochemistry, Stanford University School of Medicine, Stanford, Calif. 94305.

Two types of λ vectors are presently used in our lab in order to clone rDNA, plasmid DNA, and structural genes from yeast. Replacement vectors (λgt1, 5, 6, 7 with EcoRI ends) are too short after joining to give viable phage particles even if all genetic information is present. For growth these vectors contain an EcoRI fragment from λ (e.g. EcoRI·B or C) or E. coli (e.g. araB gene) which is then replaced by other DNA fragments. Replacement vectors are useful for producing pools of many λ hybrid phages containing fragments from 1 to 7 kb. Insertion vectors (λgt2, 3, 4 with EcoRI ends and λgt40 with SstI ends) are sufficiently long after joining to give viable phage particles. A genetic selection for phages with inserted DNA is possible by infecting pel⁻ strains (GL1, A2574). Phages with about 80% λ length plate with much lower efficiency (10^{-5}) on these strains than phages with about 100% λ length. These vectors are useful for cloning fragments of a specific size range. A method for screening several thousand plaques from one plate and a quick procedure for gel electrophoretic characterization of λ hybrid phages will also be discussed.



244 AN IMPROVED BACTERIOPHAGE λ VECTOR: CONSTRUCTION OF MODEL RECOMBINANTS CODING FOR KANAMYCIN RESISTANCE, Daniel J. Donoghue, and Phillip A. Sharp, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139.

An attenuated bacteriophage lambda has been prepared for use as an EK2 vector. This phage, designated λgt vir Jam27 Zam718-λB' can accomodate up to $11x10^6$ daltons of foreign DNA inserted through Eco R1 ends. The mutations virulence and nin 5 are present to reduce the frequency of lysogen and/or plasmid formation. The mutations Jam27 and Zam718 require a suppressor in the bacterial host. The phage recombination functions contained in the Eco R1 λC fragment have been deleted, and only the Eco R1 λB fragment remains (in reverse orientation) in the center portion of the vector. In addition, this phage adsorbs to sensitive bacteria at a significantly reduced rate, conferring another block to the escape of free phage. Model recombinants have been constructed by in vitro recombination with an Eco R1 fragment coding for kanamycin resistance (originally derived from R-factor R6-5). This fragment of DNA is $4.6x10^6$ daltons in size, contains an inverted repeat, and also appears to contain a promoter for the kanamycin resistance gene. Using this model recombinant, the rate of transfer of kanamycin resistance to permissive and nonpermissive strains of E. coli has been measured. In addition, the frequency of vector "disarming" has been measured by recombination with a heteroimmune λimm^{434} prophage.

245 SV40 AS A VECTOR, Joe Sambrook, William Topp and Michael Botchan, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724

Certain lines of human cells transformed by wild-type SV40 contain large quantities (> 1000 molecules per cell) of closed circular viral DNA. Similar cell lines have been selected after exposure to specific segments of SV40 obtained by the use of restriction endonucleases, that lack substantial portions of the late region of the viral genome. These cell lines should provide a source of cloned defective SV40 DNA molecules that have been propagated in the absence of infectious helper virus and are suitable for use as vectors. By propagating the recombinant molecules in transformed cells it should be possible to carry large segments of foreign DNA and circumvent many of the problems imposed by packing of DNA into virus particles.

246 CONSTRUCTION OF SV40 VECTORS AND EXPRESSION OF INSERTED SEQUENCES. Stephen P. Goff and Paul Berg, Dept. of Biochem., Stanford University, Stanford, Ca. 94305.

Cleavage of SV40 DNA with restriction endonucleases Hpa II and Bam I yields a fragment of the genome (0.13 to 0.735 on the SV40 map) suitable for cloning DNA segments in cultured CV-1 monkey cells. This vector (termed SVGT-1) has been joined to a fragment of phage lambda DNA by the poly(dA):poly(dT) method. The resulting hybrids were propagated in CV-1 cells in the presence of a temperature-sensitive SV40 helper, tsA58, at 41°C. The structures of the cloned hybrid molecules were determined by analysis of restriction endonuclease digests and by heteroduplex analysis.

The RNA produced by CV-1 cells infected with hybrid genomes contained virtually no lambda-specific RNA, although the hybrids replicated as well as the helper. The reason for the lack of stable lambda RNA is unknown; perhaps the linker segments of poly(dA):poly(dT) block transcription or render the transcripts highly unstable. To test this possibility, we have rejoined the two fragments of SV40 DNA produced by Hpa II and Bam I cleavages, using the poly(dA):poly(dT) method. The resulting molecule is a wild-type genome except for short sequences of poly(dA):poly(dT) at the Hpa II and Bam I cleavage sites. Complementation tests were used to determine whether these sequences block the expression of the intact late genes between them (the D and E genes).

Other fragments of SV40 DNA are also being tested for use as vectors. One such fragment, containing the SV40 origin for DNA replication and an intact D gene, will allow insertion of DNA segments up to 3 kb in length.

247 MAPPING OF SEQUENCES WITH TWOFOLD SYMMETRY ON SV40 GENOME, Che-kun James Shen and John E. Hearst, Dept. of Chem., Univ. of California, Berkeley, CA 94720

Sequences with a twofold axis of symmetry have been detected in SV40 DNA and mapped by electron microscopy by their ability of SV40 single strands to form hairpins which are stabilized by the photochemical crosslinking reagents trioxsalen(4,5',8-trimethylpsoralen). SV40 I was digested with restriction enzymes EcoRI or HpaII and the resulting linear SV40 DNA molecules were denatured and photochemically reacted with trioxsalen at 16.0 ± 0.5°C at different ionic strenths. In 20 mM NaCl, one specific hairpin 150 base pairs long was detected at 0.17 ± 0.02 map units on the EcoRI map of SV40 DNA which is an "in vitro" promoter site for E.Coli RNA polymerase. A similar relationship between "in vitro" RNA polymerase promoter sites and hairpins has been reported for fd bacteriophage DNA(PNAS, 73 , 2649 - 2653). In 30 mM NaCl, five more hairpins appeared on these denatured and crosslinked SV40 DNA molecules. Four of these were found to be at 0.26 ± 0.02, 0.68 ± 0.03, 0.84 ± 0.02, and 0.94 ± 0.01 units on the EcoRI map, respectively. The fifth one is located on or near the EcoRI cleavage site of SV40 DNA. Of these five additional hairpins, the one at 0.68 map units is near the replication origin and the hairpin at 0.94 map units is near the 5' end of the 16S late messenger RNA.

248 BIOLOGICAL PROPERTIES OF POLYOMA DNA. Malcolm A. Martin and Wallace P. Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

One type of recombinant DNA experiment that has engendered considerable concern is the propagation of oncogenic animal virus genetic information in a procaryotic system. We have been charged by the NIH Recombinant DNA Molecule Program Advisory Committee to assess the potential risks associated with such an experiment and are in the process of cloning the polyoma virus genome in E. coli using approved phage and plasmid vector systems and P4 physical containment. Cloned bacteria, phage, and purified recombinant DNA will be fed to or inoculated into mice, the natural host for polyoma virus, to evaluate the biologic effects of such materials. Since it is very likely that polyoma DNA will be replicated but not expressed in bacterial cells, we have carried out a series of animal studies to assess the infectivity and transforming efficiency of purified viral DNA. Our results indicate that polyoma DNA is relatively highly infectious by the subcutaneous and intraperitoneal routes and very poorly so following nasal, gastric or colonic instillation. The effect of DNA conformation and restriction enzyme cleavage on such infectivity assays as well as results involving a hamster transformation system will be discussed.

249 STATE OF AN SV40-*E. COLI* RECOMBINANT GENOME IN TRANSFORMED RAT CELLS AND PERSISTENTLY INFECTED MONKEY CELLS. P. Upcroft, J.A. Upcroft, and G.C. Fareed, Molecular Biology Institute and Department of Microbiology and Immunology, University of California, Los Angeles, CA 90024

The structure and fate of SV40 genomes carrying in the late gene region an *E. coli* suppressor gene have been examined both in transformed rat embryo cells and persistently infected, permissive monkey kidney cells. High molecular weight DNA obtained from cloned lines of rat embryo cells which had been transformed by the purified hybrid SV40-su$^+$III DNA was cleaved with various restriction endonucleases and fractionated by agarose gel electrophoresis. Both SV40 and *E. coli* su$^+$III DNA sequences were localized on specific DNA fragments by the Southern (1975) procedure as described by Ketner and Kelly (1976) and Botchan *et al.* (1976).

An alternative mammalian vector system utilizes permissive monkey cells which are persistently infected with SV40. We have cloned lines of the TC-7 subline of CV-1 cells that were infected with the SV40-su$^+$III hybrid DNA. These cell cultures carry appreciable amounts of free viral DNA and such cell lines may serve as a helper virus-free molecular cloning system with the capability for propagating much larger foreign DNA segments than can be accepted in the lytic system for SV40. Identification of both free and integrated hybrid genomes in these cells can be accomplished with the Southern technique.

Botchan, M., Topp, W., and Sambrook, J. (1976) Cell 9, 269-287.
Ketner, G., and Kelly, T. J., Jr. (1976) Proc. Nat. Acad. Sci. USA 73, 1102-1106.
Southern, E. (1975) J. Mol. Biol. 98, 503-518.

250 RELATIONSHIP BETWEEN THE NUCLEAR AND POLYSOMAL RNA POPULATIONS OF ACHLYA: A SIMPLE EUCARYOTIC SYSTEM. William E. Timberlake and Robert B. Goldberg, Wayne State University, Detroit, Michigan 48202 The relationship between hnRNA and mRNA in the water mold Achlya has been investigated in several ways. Sucrose density gradient centrifugation under denaturing conditions showed that nuclear and polysomal poly(A)RNA have indistinguishable size distributions. Hybridization experiments in which an excess of nuclear DNA was reacted with ^{3}H-poly(A)hnRNA or ^{3}H-poly(A)mRNA showed that both populations contain repetitive as well as single-copy transcripts, in the same proportion. Analysis of hybrids on hydroxyapatite demonstrated that repetitive transcripts represent a population of molecules distinct from the single-copy transcripts. The complexity of whole-cell, nuclear, and polysomal RNA were also determined by saturation hybridization to single-copy ^{3}H-DNA. All three populations were complementary to essentially the same fraction of DNA. Terminal hybridization values were 3.84, 3.76, and 3.76% of the DNA for whole-cell, nuclear, and polysomal RNA respectively, representing a complexity of 3.3 - 3.4 X 10^6 nucleotides. The data suggest that the hnRNA and mRNA populations are essentially identical. No evidence for selective turnover of any sequence component or size class within the nucleus was observed.

251 TRANSFER RNA BIOSYNTHESIS IN THE SILKWORM, BOMBYX MORI, Richard L. Garber, M.A.Q. Siddiqui*, and Sidney Altman, Biology Department, Yale University, New Haven, Ct. 06520, and *Biochemistry Department, Roche Institute of Molecular Biology, Nutley, N.J. 07110

Transfer ribonucleic acid is transcribed from DNA as precursor molecules greater in length than the functional species, thus requiring nuclease processing. Although the original suggestion of this biosynthetic scheme was derived from studies of eukaryotes, details of precursor RNA structures and enzymology of processing and nucleotide modification have only been attained with bacteriophage and bacteria.

From the silkworm, Bombyx mori, we have recently isolated tRNA precursors, some pure by standards of RNA fingerprinting. One of these, a precursor to alanyl-tRNA, appears to have new 5'- and 3'-termini. Nucleotide modifications are present in this precursor. Unfractionated precursors can be converted in vitro to tRNA-size products with silkgland extract. This extract contains activities resembling the prokaryotic endonuclease RNase P and a 3'-5' exonuclease, both required in bacterial tRNA biosynthesis. No polycistronic precursors have been observed in Bombyx.

252 PROFILES OF WHOLE BLOOD RNA OBTAINED FROM LSD-TREATED RABBITS, Gilles H. Cousineau, Georges Dubois, Elisabeth Lalague, Louis-Gilles Charbonneau and Marc Champagne, Laboratoire de Biologie Moléculaire, Département de Sciences Biologiques, Université de Montréal, Montréal, P.Q., H3C 3J7, CANADA.

Rabbits received LSD at 10, 20 and 25 μg/Kg weight. Whole blood was taken periodically from the heart, placed in sterilized test tubes containing 2 ml of methylsulfoxide (10%, final). The cells were routinely frozen in acetone-dry ice. The material was then treated in one of the following fashions: (1) Homogenization in 5 volumes of Buffer 1 (0.001M $MgCl_2$, 0.1M NaCl and 0.01M K-acetate, pH 5.2) and bentonite, 0.3%, final. Cold, 0.5% Na-dodecyl sulfate-phenol was used for deproteinization. The RNA was precipitated by addition of Na-acetate (20%,final) to the aqueous phase. Approximately 60 μg of RNA was dissolved in 25 μl of Buffer 2 (0.04M Tris-HCl, pH 7.4, 0.2M Na-acetate and 0.001M Na-EDTA) containing 20% sucrose, placed on top of 7.2% polyacrylamide gels and electrophoresis carried out. (2) The material was hemolysed in Buffer 3 (0.01M Tris, 0.005M $MgCl_2$ and 0.01M NaCl) and Proteinase K at 500 μg/ml. To one volume of this preparation was added 1 volume of Sarkosyl NL-97 (0.5%,final), Buffer 4 (0.02M Tris, 0.02M EDTA and 0.2M Na-bisulfite) and EtBr (50 μg/ml,final). The supernatant, saturated with KI (density of 1.62), was centrifuged at 10,000g for 65 hrs, dialysed and the precipitated RNA analysed by gel electrophoresis. Results with these methods indicate that the control preparations contain 23S, 18S, 12S and 4S RNA. Blood of rabbits injected with 10 μg of LSD show the presence of 23S, 12S and 4S RNA only. At higher LSD concentrations, with the exception of 4S RNA, all the other RNA populations were absent. These methods are precise, rapid and easily done, and allow us the possibility of determining the presence of low amounts of LSD.

253 POST-EMBRYONIC CELL LINEAGE OF THE HERMAPHRODITE GONAD IN C. ELEGANS, Judith Kimble and David Hirsh, MCDB, Univ.of Colo.,Boulder,Colo.80309

The post-embryonic lineage of the C. elegans hermaphrodite gonad has been determined by direct observation in living worms. The gonadal primordium in newly hatched worms consists of four contiguous cells. Two of them are somatic and two are germ line precursor cells. The lineage of the somatic cells proceeds according to an invariant pattern with respect to the number of cell divisions, cell migrations, and the final fate of specific daughter cells. The lineage of the germ line cells is variable. The somatic development occurs in three distinct stages. An early period of cell divisions gives rise to a small group of cells. Most of these cells subsequently migrate extensively. Finally, another period of cell divisions generates all the cells present in the adult somatic structures. No single adult structure represents a clone of one cell. Instead each structure (uterus, spermatheca, and sheath) includes daughter cells of different ancestry.

254 BIOCHEMICAL ASPECTS OF DIFFERENTIATION IN HISTOPLASMA CAPSULATUM. George Boguslawski, James S. Parr, Dean A. Stetler, and Robyn R. Meyer, Department of Microbiology, University of Kansas, Lawrence, KS 66045

The fungus, Histoplasma capsulatum, is an important human pathogen. The attractiveness of this fungus as a model organism for the study of cellular differentiation stems from the fungus' ability to undergo a completely reversible morphological change in response to temperature shifts. At 23°C H. capsulatum is mycelial (M); when the temperature is raised to 37°C, the cells convert to a yeast-like form (Y). The conversion results in a number of biochemical changes including the appearance of multiple RNA polymerase species and the loss of an inhibitor of RNA polymerase. We have recently found two other changes associated with the conversion process. The first of these is the presence in the M cells of sulfite reductase, the enzyme not detectable in the yeast phase. As a consequence, the mycelial cells are self-sufficient whereas the yeast cells require cysteine for growth. The second conversion-related change is the appearance in the Y cells of an unusual ribonucleotide polymerizing enzyme (RPase) which is not found in mycelia. The enzyme does not require a template or a primer for activity, can utilize any of the individual ribonucleoside-triphosphates, and is inhibited by pyrophosphate but not phosphate ions. Mn^{2+} ions are required for activity. RPase has a mol. wt. of 900,000 and is composed of two kinds of subunits (75,500 and 69,000) in a 1:1 ratio.

Since the only governing factor in the conversion is the temperature shift, we interpret the above results as indicative of differential gene expression in the two morphological forms. Whether this is on transcriptional or translational level is now under investigation.

255 STUDIES ON THE REGULATION OF THE E. COLI LACTOSE AND ARABINOSE OPERONS USING A HIGH RESOLUTION ELECTRON MICROSCOPIC TECHNIQUES, J. Hirsh and R. Schleif, Brandeis University, Waltham, Mass. 02154

Regulation of the Escherichia coli lactose and arabinose operons was studied using a high resolution electron microscopic technique. Regulatory proteins were visualized bound to short, homogeneous DNA fragments containing the regulatory regions from the operons. Many known properties of the lac operon were confirmed, and additional information on the conformation of the operator DNA under the bound lac repressor was obtained. Two types of complexes were observed and studied which form on the regulatory sequences of the ara DNA. Both complexes are likely to contain RNA polymerase, and require the CAP and araC proteins for their formation.

256 GROWTH INHIBITION OF TETRAHYMENA PYRIFORMIS BY ANTIBIOTICS WHICH INDUCE PROTEIN SYNTHESIS ERRORS IN VITRO, Edward Palmer, Dept. Micro., Univ. Rochester, Rochester, NY 14642.

While many aminoglycoside antibiotics cause misreading of RNA by prokaryotic ribosomes, only a few aminoglycosides are effective in causing eukaryotic ribosomes to misread RNA,(Wilhelm and Pettitt,Fed. Proc. 35:1440 (1976)). The drugs effective with eukaryotic ribosomes include Gentamycin A, Kanamycin C, Lividomycin A, Lividomycin B, Paromomine and Paromomycin. These studies have been performed in vitro because aminoglycosides do not cross the membranes of higher cells. We have screened Tetrahymena pyriformis (which feeds by phagocytosis) for sensativity to various aminoglycoside antibiotics. The aminoglycosides which inhibit the growth of Tetrahymena pyriformis are exactly the same aminoglycosides, which cause eukaryotic ribosomes to misread RNA in vitro. These observations suggest that certain aminoglycosides may induce protein synthesis errors in living Tetrahymena, and thus may be useful in the genetic analysis of this organism.

257 MAPPING THE DROSOPHILA 5S GENES IN SEVERAL RECOMBINANT PLASMIDS, Ann Sodja, N. Davis Hershey, Sue Conrad, Norman Davidson, Division of Chemistry, California Institute of Technology, Pasadena, Ca. 91125, Chris Ilgen, John Carbon, Department of Biology, University of California, Santa Barbara 93107.

Recombinant plasmids containing the Col E1 prokaryotic vector and moderate size fragments of Drosophila DNA obtained by hydrodynamic shear were constructed by the poly(A)-poly(T) tailing method. Several bacterial strains bearing plasmids containing Drosophila 5S RNA genes were selected by colony filter hybridization. Two of the plasmids, No. 9 and No. 19, were selected for further study. The former contains about 1.5 kb of Dm DNA and 3 5S genes. The Dm insert in the latter has a length of about 13 kb and contains 33 tandem repeats of the 5S gene plus spacer units. The genes have been mapped and the length of the repeat unit of the 5S genes measured by electron microscope partial denaturation mapping, electron microscope ferritin mapping, and restriction endonuclease - gel electrophoresis studies. All methods agree that the repeat length is about 380±20 nucleotides, consisting of about 260 nucleotides of spacer and 120 nucleotides of gene. Partial denaturation mapping shows that the spacer sequence is A+T rich, relative to the gene sequence and relative to most of the Col E1 DNA. Each of the enzymes, Hae III and Hha I, cuts the repeat unit in one and only one place. No. 9 contains two full repeats that differ in length by about 20 nucleotides; the larger is about 390 nucleotides in length. The 33 repeat units in No. 19 appear to have a length heterogeneity of less than 10 nucleotides. There is some tendency for excision of circular DNA molecules containing several 5S repeat units during growth and chloramphenicol amplification of the plasmids in E. Coli.

258 RAPID DEVELOPMENT MUTATIONS IN DICTYOSTELIUM DISCOIDEUM, Richard H. Kessin, The Biological Laboratories, Harvard University, Cambridge, MA 02138

Several mutations affecting the speed of slime mold development on a biochemical and morphological level have been characterized and analysed genetically. These mutants aggregate rapidly and have an aberrant fruiting structure. The mutation carried by strain FR17 is recessive and is located on linkage group IV. A selection procedure for isolating mutants of this type has been developed and new mutations have been tested for complementation. One of the new alleles causes aggregation in concentric rings. The mutant phenotypes can be nutritionally corrected.

259 BIOCHEMICAL GENETICS OF HUMAN HYPOXANTHINE PHOSPHORIBOSYLTRANSFERASE, Gregory Milman, Johns Hopkins University School of Hygiene and Public Health, Baltimore, Md. 21205.

The spot corresponding to hypoxanthine phosphoribosyltransferase (HPRT, E.C.2.4.2.8) has been identified in two-dimensional polyacrylamide gels of HeLa cell extracts. A missense mutant displays a new HPRT spot at the same molecular weight but different isoelectric focusing position. Five independently isolated revertants of the missense mutant display spots corresponding to both the wild-type and mutant proteins indicating that they synthesize HPRT from two separate genes. If the missense protein is synthesized from a mutated form of the initially active HPRT gene, then wild-type HPRT protein in the revertants must be synthesized from a newly activated but previously silent wild-type gene. The newly activated gene in the revertants of the missense mutation appears unstable producing a high frequency of spontaneous HPRT mutants.

Purified wild-type and missense mutant proteins labeled *in vivo* with radioactive lysine, arginine, or methionine were digested with trypsin and the tryptic peptides were separated by column chromatography. Less than 50 nanograms of HPRT are required to produce a tryptic peptide pattern. A methionine labeled peptide was identified as the C-terminus because it was not labeled with either lysine or arginine. The missense mutant protein has a new lysine labeled peptide, but the chromatography patterns of arginine or methionine labeled peptides were identical to those of the normal protein. The presence of a new lysine labeled peptide provides strong evidence for a structural gene mutation.

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260 Genetic Control of Sexual Dimorphism in the Nematode Caenorhabditis elegans, Michael R. Klass, Dept. Mol., Cell., & Devel. Biol., Univ. Colorado, Boulder, Col. 80302

The small free-living nematode Caenorhabditis elegans is usually found as a hermaphrodite, but occasionally true males appear in the population. Male and hermaphrodite gonads develop from morphologically identical primordia. The small primordial gonad lies on the ventral side of the worm in the coelomic cavity. The gonadial primordium contains four nuclei at parturition. As this primordium develops in a hermaphrodite, it produces a double-armed, mirror symmetrical gonad that produces first sperm and then eggs. In the male, however, this primordium develops into an asymmetrical structure composed of a ventrally located testis, a loop region, a seminal vesicle, and a vas deferens. The male gonad presents a linear sequence of nuclei in successive stages of spermatogenesis beginning with a mitotic region in the testis, followed by clearly distinguishable stages of meiosis. Normal males are genotypically XO while hermaphrodites are XX. Two major loci (tra-1 and tra-2) in C. elegans cause the sexual transformation of hermaphrodites to males. A temperature-sensitive sex transformer mutant, tsB202, has been isolated. tsB202 carries an autosomal recessive mutation in linkage group II (an allele at the tra-2 locus) that at restrictive temperature transforms an XX hermaphrodite into a phenotypic male complete with a male gonad and vestigial external genitalia but lacking mating behavior. Temperature shift experiments have determined the specific temporal sequences of gonadogenesis, oogenesis, and spermatogenesis. In tsB202 both male and hermaphrodite intersexes can be created by proper manipulation of temperature. The male intersex produces oocytes in an otherwise typical male gonad while the hermaphrodite intersex produces only sperm.

261 PRELIMINARY CHARACTERIZATION OF THE RIBOSOMAL GENES IN MICE AND MEN, Norman Arnheim and E.M. Southern, Mammalian Genome Unit, West Mains Road, Edinburgh, Scotland.

Studies on the structure of mouse and human ribosomal DNA were carried out on EcoRI or Hind III digests of total DNA using the "transfer" method. The analysis of DNA's from five inbred strains of mice (C57BL, CBA, C3H, BALB/c and CBA/H T6T6) indicated that the structure of the ribosomal gene repeats was heterogeneous within a strain and that this pattern of heterogeniety differed among certain of the strains. Tissue culture cells originally established from C3H mice (L-cells) or BALB/c mice (myeloma cells) appeared to have essentially the same pattern of heterogeniety in ribosomal gene structure as the parental strains, although some quantitative differences were observed. Studies on DNA from several different human sources indicated that the structure of the ribosomal genes in man is heterogeneous as well. Mice and men carry their ribosomal genes distributed among a number of nucleolar organizers. Our studies on mouse-human cell hybrids which contained different human nucleolar organizers suggest that certain ribosomal repeats are common to more than one nucleolar organizer.

262 CLONING OF THE GENE CODING FOR THE PLANT CO_2 FIXATION ENZYME, Stanton Gelvin, Linda L. Walker, Chris Head, and Stephen H. Howell, Department of Biology, University of California, San Diego, La Jolla, California 92093

We have cloned the gene coding for the large subunit (LS) of D-ribulose-1,5-bisphosphate carboxylase (the enzyme responsible for CO_2 fixation in plants) from the unicellular green alga Chlamydomonas reinhardi. Total C. reinhardi chloroplast DNA was digested with Eco R1 endonuclease, the resulting fragments cloned in the plasmid pMB9, and the LS gene-containing clones selected by hybridization with ^{32}P-labeled LS mRNA. These clones contain a chloroplast DNA fragment of molecular weight 3.2×10^6 daltons. This fragment also hybridizes with chloroplast 23 S and 16 S rRNA. Such data are in agreement with hybridization data of labeled LS mRNA, 16 S, and 23 S rRNA to total chloroplast DNA digested with Eco R1 endonuclease and immobilized on Southern filters. Such filters also show that these RNA species hybridize to a DNA fragment of molecular weight 3.2×10^6 daltons. We are currently doing fine structure restriction mapping and electron microscopic R-loop analysis to determine the relative positions of the genes on this fragment, and studies to determine the expression of these genes in E. coli minicells. These studies were carried out under P2-EK 1 containment conditions.

263 DNA SEQUENCE ORGANIZATION IN THE WATER MOLD ACHLYA. Robert B. Goldberg, Michael E. Hudspeth, and William E. Timberlake, University of California, Los Angeles 90024.

Achlya ambisexualis is an oomycete fungus whose sexual stage is induced by steroid hormones. In order to provide a firm foundation for investigations of the molecular mechanisms of steroid-mediated gene regulation we have determined the pattern of DNA sequence arrangement in the genome of this unique organism. The reassociation kinetics of 200 nucleotide-long DNA fragments analyzed by hydroxyapatite chromatography reveals the presence of at least three kinetically distinct classes. Classification of these classes and their rate constants ($M^{-1}sec^{-1}$)is: Single copy (2.13×10^{-2}), slow (1.42), and fast (43.4). The slow and fast classes contain approximately 70 and 2000 copies per haploid genome respectively and comprise 11% and 2% of short DNA fragments. The kinetic complexity of the single copy, slow, and fast DNA classes relative to E. coli DNA is 4.4×10^7, 8.5×10^4, and 575 nucleotide pairs. In order to determine if repetitive and single copy DNA sequences are interspersed in the genome the reassociation kinetics of 1800 nucleotide DNA fragments were studied. Those experiments failed to reveal any DNA sequence interspersion at this fragment length. This observation was confirmed by analysis of the reassociation kinetics of tracer amounts of 1400 nucleotide-long fragments of total nuclear DNA driven by an excess of 200 nucleotide-long fragments containing only the sequences of the repetitive DNA classes. We conclude from these studies that the short period interspersion pattern of DNA sequence organization found in most other eukaryotes examined to date does not occur in the Achlya genome. (Supported by NSF grant BMS74-21461 and the Brown-Haven Foundation.)

264 COMPARISON OF KINETIC PARAMETERS FOR XANTHINE DEHYDROGENASE FROM WILD-TYPE AND FROM A PUTATIVE REGULATORY VARIANT OF DROSOPHILA MELANOGASTER, E.P.M. Candido and T.C.R. Edwards, Dept. of Biochem., U. of B.C., Vancouver, V6T 1W5, and A. Chovnik, Dept. of Gen. & Cell Biol., U. of Conn., Storrs, Conn. Enzymatically active xanthine dehydrogenase (XDH) has been purified in high yield by immunoaffinity chromatography of crude extracts on a Sepharose 4B column containing covalently attached anti-XDH antibodies. A detailed kinetic analysis of wild-type XDH over a range of substrate and NAD concentrations revealed that Drosophila XDH exhibits ordered binding for the substrate and cofactor, i.e. the reaction follows a "ping-pong bi bi" mechanism, analogous to that of xanthine oxidases and dehydrogenases from vertebrate sources. The purified enzyme from $ry^{+11}i409N$ flies (wild-type), and from a strain producing increased amounts of XDH ($ry^{+4}i409H$) yielded similar K_m values for xanthine, hypoxanthine and NAD (see below). The results provide further evidence that the i409H site is responsible for increased levels of synthesis of normal XDH, rather than for synthesis of an enzyme with higher affinity for substrate or cofactor.

	Km, Moles/l		
Strain	Xanthine	Hypoxanthine	NAD
$ry^{+11}i409N$	2.4×10^{-5}	1.6×10^{-5}	4.0×10^{-5}
$ry^{+4}i409H$	2.1×10^{-5}	1.7×10^{-5}	2.6×10^{-5}

265 MUTANTS OF S49 MOUSE LYMPHOMA CELLS SHOWING CHARGE ALTERATIONS IN THE REGULATORY SUBUNIT OF CYCLIC AMP-DEPENDENT PROTEIN KINASE, Robert A. Steinberg, Department of Microbiology, University of California, San Francisco, California 94143

Among variant clones of S49 mouse lymphoma cells resistant to cytolysis by dibutyryl cAMP are a number whose cAMP-dependent protein kinases are altered in their dose-responses to cAMP activation. The lesion in these "K_a" mutants purifies with the regulatory subunit of cAMP-dependent protein kinase (Hochman et al., Proc. Nat. Acad. Sci. USA 72, 5051 [1975]). Analysis by two-dimensional polyacrylamide gel electrophoresis of the kinase regulatory subunit from more than 25 mutagen induced K_a mutant clones has revealed at least three subclasses of this mutant type. One subclass has mutant regulatory subunits which comigrate with wild type subunit in the two-dimensional gel system; one has mutant subunits which are more acidic than wild type by about one charge; and the third has mutant subunits which are more basic than wild type by about two charges. Calibration of gels for unit charge changes allows refinements of the estimates for the mutationally induced regulatory subunit charge alterations which, in turn, delimit the possible amino acid substitutions underlying the charge shifts. These studies provide strong evidence for the induction of missense structural gene mutations in this cultured animal cell system.

203 CHEMICAL METHODS FOR SEQUENCING DNA AND THE RECOGNITION OF DNA-PROTEIN CONTACTS, Walter Gilbert and Allan Maxam, Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, MA 02138

We have developed a chemical method for DNA sequencing. We break, partially, a terminally labeled DNA molecule with four different chemical reactions, each reflecting a chemistry that identifies a specific base. When the products of these four reactions are separated by size on a polyacrylimide gel, the lengths of the terminally labeled fragments released by each reaction determine the sequence. The method will sequence from one to a hundred bases in from the point of labeling.

These methods have been used to sequence the terminal region of tumor virus RNA's; some of the implications of these sequences will be discussed.

The chemical approach that we use permits us to identify some of the contact points at which proteins that interact with DNA contact the DNA sequence. Such experiments not only determine where control proteins bind to DNA but also give some insight into the way in which they bind.

204 NUCLEOTIDE SEQUENCE OF BACTERIOPHAGE ØX174 DNA, J.C. Fiddes, F. Sanger, G.M. Air, B.G. Barrell, N.L. Brown, A.R. Coulson, C.A. Hutchison III, P.M. Slocombe and M. Smith, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, England

The rapid plus and minus technique has been used to obtain an almost complete nucleotide sequence for the DNA of bacteriophage ØX174. The locations of the initiation and termination sites of the nine known ØX174 genes have been identified. Intercistronic spaces between the genes range from two to 111 nucleotides. Two pairs of genes were demonstrated to overlap, with the nucleotide sequence being read in different phases. Gene B is thus entirely contained within gene A, and E likewise is within D.

205 RAPID DNA SEQUENCING BY PARTIAL RIBOSUBSTITUTION, Wayne M. Barnes, MRC Laboratory of Molecular Biology, Cambridge, England.

A new rapid method for DNA sequence analysis has been developed. The method uses the Gilbert-Maxam-Mirzabekov principle of base-specific partial cleavage of 5'-labelled DNA in a variation suggested by Dahlberg & Abelson. In this method the cleavage is at partially substituted ribonucleotides which are introduced by DNA polymerase extension in the presence of Mn^{++}. Specificity and label incorporation are achieved by extending a restriction fragment primer with DNA polymerase I as in the Sanger & Coulson method. After a short initial incorporation with α-^{32}P-deoxynucleotide triphosphates to label the 5' region of the target sequence, the triphosphates are removed and the reaction mixture is divided four ways for a second, primed extension. The second extension is a cold chase in the presence of Mn^{++}, all four deoxynucleotides and one of the four ribonucleotides under conditions that result in about 2% ribonucleotide substitution at each position. After cleavage at the restriction site and alkali cleavage at the positions of partial ribosubstitution, each reaction mixture is analysed by electrophoresis on a high resolution denaturing acrylamide gel. As in the other rapid DNA sequencing methods, the extent of DNA sequence that can be determined from a single experiement is limited only by the resolution of the analysing gels. At present some 100 nucleotides of sequence can be determined from a single priming reaction. The application of this technology to the identification of protein binding sites on DNA will also be discussed.

206 REGULATION OF THE HIS4 REGION OF YEAST, Gerald R. Fink, Helen Greer, Joseph Keesey, Ramunas Bigelis, Department of Genetics, Development and Physiology, Cornell University, Ithaca, N.Y. 14853

The his4 region of yeast is a fungal gene cluster which specifics three steps in the pathway of histidine biosynthesis. The complementation behavior of missense mutations indicates three separate cistrons in the order his4A, his4B, and his4C encoding the information for the 3rd, 2nd, and 10th steps in the pathway respectively. All three types of nonsense mutations (UAG, UAA, and UGA) as well as frameshift mutations show polarity from A —> C. The independent functioning of these regions is dramatized by several observations (1) deletions of the AB region have a functional C protein (2) nonsense and frameshift mutations in C have a functional A and B protein.

All three enzymatic activities specified by his4 are in an aggregate of 180-190,000 MW as determined by elution from an agarose column. Co-purification of the three activities is achieved by chromatography first on AMP-Sepharose and then DEAE cellulose. Analysis of the purified protein by SDS-acrylamide gel electrophoresis shows a single protein of 95,000 MW. In addition, immunological and genetic evidence support the conclusion this 95K protein is the gene product of the his4 region. All three enzymic reactions are carried out by this protein which is presumably a dimer of two identical 95K chains. The partial activities of deletion, and nonsense and frameshift mutations can be explained if the three his4 activities are located at distinct regions of the 95K protein and these regions can function independently.

Analysis of regulatory mutants affecting the expression of this region show that aberrant regulation is a consequence of (1) unlinked mutations (2) linked dominant mutations (3) a deletion through the his4 end of the his4 region.

207 GENETIC CONTROL OF 2μ DNA REPLICATION IN S. CEREVISIAE, Dennis Livingston and Leland Hartwell, Department of Genetics, University of Washington, Seattle, Washington 98195

A yeast cell contains three forms of DNA, nuclear, mitochondrial and 2-micron. The latter is a well defined circular plasmid containing an inverted repeat. Strains harboring different forms of 2-micron DNA have been discovered by comparing restriction enzyme patterns from inter-fertile yeast strains and two strains have been found that lack 2-micron DNA. Crosses between these strains show that 2-micron DNA is inherited cytoplasmically, that there is no restriction between different forms, and that the strains which lack 2-micron DNA have a nuclear genome capable of maintaining the replication of this plasmid. Furthermore, 2-micron DNA can be transmitted independently of both nuclear and mitochondrial genomes in crosses producing heteroplasmons and the frequency of its transfer suggests that the 50-100 copies are localized in one or a few cellular organelles.

The initiation of nuclear DNA replication requires the products of genes cdc 28, 4, and 7 and the elongation of nuclear DNA requires the products of genes cdc 2, 8 and 21. Mitochondrial DNA elongation requires the products of genes cdc 8 and 21 but its replication is not dependent upon the nuclear initiation genes, cdc 28, 4, 7. Despite its apparent cytoplasmic location, 2-micron DNA replication requires the same gene products as are necessary for initiation and elongation of nuclear DNA replication.

The 2-micron DNA plasmid may be useful for investigations of the mechanisms involved in nuclear DNA initiation and replication and as a vector for studying gene expression of interspecies recombinant DNA molecules.

208 REGULATION OF QUINATE CATABOLISM IN NEUROSPORA: THE QA GENE CLUSTER, J.W. Jacobson, J.A. Hautala, M.C. Lucas, W.R. Reinert, P. Strøman, J.L. Barea, M.E. Case, and N.H. Giles, Genetics Program and Department of Zoology, University of Georgia, Athens, GA 30602

The expression of the three linked structural genes (qa-2, qa-3, and qa-4) encoding the enzymes functioning in quinic acid catabolism in Neurospora is controlled by the protein product of a tightly linked regulatory gene (qa-1) which appears to act with the inducer quinic acid as a positive regulatory effector (1). Mutants in the qa-1 gene are non-inducible for all three enzyme activities. No conclusive evidence exists that the gene cluster is transcribed as a polycistronic messenger RNA. Identification and purification of the qa-1 gene product has as yet had little success since it is apparently present at very low intracellular concentrations. Since current complementation data suggest that the qa-1 protein functions primarily in the nucleus in which it is encoded, experiments are underway to detect the regulatory protein in extracts of isolated nuclei and to see if it fits the criteria of a nonhistone chromatin protein.

In order to elucidate the mechanism of regulation, two of the enzymes under the control of the qa-1 gene have been purified and partially characterized. Dehydroquinase, encoded in the qa-2 locus, is a multimeric enzyme of 20-22 identical 10,000 M.W. subunits (2). Mutants in the qa-2 locus which affect either the catalytic site of the enzyme or its aggregation have been characterized (3). Quinic dehydrogenase, encoded in the qa-3 locus, appears to be a 40,000 M.W. enzyme. The amino acid sequence of the qa-3 gene product is being determined. Thus far the qa-2 gene product has been resistant to sequence analysis.

Attempts are underway to isolate mRNA transcribed from the qa region. Messenger RNA from induced cultures has been isolated and characterized. Whole cell RNA, polyadenylated and non-polyadenylated mRNA fractionated by oligo(dT)-cellulose chromatography, as well as various size classes of mRNA fractionated by Sepharose CL-4B chromatography have been translated in the cell-free wheat germ system. Products synthesized in vitro range in M.W. from 10,000 to over 100,000 as determined by SDS gel electrophoresis. Experiments are underway to identify the fraction of RNA containing the specific qa mRNA by double immunoprecipitation of either qa-2 or qa-3 gene product synthesized in vitro in the wheat germ system.

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209 GENETIC CONTROL OF PHOSPHORUS METABOLISM IN NEUROSPORA, Robert L. Metzenberg, Department of Physiological Chemistry, University of Wisconsin, Madison, WI 53706

Several enzymes involved in the acquisition of phosphorus are not made in appreciable amounts by wild type Neurospora when there is adequate phosphorus in the medium, but are made in large amounts when the cells are growing in low phosphate medium. One member of the family, alkaline phosphatase, approaches 2% of the extractable protein in phosphorus-starved cells, but is about 20,000-fold lower in phosphorus-replete cells. The gene for this enzyme, and for acid phosphatase have been mapped. They are unlinked to one another, and to the regulatory genes. A model that satisfactorily fits the properties of mutants in these genes may be summarized as follows.

(1) The normal product of the nuc-1 gene is required to turn on expression (transcription) of the structural genes of the phosphorus family. Thus the immediate control over the structural genes is positive.

(2) The normal product of the preg gene is required to nullify the nuc-1 product.

(3) The normal product of the nuc-2 gene is required to nullify or destroy or prevent the synthesis of the preg product.

(4) Phosphate or something derived from it nullifies or destroys or prevents the synthesis of the nuc-2 product.

Genetic analysis of this system has come to the point at which one may hope to develop an assay for the hypothetical nuc-1 product, and perhaps ultimately isolate it. The fact that nuc-1 product is almost an absolute requirement for synthesis of alkaline phosphatase *in vivo* suggests that if $nuc\text{-}1^+$ product could be introduced into a $nuc\text{-}1^-$ cell, even in very small amounts, it might be possible to detect the synthesis of alkaline phosphatase and thus bioassay the nuc-1 product. Some preliminary approaches to inserting proteins into wall-less "slime" cells of Neurospora, using liposomes, will be described. An alternate approach takes advantage of the fact that the nuc-1 product may be expected to be concentrated in the nucleus. Nuclei can be isolated very easily from "slime". A double-label autoradiographic system suitable for use with high-resolution two-dimensional gels has been developed, and it is hoped that this will allow the detection of alterations in the nuc-1 product in suitable mutants.

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266 ISOLATION AND CHARACTERIZATION OF PLASMIDS CONTAINING GENES FOR THE STABLE YEAST RNA SPECIES, Jacques S. Beckmann, Peter F. Johnson, John N. Abelson and Shella A. Fuhrman, Dept. Chem., Biol., Univ. Calif. San Diego, La Jolla, Ca. 92093

Recombinant plasmids were constructed by insertion and ligation of yeast DNA within the tet^R gene of the E. coli plasmid pBR313 (Bolivar, Rodriguez, Betlach and Boyer, manuscript in preparation). The plasmid tet^R gene was split by endonucleases R BamI or HindIII yielding GATC and AGCT cohesive ends, respectively. Yeast DNA cut with endo R BglII (GATC) was recombined with the former and endo R HindIII cleaved DNA with the latter. Some 4000 $amp^R tet^S$ clones were isolated from more than 30 independent transformations, all of which were then tested for the presence of yeast specific genes for 4S, 5S or 5.8S RNAs. We have modified the screening method described by Grunstein and Hogness (PNAS US 72, 3961-5, 1973) so that it is now possible to rapidly replica plate and screen several thousand clones. Approximately 200 clones carrying yeast tRNA genes were recognized by hybridization with electrophoretically purified ^{32}P-labeled 4S RNA. Some of these clones were further subdivided into smaller families according to their hybridization patterns to individual tRNA species, purified by two dimensional gel electrophoresis (Piper, Wasserstein, Engbaek, Kaltoft, Celis, Zeuthen, Liebman and Sherman, Nature 262, 757-61, 1976). The DNA organization of some of these clones as analysed by restriction mapping will be reported. About 125 clones containing either a 5S or a 5.8S gene were identified following the same hybridization method with ^{32}P-labeled 5 or 5.8S yeast RNA. A detailed study of the restriction map of one of these clones was undertaken.

267 ORGANIZATION OF YEAST RIBOSOMAL DNA, J.H. Cramer, F.W. Farrelly, J.T. Barnitz and R.H. Rownd, Department of Biochemistry and Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisc. 53706

We have constructed hybrid plasmids between EcoRI partial digestion fragments of purified Saccharomyces cerevisiae ribosomal DNA and the plasmid RSF 2124, which has a ColE1 replicator and is ampicillin resistant. The hybrid plasmids have been used to map the EcoRI, HindII, HindIII and SmaI restriction sites in the yeast rDNA repetitive units. An analysis of a large number of plasmids has shown that the repetitive units are homogeneous in size and composition, and that they are not separated by large heterogeneous spacer regions. Some of the plasmids contain EcoRI restriction fragments that are not part of the rDNA repetitive unit. These sequences appear to represent "saddle fragments" which span the interface between rDNA and the remainder of the nuclear DNA. Their characteristics are under investigation. From a comparison of the restriction endonuclease and denaturation maps of the yeast rDNA, we have been able to position the ribosomal RNA coding regions on the denaturation map and have determined that the spacer regions are A-T rich. Similar studies are being conducted with the rDNA of the closely related yeast Saccharomyces carlsbergensis.

268 THE SYNTHESIS OF YEAST RIBOSOMAL PROTEINS IN VITRO, Jonathan R. Warner and Charles Gorenstein, Albert Einstein College of Medicine, Bronx, N. Y. 10461.

We have demonstrated that a wheat germ extract, primed with yeast RNA, synthesizes at least thirty five yeast ribosomal proteins. These proteins are all coded by mRNA molecules which contain poly A. By quantitative analysis of the products formed under limiting mRNA concentrations, we have measured the level of mRNA for the individual ribosomal proteins under various conditions. In confirmation of studies in vivo (Gorenstein and Warner, PNAS 73, 1547, 1976) we find that the synthesis of mRNA for the different ribosomal proteins is coordinately regulated. Within five minutes after wild-type cells are shifted to from 23° to 36° the synthesis of mRNA for most of the ribosomal proteins ceases. The mRNA that is present decays with a half life of 8 to 10 minutes. The synthesis of ribosomal precursor RNA and of mRNA for non-ribosomal proteins is unaffected. After 15 to 20 minutes the synthesis of mRNA for ribosomal proteins resumes, and by 60 minutes the production of ribosomal components has equilibrated at the increased temperature. In a set of mutants ts for ribosome synthesis, the resumption of synthesis of mRNA for ribosomal proteins never occurs, and the cells are then unable to form new ribosomes at the restrictive temperature, although transcription of ribosomal precursor RNA continues.

Further studies have demonstrated that under conditions of amino acid starvation, the synthesis of ribosomal RNA and of mRNA for ribosomal proteins are coordinately reduced to 20-30% of control levels, whereas synthesis of tRNA and of many other mRNA species continues.

This research was supported by grants from the NSF and the ACS.

269 CONTROL OF CELL TYPE IN SACCHAROMYCES CEREVISIAL. Ira Herskowitz, Jeffrey Strathern, James Hicks, and Jasper Rine. Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403.

Haploid yeast cells are either of mating type a or of mating type α. Cells of one mating type can switch to the other mating type at low frequency in ho strains and at high frequency in HO strains. Our studies have identified 3 points of interest with respect to eucaryotic gene control: (1) We propose that cell type (mating type a or mating type α) is determined by insertion of a bloc of genes into the mating type locus. In this "cassette model", hMa and hMα loci are silent copies of α and a mating type loci, respectively, which become activated by insertion of these genes (or copies of these genes) into the mating type locus. (2) Strains carrying the HO gene switch mating types as often as every cell division cycle and exhibit a specific pattern of switching. Cells differ in their competence to switch mating types: experienced cells (those which have undergone at least one cell cycle) give rise to switched cells 80% of the time, whereas inexperienced cells switch rarely if at all. This pattern of switching gives rise to a population of cells which has similarities to a stem cell/differentiated cell population. (3) The mating type locus, at least of an α cell, may be a gene cluster. From analysis of non-mating mutants defective at the α mating type locus, there appear to be at least two complementation groups. One is formally a positive regulator of α-specific functions, and the second is formally a positive regulator of sporulation and a negative regulator of a-specific functions.

270 CHROMATIN REPLICATION IN VITRO, Ronald L. Seale, Division of Molecular and Cellular Biology, National Jewish Hospital and Research Center, 3800 E. Colfax Ave., Denver, CO 80206.

An in vitro replication system, using HeLa cell nuclei, was characterized for the study of chromatin replication. Isolated nuclei incorporated ^{3}H-TTP for at least 30 min into a DNA product, and this DNA was synthesized from active replication forks. The DNA replicated in vitro accumulated as immature DNA precursor fragments; the ligation functions are extracted into the cytosol during cell fractionation.

The association of DNA replicated in vitro with nucleosomes was investigated by micrococcal nuclease digestion. The in vitro DNA was degraded to 75-80% acid soluble material, compared to 50% in controls, indicating that half the new DNA is associated with histones. The nuclease-resistant DNA was shown to be associated with nucleosomes since early digestion products were multiples of the 180 bp unit particle and limit digest products were the complete set of oligonucleotides of 30-130 bp, as in controls. Analysis of the sizes of oligonucleotide digestion intermediates showed that nucleosomes are partitioned to one arm of the replication fork.

Since nuclei in vitro accumulated DNA precursor fragments, it was possible to isolate nucleosome-associated Okazaki fragments and demonstrate the association of these precursors with the nucleosome. By denaturation of monomer, dimer, trimer and tetramer-sized double-stranded units of in vitro DNA, the Okazaki fragment termini were shown to map outside the nucleosome.

271 DEVELOPMENTAL REGULATION OF XANTHINE DEHYDROGENASE IN Drosophila melanogaster, Janis O'Donnell, Stephen Clark, and Arthur Chovnick, Genetics and Cell Biology, University of Connecticut, Storrs, Conn. 06268

The rosy locus which codes for the enzyme xanthine dehydrogenase (XDH) in Drosophila melanogaster is being analyzed in an attempt to understand its functional organization. The genetic limits of the structural element have been described (Gelbart et al., Genetics 78:869, 1974). Variants affecting XDH activity have been detected and mapped to a region contiguous to the structural element (Chovnick et al., Genetics 84:233, 1976; McCarron et al., In preparation). These control element variants were detected in stocks derived from natural populations. Mutagenesis programs based upon prokaryote models have thus far failed to produce regulatory mutants. These observations suggest that there are features unique to eukaryote control regions which require different approaches. In order to further analyze regulation of the rosy region we have been studying the pattern of XDH activity during the development of the insect. The "normal" developmental pattern of XDH in several isoallelic lines derived from wild-type stocks has been determined and will be discussed. Other isoallelic lines and induced XDH mutants are being examined for possible deviations from this developmental profile. Any developmental variants detected in this manner may be subjected to genetic fine structural analysis. Schemes which permit screening of X-ray and chemically mutagenized individuals for other developmental variants are being devised.

272 A COLD-SENSITIVE MUTANT STRAIN OF NEUROSPORA CRASSA DEFECTIVE IN THE PRODUCTION OF 17S rRNA FROM RIBOSOMAL-PRECURSOR RNA. Peter J. Russell and Eric U. Selker, Biology Department, Reed College, Portland, OR 97202.

The biosynthesis of ribosomal RNA (rRNA) in wild-type Neurospora crassa was studied by labeling experiments using [5-^{3}H]uridine and was shown to occur by a pathway similar to that shown for other eukaryotic organisms. The first RNA molecule to be synthesized in significant quantities is the 2.4x10^6-dalton ribosomal precursor RNA (pre-rRNA). This RNA is cleaved to produce two species of RNA with weights of 0.7 and 1.4x10^6 daltons. The former is the mature 17S rRNA of the 37S ribosomal subunit, and the latter is the precursor to the 1.27x10^6 (25S) and 61,000 dalton (5.8S) rRNAs of the 60S ribosomal subunit.

The cold-sensitive mutant strain of N. crassa, crib-1, has been shown previously to synthesize unequal amounts of 25S and 17S rRNA molecules at 10°C, resulting from a greatly reduced accumulation of stable 17S rRNA. Synthesis of these two rRNA molecules in the crib-1 strain at 25°C is almost normal. Labeling experiments have shown that, at 10°C, crib-1 synthesizes the 2.4x10^6-dalton pre-rRNA and that cleavage of this molecule to produce the 1.27x10^6-dalton rRNA proceeds normally, whereas very little stable 0.7x10^6-dalton rRNA is produced. At 25°C, synthesis and processing of pre-rRNA in crib-1 is almost normal. We conclude that crib-1 has a conditional defect in the processing of pre-rRNA to the mature rRNAs and we hypothesize that the primary molecular lesion in this mutant strain involves one of the components required for ribosome biosynthesis, for example, a ribosomal protein, methylase or nuclease.

273 ECTOPIC PEPTIDE HORMONES AND ONCOPLACENTAL ENZYMES IN CULTURED HELA CELLS, Rody P. Cox and Nimai K. Ghosh, New York University Medical Center, 550 First Avenue, New York, NY 10016

Human cervical cancer cells, HeLa65 and HeLa71 ectopically synthesize glycopeptide tropic hormones - human chorionic gonadotropin (HCG), luteinizing hormone (LH), follicle stimulating hormone (FSH) and their subunits. These HeLa cells, however, do not produce other glycotropic and pregnancy-specific hormones - thyroid stimulating hormone (TSH) and human chorionic somatomammotropin (HCS). All five hormones were measured by radioimmunoassays using antibodies against corresponding highly purified hormones and ^{125}I-labeled antigens as tracers. A second antibody against γ-globulin was used to isolate radioactive antigen-antibody complexes in assays for HCG, LH, FSH and TSH, whereas charcoal adsorption technique was used in HCS measurement. Production of HCG, LH and FSH is stimulated several hundred fold by aliphatic monocarboxylates, C$_4$-butyrate being most effective. Those fatty acids that mediate increased production of glycopeptide hormones inhibit cell growth and DNA synthesis without apparently altering RNA synthesis. Agents that induce glycopeptide hormone synthesis in HeLa cells cause a simultaneous increase in the activity of the oncofetal alkaline phosphatase. HeLa cells also have an aldolase similar to that found in placenta. Our findings provide further support for the carcinoplacental enzymic constitutions of HeLa cells. These cells apparently have the potential to produce peptide hormones that are eutopically synthesized by human pituitary and/or placenta. This information may have relevance to studies on differentiated eukaryotic gene expressions in neoplastic and embryonic cells. (Supported by the USPHS, NIH Grant)

274 ROLE FOR PYR·PUR DNA'S IN CHROMOSOME CONDENSATION, A. Richard Morgan and Douglas Johnson, Biochem. Dept., University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

Long pyrimidine·purine stretches of DNA of unknown function occur in eukaryotic chromosomes. Synthetic DNAs of repeating sequence indicate pyr·pur DNAs from a unique class. For example they form triplexes with RNA utilizing Hoogsteen pairing in the major groove of the duplex. (Morgan and Wells, (1968) J. Mol. Biol. 37:63) The DNAs $d(TTC)_n \cdot d(GAA)_n$, $d(TC)_n \cdot d(GA)_n$, $d(TCC)_n \cdot d(GGA)_n$ and $dC_n \cdot dG_n$ all show a transition in the presence of 1mM $MgCl_2$, pH<6 to a new structure characterized by increases in T_m and buoyant density and the uptake of one proton/8 nucleotides for $d(TC)_n \cdot d(GA)_n$. The kinetics of formation of the new complex are strongly concentration dependent. A specific and compact tetraplex can be made by, e.g. adding $d(GA)_n$ to the remaining space in the major groove of the triplex $d(TC)_n \cdot d(GA)_n \cdot d(TC)_n$. Double isotope and buoyant density labelling experiments prove the complex contains an even number of strands. A Hoogsteen duplex or conformational change can be eliminated on the basis of the kinetics and that DNAs such as $d(TG)_n \cdot d(CA)_n$ and the random pyr·pur DNA $d(T,C)_n \cdot d(G,A)_n$ do not form new structures. Chemical probes and EM data are consistent with a tetraplex. A biological role is suggested by the in vitro replication of pyr tracts from L cells to give pyr·pur DNAs of repeating sequence with the same properties as the synthetic repeating polymers. The putative tetraplexes, once formed, are stable at pH 7 in the presence of Mg^{++}. The formation is strongly inhibited by intercalating agents such as ethidum and m-AMSA. The latter has recently been shown to act in G2 phase, possibly on chromosome condensation (Baguely and Ralph). Supported by MRC Canada)

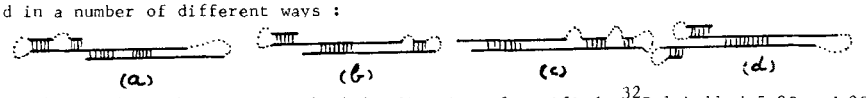
275 PRESENCE OF mRNA SEQUENCES IN HIGH MOLECULAR WEIGHT hnRNA. William E. Hahn, John A. Bantle & Ian H. Maxwell, Dept. of Anatomy, Univ. of Colo. School of Medicine, Denver, Colo. 80262

Identification of primary transcripts (PTs) for mRNA is fundamental to understanding the organization and regulation of the eukaryotic genome. Ovalbumin and silk fibroin mRNAs are apparently about the same size as their putative nuclear RNA precursors, but the precursor of globulin mRNA appears to be 2-3 times larger than the functional mRNA. The size relationship between the diverse, lower copy frequency mRNAs and their respective PTs is not known. To determine whether high molecular weight hnRNA molecules contain sequences homologous to those found in poly(A)mRNA, we prepared a probe DNA, "mDNA". mDNA was obtained by hybridizing labeled unique sequence DNA to poly(A)mRNA prepared from mouse brain polysomes. Poly(A)mRNA in the size range of 2000 NT or less was used. The complexity of the mDNA was equal to about 35,000 different average size mRNAs. mDNA incubated with hnRNA molecules which were mostly 5000 NT or greater in length (number ave. size=9000 NT) hybridized to nearly the same extent as was observed in "back" hybridization with poly(A)mRNA. The Cot ½ was greater than when poly(A)mRNA was used as driver, but this is expected since many sequences not complementary to the mDNA are present in hnRNA. Electronmicroscopy indicated little (2% of mass) size overlap between the hnRNA and poly(A)mRNA used in these experiments. Therefore, we conclude that hnRNA molecules 5000 NT or greater contain most of the sequences present in a diverse population of mRNA. These results imply that PTs are, in general, considerably larger than respective mRNAs. It is not known whether hnRNA molecules contain more than one mRNA sequence.

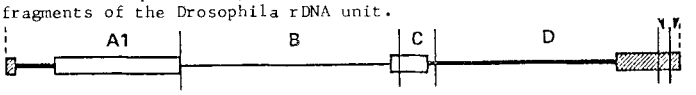
276 LOCALIZATION OF THE SEQUENCES CODING FOR 5.8S AND 2S RNAS ON A CLONED DROSOPHILA rDNA REPEATING UNIT.

B.R. JORDAN and D.M. GLOVER, CBM/CNRS, Marseille, France and Imperial College, London, England

Mature Drosophila 26S rRNA consists of four polynucleotide chains (18S, 18S, 5.8S and 2S) held together by hydrogen bonding. This structure arises through post-transcriptional processing of an originally continuous RNA molecule. The four polynucleotide chains could be arranged in a number of different ways :



We have solved this question by hybridization of purified, ^{32}P-labelled 5.8S and 2S RNAs to cloned fragments of the Drosophila rDNA unit.



Saturation hybridization experiments show that one copy of 5.8S and one copy of 2S hybridize to fragment A1 (and fragment A1 only) thus eliminating model (d). Further digestion of the rDNA unit by restriction enzymes Sma and Hae III followed by agarose gel electrophoresis and Southern hybridization to either 5.8S or 2S RNAs have shown that both these RNAs hybridize to the "transcribed spacer" region between 18S and 26S, thus eliminating models (b) and (c).

277 DYNAMICS OF RNA POLYMERASE B DISTRIBUTION IN POLYTENE CHROMOSOMES,

Arno L. Greenleaf, Milan Jamrich, and Ekkehard K.F. Bautz, University of Connecticut, Storrs, CT and University of Heidelberg, Germany.

Indirect immunofluorescence data to be presented show that RNA polymerase B (or II) is found almost exclusively in interbands and puffs in Drosophila melanogaster salivary gland polytene chromosomes, while histone H1 is found essentially only in bands. Following "heat shock," RNA polymerase molecules redistribute, becoming associated with the newly induced puffs and dissociating from previously active sites.

Results of studies in progress on RNA polymerase B distribution during puff formation and regression as influenced by various experimental manipulations, as well as a model of the functional organization of Drosophila polytene chromosomes, will also be presented.

278 ISOLATION AND CHARACTERIZATION OF NUCLEI AND CHROMATIN FROM NEUROSPORA CRASSA, J.A. Hautala and N.H. Giles, Genetics Program and Department of Zoology, University of Georgia, Athens, GA 30602

A procedure has been developed for isolating nuclei from either the conidial or germinated conidial growth phase of Neurospora crassa. The conidia were lysed by a French press of a frozen cell suspension, and the nuclei were freed from the broken cells by repeated homogenization in an Omni-mixer. Pure nuclei were obtained from the crude nuclear fraction by density banding in a Ludox gradient. The final nuclear yield was 20-30%. The nuclei had a DNA:RNA:protein ratio of 1:3.5:7 and were active in RNA synthesis. The nuclei, stained with the fluorescent DNA stain DAPI, appear under fluorescence microscopy as bright blue spheres, 1 μ in diameter, essentially free of cytoplasmic attachments. Chromatin extracted from the nuclei in 70-75% yield by dissociation with 2 M sodium chloride and 5 M urea had a DNA:RNA:protein ratio of 1:1.05:1.7. Chromatin reconstituted from this preparation exhibited a level of RNA polymerase template activity lower than that of pure Neurospora DNA, but the maximum degree of reconstitution obtained was only 10%. Fractionation of Neurospora chromatin on hydroxylapatite separated the histones from the chromatin acidic proteins. The normal complement of histone proteins was present in both the reconstituted and dissociated chromatin preparations. The acidic protein fraction exhibited a variety of bands on sodium dodecyl sulfate gel electrophoresis ranging in molecular weight from 15,000 to 70,000. The gel pattern was much more complex for total dissociated chromatin than for reconstituted chromatin.

279 SMALL STABLE RNA MOLECULES IN THE NUCLEUS: POSSIBLE MEDIATORS IN GENE EXPRESSION, David Apirion, Bikram S. Gill and Uma S. Podder, Washington University Medical School, St. Louis, MO 63110

To probe the possibility that small stable nuclear (ssn)RNAs are involved in gene expression, first we tested how much of these molecules are retained in chromatin. We found that large quantities (as much as 10,000 molecules/nucleus), are retained in chromatin from mouse L cells prepared by three different procedures. In order to achieve differential gene expression mediated by ssnRNA it is only necessary to assume different distributions of these molecules along the genome (association with middle repetitive DNA). Thus neither qualitative nor quantitative differences in ssnRNA patterns among different cell lines from the same organism are required to achieve specificity. We compared the ssRNAs from a large number of cell lines from a number of mammalian species. While species specificity was detected, diverse lines from the same species have qualitatively similar molecules. Quantitative analysis suggests that differences in number of specific molecules per nucleus exist between lines. However, when the ssRNA of organs were compared, (liver and kidney from mouse) they show great similarities but not identical patterns. These patterns are radically different from those observed in mouse cell lines. Moreover, in the regenerating liver new ssRNA molecules appear. Thus, while these experiments do not establish the function of ssRNA molecules, they suggest that they could have a role in gene expression. Most important, these experiments do not support the prevailing concept that all these molecules have a non specific general function, such as being part of a nuclear backbone, or participating in transport of RNA within the nucleus, or from the nucleus to the cytoplasm.

280 PHYSICAL MAPPING OF MITOCHONDRIAL GENOME IN MAMMALIAN CELLS. K.Koike, M.Kobayashi, S.Tanaka and H.Mizusawa, Cancer Institute (JFCR), Toshima-ku, Tokyo 170, Japan

We are seeking insights into mechanisms which control the replication and the expression of the genetic informations encoded in the mitochondrial genome in mammalian cells. In recent years, extensive use has been made of bacterial restriction endonucleases in the analysis of DNA. We applied the technique and examined the specific fragments of mtDNA, generated by restriction enzymes. As previously reported [Koike *et al* (1975)], the restriction enzyme EcoRI created six double-stranded breaks at specific sites in the rat mtDNA molecule. On the other hand, two or three restriction sites were obtained in mouse, rabbit and human mtDNAs. By analyzing fragments from pulse-labeled replicative intermediates, it was demonstrated that discontinuous replication of rat mtDNA begins at the fixed origin and proceeds unidirectionally around the circular genome, terminating at a region adjacent to the initiation origin. In this report, we describe our recent results concerning the physical mapping of the mitochondrial genome. Rat mtDNA was cleaved into unique fragments by restriction enzymes EcoRI, HhaI and HindIII from *E.coli*, *H.haemolyticus* and *H.influenzae*, respectively. Sizes of the fragments were determined with data obtained from the electron microscope and the agarose gel electrophoresis. On the basis of these data and estimates of the sizes of the partially-digested products, the physical map was constructed. We also used the technique in a comparative study of mtDNAs of rat, mouse, rabbit and human and found that there was a considerable base-sequence diversion between the species, indicating evolutionary diversification. Further informations about the physical mapping are being accumulated in those mammalian cells. The maps are currently in use for the analyses of *in vitro* plasmid-mtDNA recombinants grown in *E.coli*.

281 INDUCTION OF HEAT SHOCK PROTEINS IN DROSOPHILA MELANOGASTER, L. Moran. Département de Biologie moléculaire, Université de Genève, Geneva, Switzerland.

We have characterized the tryptic digests of proteins induced by heat shock in larval salivary glands and a Kc cell line adapted to grow in suspension. Of the major heat shock polypeptides only six have distinctly different fingerprints and thus are presumably coded for by different genes. The six distinct polypeptides have molecular weights of 84K, 70K, 68K, 26-27K, 23K and 22K daltons on SDS polyacrylamide gels.

Poly(A) containing RNA has been isolated from salivary glands and tissue culture cells. In both cases the RNA induced after heat shock codes for the heat shock proteins in vitro. Polysomal, poly(A) containing RNA which has been purified from heat shocked cells can be resolved into six species on acrylamide gels after removal of the poly(A) tails. Evidence will be presented that each of these messenger RNA's codes for one of the six distinct polypeptides.

282 CARCINOGENESIS IN MOUSE & MAN:A "CONCILIATORY" GENETIC MODEL. John E. Byfield, Div. Radiation Oncology, Univ. Calif., San Diego,Cal. 92103.

A convergence of data is developing for those physico-chemical agents which both cause and cure cancer in rodents and man. Recent studies on in vitro transformation of rodent cells indicate that many or all agents that induce DNA strand breaks can also induce transformation and that the induction of either mutations or DNA excision repair is probably not an obligatory step. Similarly, "turning on" of RNA virus-related gene expression is also not obligatory. However, such cells probably always contain viral information. The extreme difficulty in obtaining unequivocal evidence for viral involvement in human cancer together with the abundant clinical evidence that transformation of human cells by "clean" DNA-damaging agents (anthracyclines,actinomycins, halogenated pyrimidines) is an extremely rare event suggests that a qualitative difference in the mechanism of carcinogenesis may exist for most human versus rodent cells. My "conciliatory" model proposes that rodent cells contain the genetic information requisite for transformation (i.e. the familiar oncogene theory) and that this can be activated by a genetic reorganization induced by simple DNA strand breakage not involving mutation per se. "Chemical" carcinogenesis may therefore occur in rodents by 2 processes:(a) structural gene reorganization needing only endonucleolytic activity or (b) true mutation at the normal oncogene site. In human cells only the latter type occurs since the oncogene is absent. The gene may be the surface protein creating "top & bottom"

283 SELECTION AND CHARACTERIZATION OF ALCOHOL DEHYDROGENASE MUTANTS IN C. ELEGANS, David L. Baillie and Raja Rosenbluth.

Alcohol dehydrogase minus mutants of C. elegans have been screened for using resistance to pentene-3-ol and pentyne-3-ol (both reagents have been successfully employed for this purpose in D. melanogaster by Sofer). Pentene-3-ol would not kill the wild type even at high concentrations, whereas pentyne-3-ol was lethal to wild type after 12 hr incubation in .1% solution of pentyne-3-ol. Using this concentration F_2 individuals from EMS (.05M, 4 hr) treated P_o have been screened and several putative ADH^- mutants have been isolated. Some of these individuals show a sensitivity to ethanol which is analogous to that demonstrated in D. melanogaster. Complementation tests have been performed on these mutants and mapping to linkage group is in progress. It is hoped that selection on ethanol will provide a selective procedure for fine structure mapping and a means by which overproducer mutants for alcohol dehydrogenase may be obtained.

284 USE OF STEROID-RESISTANT VARIANTS TO ANALYZE THE INTERACTION OF STEROID HORMONES AND CYCLIC AMP IN HTC CELLS, Daryl Granner and E. Brad Thompson, Depts. Med. and Biochem. Univ. Iowa and VA Hosp., Iowa City IA 52242, and Lab. Biochem. NCI, NIH, Bethesda MD

Maximal induction of tyrosine aminotransferase (TAT) in intact liver and fetal liver organ culture requires the presence of both glucocorticoid hormones and cyclic AMP. We have studied this process in HTC cells and find that: 1) dibutyryl cAMP (Bt_2cAMP) is ineffective unless the cells have been incubated in inducing concentrations of dexamethasone (DEX) for several hours; 2) removal of DEX results in a rapid loss of response of HTC cells to Bt_2cAMP; 3) steroids which are suboptimal or antiinducers of TAT have like effects on Bt_2cAMP reinduction. This requirement for steroid induction has been explored using steroid-resistant variants of HTC cells that have normal amounts of cytosol receptor which can be activated, translocated into the nucleus, and then bound to chromatin. TAT induction, however, does not result. The following table shows that reinduction also does not occur in these variants.

	TAT Specific Activity			
cell type	S77 Medium		S77 + DEX	
	$-Bt_2cAMP$	$+Bt_2cAMP$	$-Bt_2cAMP$	$+Bt_2cAMP$
HTC wild type	3.9	4.7	30.5	49.7
HTC-M714H	2.0	2.3	2.5	2.4
HTC-719C	4.2	4.4	7.3	5.8
HTC-263E	4.1	3.7	7.0	6.0

Conclusion: These studies suggest that Bt_2cAMP reinduction requires the formation of a product of the steroid hormone, possibly TAT-mRNA. Definition of the site of the defect in the variant cells should help localize the site of steroid-cAMP interaction.

285 MUTATIONS OF S. CEREVISIAE CAUSING OVERPRODUCTION OF DIHYDROFOLATE REDUCTASE, Christopher G. Goff, Judith Fox, and Gary Otto, Haverford College, Haverford Pa.

Dihydrofolate reductase (FH_2Rase) is required for synthesis of tetrahydrofolate, and thus for one-carbon transfer reactions, in essentially all organisms. Thus folate analogs like aminopterin, which specifically inhibit FH_2Rase, block growth of normal cells. Mutants resistant to folate analogs can be selected, and in both bacteria and mammalian cells the mutations usually affect FH_2Rase, either by altering its active site (thus preventing folate-analog binding) or by causing overproduction of FH_2Rase (thus titrating out the intracellular analog). At least in bacteria, this enzyme may regulate its own synthesis; many mutations appear to affect folate-analog binding and FH_2Rase synthesis simultaneously.

We are attempting to select both these types of FH_2Rase mutants in haploid S. cerevisiae, with the aim of studying mechanisms regulating gene expression in a genetically well-defined eucaryote. Yeast cells are relatively impermeable to folate analogs, so we are using indirect methods. We mutagenize cells, enrich for FH_2Rase mutants by growing out in inhibitory levels of aminopterin, plate, and assay colonies in situ for FH_2Rase activity using a coupled dye-reduction color assay. To date we have isolated several low-level overproducers (3-fold more activity than wild type). We hope eventually to genetically map "overproducer" and "analog resistant" mutations in detail and to physically map the FH_2Rase protein. We should then be able to locate regulatory mutations relative to the structural portion of the FH_2Rase gene. We have purified wild-type yeast FH_2Rase and are beginning peptide mapping and immunological studies.

210 GENE EXPRESSION DURING EARLY DEVELOPMENT OF C. ELEGANS, David Hirsh, Department of M.C.D. Biology, U. of Colorado, Boulder, CO. 80309

Caenorhabditis elegans is a self-fertilizing hermaphroditic nematode. True males occur spontaneously and are used for genetic crosses. Self and cross fertilizations occur internally. Embryogenesis from fertilization to a 500 cell larva occurs within an egg case. Zygote defective mutants die during embryogenesis prior to emergence of the larva. We have studied genetically 25 temperature sensitive zygote defective mutants to determine the times of expression of genes whose products are required for completion of embryogenesis. In 21 of the 25 mutants, homozygous progeny of heterozygous parents complete embryogenesis at restrictive temperature indicating that parental gene expression is sufficient for survival of zygotes. In 10 of these 21 mutants, progeny from homozygous mothers mated to wild type fathers die as zygotes. Therefore, these 10 mutants are strict maternals. Parental genome expression must occur for zygote survival. In the remaining 11 mutants, zygotes from homozygous mothers are rescued by wild type fathers. Therefore, in these 11 mutants, either parental or zygotic genome expression allows completion of embryogenesis. In 2 of these 11 mutants, paternal genome expression supplies a sperm cytoplasmic factor that rescues homozygous zygotes, since heterozygous fathers mated to homozygous mothers produce both homozygous and heterozygous viable progeny. Among the 4 mutants that cannot complete embryogenesis as a result of parental genome expression, 3 mutants are non-maternals strictly dependent on zygotic genome expression. Only one mutant produces progeny that die in both of the original genetic tests. Therefore, zygote survival requires both parental and zygotic genome expression.

Temperature-shift experiments so far show that all strict maternal mutants are temperature sensitive before the 16 cell embryonic stage, strict non-maternals are temperature sensitive late in development, and mutants dependent on either parental or zygotic genome expression are temperature sensitive after the 16 cell stage except in the two cases where sperm cytoplasm rescues the zygotes. These two mutants are temperature sensitive early in embryogenesis.

286 STUDIES ON TWO BODY-WALL MYOSINS IN WILD TYPE AND MUTANT NEMATODES, Frederick H. Schachat, Robert L. Garcea and Henry F. Epstein, Stanford U., Stanford, CA 94305

Native myosin purified from the wild-type, N2, and a body-wall defective mutant, E675 of the nematode contains two myosins, each homogeneous for different heavy chains. These myosins can be resolved from one another on hydroxyapatite and, when cleavaged with CNBr, they yield different peptide-fragments. The ratio of the two myosins is about 2:1 in E675 and is similar in N2.

In E190, one of the homogeneous myosins is absent. e190 and e675 are alleles of the same gene, unc-54. The myosin lacking in E190 is the same one affected in E675. This suggests that unc-54 is the structural gene for a myosin heavy chain.

In order to determine the role of these different myosins, we are using antibodies to locate the myosins on thick filaments from body-wall muscle. Additionally, we are studying the patterns of synthesis and degradation of the two myosins in the wild-type and muscle-defective mutants in order to discover how the observed stoichiometry is maintained.

287 ON STUDYING RECESSIVE LETHAL AND STERILE MUTANTS OF CAENORHABDITIS ELEGANS, R. K. Herman and P. M. Meneely, Dept. of Genetics & Cell Biology, University of Minnesota, St. Paul, MN 55108

We are concentrating on the isolation and genetic characterization of recessive lethal and sterile mutants of C. elegans in the hope that the anatomical and biochemical study of these mutants will tell us something about the genetic control of development. Obviously, for maintenance and genetic manipulation, recessive lethal and sterile mutations must be balanced in suitably marked heterozygotes. We are thus also engaged in the production and identification of balancer chromosomes, which generally carry chromosome rearrangements in order to suppress crossing over. Both X-rays and triethylenemelamine have been used to generate chromosome rearrangements. One of the rearrangements, a duplication of the X chromosome translocated to linkage group V, is being used to balance EMS-induced lethals and steriles falling in a small region of X. We have thus far found 12 independent lethals and steriles in this region. The mutations have been classified into 10 complementation groups, with repeats in 2 genes. The phenotypes of the mutants are varied. Some die during embryogenesis. Others are arrested at various larval stages. One becomes an adult but is sterile because of a sperm defect. Still another is fertile, but all of its offspring die as larvae. We have also selected directly for suppressors of crossing over in linkage group II after X-ray treatment and have used one such crossover suppressor to balance several dozen EMS-induced recessive lethal and sterile mutations.

288 EMBRYONIC DEVELOPMENT OF THE INTESTINAL TRACT, K. K. Lew. S. Ward Dept. of Biological Chemistry, Harvard Medical School, Boston, Mass. 02115

The entire embryonic cellular lineage which give rise to the intestine of C. elegans, a free living soil nematode, has been determined by light microscopic observation from the fertilized egg to the newly hatched animal. The cells which give rise to the intestine undergo both symmetrical and assymetrical cellular divisions. Besides cellular lineage analysis the cells which produce the intestine were studied for biochemical differentiation. It appears that the gut cells biochemically differentiated well before the intestinal tract is formed. The completely developed embryonic intestinal tract is composed of 20 cells and the time course for this development is about 9 hours at 20-22°C. The analysis of wild type development will provide us with the background in the study of gut embryonic mutants.

289 A GENETIC PATHWAY FOR DAUER LARVA FORMATION IN CAENORHABDITIS ELEGANS, Donald L. Riddle, Division of Biological Sciences, University of Missouri, Columbia, Missouri 65201.

The dauer larva is a specialized third-stage larva formed under conditions of starvation. It neither feeds nor grows; it possesses a specialized impermeable cuticle and exhibits a unique morphology and behavior. If food is encountered, the dauer larva is capable of recovery to resume development. A series of mutants affected in dauer larva formation is being used to determine the nature of the genetic program for this discrete aspect of development. Mutant genes have been ordered with respect to one another in a genetic pathway based on analysis of the epistatic relationships between the genes. The genes specify a signal pathway for dauer larva formation. One class of mutant already characterized exhibits morphological defects in specific sensory neurons as determined by electron microscopy.

290 INTERACTIONS BETWEEN THE MITOCHONDRIAL AND NUCLEAR GENOMES IN NEUROSPORA, David L. Edwards, Dept. Biol. Sciences, State University of New York, Albany, N.Y. 12222

The cyanide-insensitive respiratory system of Neurospora mitochondria requires genetic input from both the mitochondrial and nuclear genomes in order to function correctly. Mutations in either genome can cause abnormal function of the pathway. A series of nuclear mutants has been isolated that cannot produce the cyanide-insensitive pathway under conditions where it is induced in wild-type cells. These mutants cannot grow in the presence of antimycin A or azide whereas wild-type cells grow by means of the cyanide-insensitive pathway in the presence of these drugs. These antimycin A-sensitive mutants can be assorted into at least two groups by complementation analysis.

An extranuclear mutant, cni-3, has been isolated which has the cyanide-insensitive pathway present constitutively. Mitochondria from cni-3 have normal cytochrome content, NADH-and succinate oxidases and normal oxidative phosphorylation. Titration of cni-3 with cyanide and salicyl hydroxamic acid indicates that the cyanide-insensitive pathway in these cells is not utilized in vivo.

These data support a model for the regulation of the cyanide-insensitive pathway based on a mitochondrial gene product acting in a negative manner to regulate structural genes for the pathway located in the nucleus. Preliminary evidence indicates that this regulation is at the level of the transcription of nuclear DNA. (Supported by research Grant GM-19410 and Research Career Development Award GM-00018 from NIH.)

291 THE PROPOSED INVOLVEMENT OF AN INTERNAL PROMOTER IN REGULATION AND SYNTHESIS OF THE MITOCHONDRIAL AND CYTOPLASMIC LEUCYL-tRNA SYNTHETASES OF NEUROSPORA, S.R.Cross, Patricia Beauchamp and Edith Horn, Genetics Division, Biochem. Dept. Duke University, Durham, N. C. 27710.

The leu-5 region of linkage group 5 of Neurospora contains the structural information for the cytoplasmic and mitochondrial leucyl-tRNA synthetases. Antigenic analysis indicates that there is little, if any, structural homology between the two enzymes. The synthesis of the two enzymes appears to be reciprocally regulated in response to a regulatory element of mitochondrial origin. These data plus the results of genetic analyses of a temperature sensitive pleiotropic leu-5 mutant and its revertants as well as a variant that produces and electrophoretically distinguishable mitochondrial enzyme suggested a model of the leu-5 region the essential feature of which is the proposal that initiation of transcription of the gene for the mitochondrial enzyme depends upon a structural element (promoter) within the adjacent structural gene for the cytoplasmic enzyme.

292 REGULATION OF ARGININE METABOLISM IN NEUROSPORA, Richard L. Weiss, Biochemistry Division, Department of Chemistry and Molecular Biology Institute, University of California, Los Angeles, California 90024

Neurospora is capable of endogenous arginine biosynthesis. In addition, the organism possesses enzymes capable of degrading arginine to ammonia, carbon dioxide, and glutamic acid. During growth in minimal medium, a large arginine pool coexists with a significant level of the first two degradative enzymes, arginase and ornithine aminotransferase. No catabolism occurs. During growth in arginine-supplemented medium, these enzymes are induced 3-4 fold and the arginine pool expands approximately 7 fold. Catabolism of arginine proceeds rapidly. When arginine is added to cells growing in minimal medium, a 30-40 min lag precedes the appearance of increased levels of the catabolic enzymes. The delayed response appears to represent the time required for transcription, processing, and expression of new mRNA molecules. Catabolism, in contrast to enzyme induction, is initiated and saturates the available enzyme within minutes. Metabolic and regulatory responses to removal of arginine from the growth medium are equally rapid. These observations are consistent with a compartmentation model in which the majority of the intracellular arginine pool is sequestered within a membrane-enclosed organelle. The significance of compartmentation as an influential contributor to metabolic regulation in eucaryotes will be discussed. Particular attention will be paid to molecular approaches which are potentially powerful tools in the continuing effort to understand biological regulatory processes in eucaryotes.

293 AN IN SITU ASSAY FOR CLONED GENE TRANSLATION IN PHAGE PLAQUES AND BACTERIAL COLONIES. A. Skalka and L. Shapiro. The Roche Institute of Molecular Biology, Nutley, N. J. 07110.

A series of in situ immunoassays have been developed which can be used in screening for translation products of genes cloned in in vitro recombination experiments with either phage or plasmid vectors. Antigen-antibody complex formation occurring within a vector phage plaque can be used to detect the production of a specific protein from an amplified gene which is transcribed at normal efficiency. Immunoassays of individual bacterial colonies lysed in situ either by λ prophage induction or by biochemical means afford an even higher level of sensitivity than the plaque assay, probably adequate to detect the production of a few molecules of protein per cell.

294 PREPARATIVE AND ANALYTICAL HORIZONTAL SLAB GEL ELECTROPHORESIS OF DNA, Donald Kaplan and Gary Wilcox, University of California at Los Angeles, Los Angeles, California 90024

Horizontal slab gel electrophoresis systems have many advantages which are not inherent in a vertical slab gel apparatus. We have developed a horizontal apparatus which has the following features: 1) a capacity of 108 samples, 2) lower percentage agarose (0.2%) and polyacrylamide (2%) gels can be used, 3) DNA restriction fragments may be visualized during electrophoresis, 4) large capacity preparative agarose gels up to 2-1/2 cm thick have been used for the purification of DNA restriction fragments, 5) different percentage gels may be run simultaneously, and 6) rapid and simple preparation of the apparatus for use.

The key design feature of the horizontal slab gel apparatus is the use of vertical agarose wicks which replaces conventional, less reliable current carriers. The agarose wicks are re-usable, can carry up to 200 ma, and do not dry out or produce variations in current across the gel.

295 GENE STRUCTURE AND TRANSCRIPTION IN DICTYOSTELIUM, R.A. Firtel, K. Kindle, W. Roewekamp, A. Cockburn, G. Frankel, M. McKeown, W. Taylor and M.J. Newkirk. Department of Biology, University of California, San Diego, La Jolla, California 92093.

We have been using several different cloning techniques to approach the question of gene structure and transcription in Dictyostelium discoideum. The approaches have involved cloning of (1) restriction and randomly sheared nuclear DNA fragments and (2)cDNA copied from mRNA.

The structure of the Dictyostelium ribosomal RNA cistron has been elucidated using hybridization of labelled rRNA to restriction digests of total Dictyostelium DNA and cloning of rDNA fragments. The data from these studies indicate a very large repeat unit (47Kb) which contains the coding regions for the 17S, 26S, 5.8S and 5S RNAs. This repeat is present as an antiparallel dimer; experiments to determine whether or not the region is extrachromosomal are under way. Clones that contain Dictyostelium coding sequences were constructed with randomly sheared DNA, then screened for hybridization to poly(A)$^+$ RNA by the colony filter hybridization technique. We are now studying three clones of nonmitochondrial origin which have been shown to hybridize to a discrete size or class of messages. The location of the mRNA complementary region and repeat and single-copy sequences have been identified. The clones are also being used to examine changes in specific messenger expression throughout the development cycle and to look for the existence of a nuclear precursor to the message. A third approach in the lab is the cloning of the cDNA made from messenger RNA. This tack has the advantage of allowing one to look for clones complementary to RNA that has been pre-selected, e.g., a semi-purified RNA, or a developmental class of RNA. The cDNA clones can then be used as pure probes for screening for DNA clones complementary to the message of interest.

296 HOW MANY DEVELOPMENTAL GENES ARE THERE IN *DICTYOSTELIUM?* William F. Loomis, Univ. of California, San Diego, La Jolla, California 92093.

When considering the dependence and interrelationship of developmental enzymes in *Dictyostelium* it is central to have some idea how many genes may be involved. Several studies have attempted to estimate the number of genes dispensible for growth but essential for aggregation or later stages of morphogenesis. These include determining the number of genetic loci which play major roles in aggregation, the number of RNA transcripts which accumulate significantly during various stages of development, and the mutational target size of developmental genes. Each of these approaches have indicated that fewer than 300 genes may play significant roles during the whole process of development. The various estimates will be compared and discussed.

297 CELL INTERACTION AND GENE EXPRESSION IN DICTYOSTELIUM. Maurice Sussman. Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania.

Progress from the aggregation stage to the slug and from the slug stage to the culmination stage is controlled by diffusable molecules produced within the cell mass. Ammonia concentration appears critical to the slug to culmination transition. Mutant strains unable to progress past the slug stage can be carried through if mixed with wild-type cells or with extracts of such cells. The biochemical basis of this synergistic development will be discussed.

298 USING CLONED DNA TO REVEAL THE GEOGRAPHY OF THE DICYTOSTELIUM GENOME
Nancy Maizels, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Analysis of plasmids which amplify fragments of Dicytostelium DNA is generating a picture of the organization of the Dictyostelium genome. Dictyostelium's ribosomal DNA repeated unit comprises 38,000 base pairs --the longest yet reported; the 17s + 25s coding region is linked to 5s DNA, as in yeast and the prokaryotes. Current work is focusing on regions of DNA which are specifically transcribed during development.

299 PARASEXUAL GENETIC ANALYSIS OF AGGREGATION LOCI IN DICTYOSTELIUM DISCOIDEUM, M.B. Coukell, Dept. Biology, York University, Toronto, Ontario

Preliminary genetic studies on the developmental (agg) mutations in a group of 40 independent aggregation-deficient (Agg^-) mutants of D. discoideum identified 5 loci essential for aggregation (CG1-5) and revealed that a large number of the agg mutations reside in linkage group II (Coukell, M.B.: Molec. gen. Genet. 142, 119, 1975). We have now extended the linkage analysis of these mutations and have assigned agg mutations in 28 of the 40 mutants to 4 linkage groups: 16 in group II, 1 in group III, 10 in group IV, and 1 in group VI. None of the agg mutations analyzed appeared to map in linkage group I. In addition a new temperature-sensitive growth locus, tsgJ, has been mapped in group III. Linkage analysis of the agg mutations was aided greatly by the discovery that diploid strains are readily induced to undergo haploidization when grown on 0.1% p-fluorophenylalanine (PFP) at elevated growth temperatures (e.g. 25.5°C). Growth of diploid strains on PFP had no effect on the type of segregant classes obtained, the subsequent growth and/or development of the segregants, or the ability of the segregants to reform stable diploids. When 5 Agg^- strains carrying agg mutations representing loci CG1-5 were fused with 5 strains carrying mutations at aggregation loci identified in another study (Williams, K.L., Newell, P.C.: Genetics, 82, 287, 1976), all 25 crosses produced Agg^+ diploids. This result suggests that the 10 aggregation loci identified in the two studies are different. These loci have been designated aggA-J. (Supported by the NRC and the NCI of Canada.)

300 RNA POLYMERASE II REGULATION IN AMANITIN RESISTANT RAT MYOBLAST MUTANTS, Mark L. Pearson and Michael M. Crerar, Department of Medical Genetics, University of Toronto, Toronto Canada M5S 1A8.

A series of independent diploid and tetraploid α-amanitin resistant mutants of L6 rat myoblasts have been shown to possess both a mutant form of RNA polymerase II defective in amanitin binding, and wild type enzyme. Mutant cells grown in the presence of amanitin respond by maintaining their total RNA polymerase II activity levels constant. Inactivation of the wild type enzyme under these conditions is compensated by an increase in the specific activity of the mutant enzyme. Determination of the amount of ^3H-γ-amanitin bound to the wild type form when these cells are grown in amanitin serves to measure the amount of this enzyme remaining. Inactivation of this amanitin binding capacity declines in parallel with the loss of nucleotide incorporating activity; the intracellular concentration of the wild type enzyme falls. These results are consistent with the possibility that RNA polymerase II levels are regulated in mammalian cells, as in bacteria, by RNA polymerase acting as a repressor of its own synthesis.

Surprisingly, some of the amanitin resistant myoblast mutants also appear to be defective in myotube formation, and the severity of the defect can be correlated with the level of mutant RNA polymerase II activity. Perhaps mutations in RNA polymerase II structural genes can lead to pleiotropic defects specific for the transcription of genes essential to myotube formation as opposed to those essential for cell proliferation.

301 ISOLATION AND CHARACTERIZATION OF RESPIRATION-DEFICIENT CHINESE HAMSTER CELL Mutants, I. E. Scheffler, G. Ditta and K. Soderberg. Dept. of Biology, University of California, San Diego, B-022, La Jolla, Calif. 92093.

Based on the nutritional requirements of a fortuitously isolated Chinese hamster cell line with a defect in NADH-coenzyme Q reductase which has been characterized by our laboratory (DeFrancesco et al., J. Biol. Chem. 251:4588-4595, 1976) we have devised a selection scheme which has permitted us to isolate a whole series of mutants defective in oxidative energy metabolism. Two such mutants have now been characterized further: 1) Mutant CCL16-B9 appears to be severely deficient in succinate dehydrogenase (SDH) activity, based on the following observations: a) Oxygen consumption by isolated mitochondria could be stimulated by α-glycerolphosphate but not by succinate, b) several assays of SDH in isolated mitochondria with either $Fe(CN)_6^{-3}$ or phenazine methosulfate (PMS) as electron acceptors were negative, in contrast to the experiments with wild type mitochondria. 2) Mutant V79-G7 has a defect which leads to a significant reduction of mitochondrial protein synthesis, as seen by polyacrylamide gel electrophoresis of selectively labeled proteins. The activities of two mitochondrial enzyme complexes are reduced at least tenfold relative to wild type: cytochrome oxidase, and the oligomycin-sensitive ATPase. Spectrophotometrically cytochrome a and a_3 are not detectable.

Additional mutants are under study which belong to different complementation groups as studied by somatic cell hybridizations.

302 CHARACTERIZATION OF CHO-CELL MUTATIONS FOR SIX DIFFERENT AMINOACYL-tRNA SYNTHESES, Larry H. Thompson, Gerald M. Adair, Don J. Lofgren, and Patricia A. Lindl, Biomed. Sciences Div., Lawrence Livermore Laboratory, P.O. Box 808, Livermore, CA 94550

We have been developing selection procedures for the isolation of conditionally lethal mutations affecting protein synthesis in Chinese hamster CHO cells. The method enriches for mutants by treatment of a population with ^{3}H-a.a. under restrictive conditions (39.5°C and reduced a.a. concentrations). In initial growth tests, most mutants had an increased requirement at 34° and/or 39.5° for one of the following a.a.: arg, asn, gln, his, met, or leu. A few of the mutants were simply temperature sensitive. Biochemical characterization of the 6 phenotypic classes revealed defects in each aminoacyl-tRNA synthetase (RS) cognate for the phenotypic modifier a.a. Mutants in each class had reduced recoverable activity in vitro for extracts prepared from cells grown under permissive conditions (34°; normal a.a.). In addition, most mutants tested had impaired aminoacylation in vivo under restrictive conditions which inhibited protein synthesis. One mutant, Arg-1, lacked any measurable ArgRS activity in vitro (<0.4% of WT). In thermolability studies in progress, the GlnRS activity at 34° of the mutant Gln-2 was only 16% of WT and was inactivated very rapidly at 40° compared to WT. Cell-hybrid complementation analysis has defined 6 genetic classes corresponding to the 6 RS classes. LeuRS and AsnRS mutants were by far the most common. There were 4 different mutants in the MetRS class, and 3 mutants for GlnRS with different properties. Only 1 mutant each was obtained for HisRS and ArgRS. All of the mutants are genetically stable and probably represent point mutations in the structural genes of the RSs. (Supported by U.S.ERDA Contract No. W-7405-ENG-48).

303 GENETIC CONTROL OF ARGININOSUCCINATE SYNTHETASE IN HUMAN LYMPHOBLASTS. Joseph D. Irr & Lee B. Jacoby, Genetics Unit, Mass General Hospital & Dept. of Pediatrics, Harvard Medical School, Boston, Ma. 02114.

The activity of argininosuccinate synthetase, one of the arginine biosynthetic enzymes produced by established lines of human lymphoblasts, shows marked derepression when cells are transferred to an arginine-deficient medium. This effect was seen in cells derived from normal individuals and in those of a person deficient in argininosuccinate lyase, the next enzyme of the pathway. The derepression was prevented by cycloheximide suggesting that protein synthesis is necessary for the response. The fully repressed level of the enzyme was obtained by the re-addition of arginine to the growth medium and partial repression occurred in normal cells growing in medium containing citrulline in place of arginine. Two types of variant clones isolated from normal cells by different selection systems have synthetase activities which are not repressed by arginine as extensively as it is in normal cells. One clone has an activity nearly 200-fold greater than its progenitor when both are grown in the presence of arginine. The synthetase activity was correlated with the amount of specific synthetase protein present in the various cells and in normal cells grown under conditions of synthetase repression and derepression and found to be in good agreement. These results are compatible with an hypothesis suggesting that arginine controls the rate of expression of the genes coding for synthetase, and the variants appear to be regulatory gene mutants.

304 CYCLIC AMP-INDUCED CYTOLYSIS IN S49 CELLS: SELECTION OF A "DEATHLESS" MUTANT, Philip Coffino and Irma Lemaire, University of California, San Francisco, CA 94143
Wild type S49 lymphoma cells respond to cyclic adenosine 3', 5'-monophosphate (cAMP) by inducing cAMP phosphodiesterase, halting growth in the G_1 phase of the cell cycle and subsequently dying. By using a counter selection procedure, we have isolated a new class of mutants of S49 cells termed "deathless" that are resistant to cytolysis, but otherwise respond like the wild type cells to cAMP. Upon removal of the cyclic nucleotide, D^- cells resume their normal growth. Unlike all other cAMP resistant mutants of S49 cells isolated until now, the D^- mutant has a functionally normal cAMP-dependent protein kinase and retains normal ability to induce phosphodiesterase and arrest cell growth in G_1. It is probable that the altered gene product of the D^- mutant is distal to protein kinase and in a biochemical pathway separate from that of cAMP induction of phosphodiesterase or growth arrest. The D^- mutant should facilitate studies of the mechanism of cAMP-induced cytolysis and growth regulation in S49 cells.

305 CONTROL OF INTRACELLULAR PROTEIN DEGRADATION BY THE CELL CYCLE ARREST SIGNAL AND INHIBITION OF PROTEIN SYNTHESIS IN SACCHAROMYCES CERVISIAE. Terrance Cooper, Dept. of Life Sciences, University of Pittsburgh, Pittsburgh, PA 15260.
Many eucaryotic organisms control their cell division cycle in response to the availability of organic and inorganic nutrients. Saccharomyces cerevisiae responds to deprivation of a nitrogen source by arresting cell division at the unbudded G1 stage. Cells situated outside of G1 at the time of deprivation, complete the cycle before arresting. This prompted us to investigate the source of nutrients used by these cells to complete division and the mechanisms controlling nutrient availability. We have found a close correlation between accumulation of unbudded cells and loss of previously formed allophanate hydrolase activity following nitrogen starvation. These losses are not specific to the allantoin system, since similar degradation can also be seen when cellular protein levels are monitored with ^{3}H-leucine. Observations of Unger and Hartwell, that arrest of the cell division cycle results from the loss of an unidentified event occurring subsequent to charging of tRNA, lead us to determine whether or not protein degradation was elicited by an inhibition of protein synthesis initiation. We have observed such degradation upon adding cycloheximide (10 µg/ml) to a culture of Saccharomyces. At this concentration, cycloheximide has been shown (J. Biol. Chem. 251:7278) to specifically inhibit initiation of yeast protein synthesis. These data raise the possibility that degradation of intracellular proteins may be another cellular process controlled by the G1 arrest signal. Concurrent onset of protein degradation and cell cycle arrest likely represents a protection mechanism providing cells with an internal means of obtaining nutrients needed to complete cell division in the face of an adverse external supply of these compounds. Supported by Grants GM19386 and GM20693.

306 CLONING AND CHARACTERIZATION OF A COMPLEX SATELLITE DNA FROM DROSOPHILA MELANOGASTER, Marian B. Carlson and Douglas L. Brutlag, Dept. Biochem., Stanford Med. Ctr.,Stanford, CA 94305
A highly repeated satellite DNA of Drosophila melanogaster, called the 1.688 satellite from its buoyant density, was found to have a much higher complexity than the other simple-sequence satellites. The 1.688 satellite separated poorly from the bulk of the DNA in various buoyant density gradients, and the fraction recovered was only 77% pure. Isolation of 1.688 satellite sequences was achieved by molecular cloning in the plasmid vector pSC101. Digestion of 1.688 satellite DNA by either HaeIII or Hinf restriction enzymes generates a series of fragments with lengths which are integral multiples of about 365 base pairs (monomer, dimer, and so on; Manteuil et al., Cell 5, 413, 1975; Shen et al., Nucleic Acids Res. 3, 931, 1976). Digestion of one of the hybrid plasmids with either enzyme produces 16 monomer fragments from the inserted satellite DNA. This cloned satellite DNA has been used as a hybridization probe to detect homologous fragments present in low yield among the HaeIII or Hinf digestion products of gradient-purified satellite DNA. The presence of long oligomers resistant to cleavage indicates that some regions of satellite DNA have many fewer recognition sites than expected from a random distribution of sites. Two lines of evidence suggest a complexity of 365 base pairs for the 1.688 satellite. First, besides the HaeIII and Hinf sites, two sites for the AluI restriction enzyme were also found to be spaced at 365 base pair intervals. Second, satellite DNA renatures with the HaeIII sites in register. In its complexity, the 1.688 satellite bears more resemblance to mammalian satellites than to the simple-sequence satellites of D. melanogaster. (Supported by NIH-GM21498 and National Foundation 5-64)

307 SIMPLE TECHNIQUES FOR THE MANIPULATION OF NEUROSPORA "SLIME". Robert E. Nelson, University of Nebraska, Lincoln, Nebraska 68588. Genetic and microbiological techniques have been developed for handling the cell wall-less variant of Neurospora crassa commonly known as "slime". Slime cells are multinucleated protoplasts and, unlike all other eucarvotic protoplasts, can be routinely maintained as a self-propagating microorganism. In liquid medium, growth is exponential; on solid medium, growth is clonal, and therefore, protoplasts can be easily mutagenized, cloned, and replica-plated. Protoplasts can be bred to filamentous strains of N. crassa with recovery of protoplast progeny of any desired genotype. Unlike walled plant cells and many animal cell types, the osmotically-sensitive protoplasts are particularly suitable for cell fractionation; for example, clean, apparently intact nuclei can be rapidly isolated in quantities necessary for biochemical studies. Protoplasts can be induced to fuse with each other or to fuse with phospholipid vesicles, and protoplast-vesicle fusion apparently can be used to transfer macromolecules into the protoplasts to assay their biological effects. These properties indicate that slime protoplasts will be a useful tool for certain molecular approaches to the study of gene control in a simple eucaryote.

308 THE IN VITRO SYNTHESIS AND PROPERTIES OF THE OVALBUMIN STRUCTURAL GENE, John J. Monahan, Larry McReynolds, Savio L.C. Woo, Charles D. Liarakos, and Bert W. O'Malley, Dept. of Cell Biology, Baylor College of Medicine, Houston, TX., 77030.

Using purified ovalbumin mRNA, a complete (1850 NT) double-stranded complementary DNA ($ds\text{-}cDNA_{ov}$) was synthesized using AMV reverse transcriptase. The $ds\text{-}cDNA_{ov}$ had a high Tm on hydroxylapatite (89°C), and had a bouyant density in CsCl gradients of 1.700 gms/cm^3. It renatured after heat denaturation, with a $C_0t_{1/2}$ value of less than 2 x 10^{-6} moles sec. l^{-1}. When the closed loop of $ds\text{-}cDNA_{ov}$ was opened with S_1 nuclease, the denatured complementary strands then renatured with the appropriate second order kinetics and a $C_0t_{1/2}$ value of 1.89 x 10^{-3} moles sec. l^{-1}. We have used this full-length, $ds\text{-}cDNA_{ov}$ to assay the action of 35 restriction endonucleases upon this DNA sequence. Some 22 enzymes: Bal I, Bam I, Blu I, Hsu I, Uba II, Xba I, Taq I, Sal I, Bgl I, Bgl II, Xho I, Kpn I, Sst II, Hae II, Hpa I, Hap II, Hha I, Hind II, Hind III, Hin II, Eco RI, and Sma I failed to cut the $ds\text{-}cDNA_{ov}$. However, 13 enzymes did cut the DNA. They were: Hae III, Pst I, Xma II, Sst I, Hga I, Xho II, Hph I, Eco RII, Hinf I, Mnl I, Mbo I, Mbo II, and Alu I. A detailed map of the restriction enzyme recognition sites for eleven of these enzymes was determined. Since the amino acid sequence for the first 85 N-terminal amino acids of hen ovalbumin is known, it was therefore possible to construct a partial nucleotide sequence corresponding to this peptide fragment. Such a theoretical sequence would be cut by the three enzymes, Eco RII, Hinf I, and Hph I, in close proximity to each other. Since these three enzymes did in fact cut the $ds\text{-}cDNA_{ov}$ in close proximity, we have therefore, tenatively assigned the coding portion of the ovalbumin gene (1161 NT) to a region which begins least 221 nucleotides from the 5'-terminal end of the gene.

309 CLONING OF INTERSPERSED REPETITIVE DNA SEQUENCES FROM THE SEA URCHIN USING SYNTHETIC EcoRI SEQUENCES, Richard H. Scheller, Terry L. Thomas, Amy S. Lee, William H. Klein, Walter D. Niles, Richard E. Dickerson, Roy J. Britten, Eric H. Davidson, California Institute of Technology, Pasadena, CA 91125.

Chemically synthesized EcoRI cohesive ends were added to repetitive DNA from the sea urchin by means of blunt end ligation. Interspersed repetitive DNA sequence was isolated by reassociation of sheared 2000 nucleotide DNA from sperm to C_0t 40, followed by destruction of nonrepetitive single-stranded tails with SI nuclease. T4 DNA ligase was used to add a symmetrical decamer containing the EcoRI endonuclease recognition site to the repetitive DNA sequences. After ligation, fragments terminated by cohesive RI ends were generated by cleavage with EcoRI. These sequences were inserted at the RI site in the plasmid vector RSF2124 by conventional means, and cloned. Since each cloned repetitive sequence terminates in RI sequences, they may be excised from the plasmid DNA at will by treatment with EcoRI endonuclease. These clones will provide a source of individual repetitive DNA sequences with which to probe the features of the eucaryotic genome. This method of cloning requires the addition of only a few base pairs to the original DNA and allows the amplification of any DNA sequence while providing the opportunity to recover the cloned sequence from the vector.

310 INSERTION AND TRANSCRIPTION OF OVALBUMIN GENE SEQUENCES IN CHIMERIC BACTERIAL PLASMIDS
Larry McReynolds, James F. Catterall, Winston Salser, Robb Moses, and Bert W. O'Malley.
Double-stranded ovalbumin DNA was amplified and purified by the cloning of bacterial transformants. The double-stranded DNA was synthesized from a complete complementary DNA transcript of ovalbumin mRNA using avian myeloblastosis virus reverse transcriptase and the self-priming ability of the initial transcript. After S_1 nuclease treatment, poly(dA) was added to the 3' termini with deoxynucleotidyl transferase and the ovalbumin gene was hybridized to a linear plasmid DNA, pMB9, containing 3' poly(dT) termini. This hybrid molecule was used to transform the E. coli strain X1849. The cloned transformants contained from 30% to 53% of the complete ovalbumin DNA as determined by hybridization with full-length $cDNA_{ov}$. The length of the inserts was confirmed by treatment of the isolated plasmids with the restriction enzyme Hha. Separation of the fragments by agarose gel electrophoresis demonstrated that the amount of inserted DNA in the clones tested varied from 680 to 1090 base pairs. Recent experiments using the full length $cDNA_{ov}$ has resulted in the insertion of a piece of DNA 1850 nucleotides long into X1776. The transcription of chimeric pDNA was studied both in vivo in minicells isolated from X1849. RNA was isolated from the minicells of clone pOv4 and hybridized to DNA probes representing both the coding and anticoding strands of ovalbumin. The results indicated that both the coding and anticoding strands of ovalbumin DNA were transcribed in the intact minicells. The RNA transcribed from the coding strands was complementary to 50% of the inserted ovalbumin DNA. However RNA transcribed from the anticoding strand was complementary to only 20% of the inserted DNA. In contrast, RNA synthesized in vitro using purified pOv4 DNA revealed symmetric transcription of the ovalbumin gene.

311 DETERMINATION OF THE NUCLEOTIDE SEQUENCE OF HUMAN GLOBIN mRNA.
Wilson, J. T., Marotta, C., Forget, B. G., Weissman, S. M., Dept. of Human Genetics Yale University School of Medicine, New Haven, CT 06510.
We have used various methods of RNA and DNA sequencing to analyze the nucleotide sequence of human globin mRNA and/or the cDNA prepared from the mRNA with reverse transcriptase. We have determined the complete sequence of the translated portion of the beta chain mRNA and of the untranslated region between the termination codon and the polyadenylic acid. We have also determined most of the sequence of the translated portion of the alpha globin mRNA and the entire untranslated sequence at the 3' end of this RNA. There are 131 nucleotides between the termination codon to polyadenylic acid and human beta globin mRNA and 109 nucleotides in alpha globin mRNA. Except for the previously noted partial homology between the 3' end of SV40 late mRNA and the beta globin mRNA, and for the common hexanucleotide AAUAAA, there were no clear sequence homologies between the 3' untranslated sequences of alpha-beta SV40 early and SV40 late mRNA. Our nucleotide sequence analysis confirms the fact that the various mutations that lead to single amino acid changes in human hemoglobin could all have arisen by single base changes. In particular, we have demonstrated a substitution of uridine for adenine in the sixth codon of signal cell mRNA. These analyses were performed without the use of cloned DNA but human globin DNA has been cloned in bacteria for other studies.

312 REGULATION OF ALCOHOL DEHYDROGENASE IN THE NEMATODE Panagrellus redivivus.
BURKE, Dan, KRIGER, Frank, and SAMOILOFF, Martin, University of MANITOBA, WINNIPEG, MANITOBA, CANADA.

Exposure of populations of P. redivivus to alcohols results in rapid increases in levels of alcohol dehydrogenase and aldehyde dehydrogenase. The level of alcohol dehydrogenase is regulated at the translational level as demonstrated by inhibitor studies. Mutants with altered enzyme function and regulation support a model involving post transcriptional control of ADH.

314 GENE ACTION AT THE FINAL MOULT OF THE NEMATODE Panagrellus redivivus. SAMOILOFF, Martin, University of MANITOBA, WINNIPEG, MANITOBA, CANADA.

At the final moult in Panagrellus redivivus a requirement for nuclear steroid binding and polyatenylated RNA is first manifested. Specific behaviours associated with reproduction and the setting of the aging-clock also occur at this stage.

315 ISOLATION AND IDENTIFICATION OF IMMUNOGLOBULIN LIGHT AND HEAVY CHAIN GENE SEQUENCES, Leona C. Fitzmaurice, Jacki N. Bennett and Alan R. Williamson, The University of Glasgow, Glasgow G12 8QQ, Scotland.

Our recent research effort has been directed towards the identification and isolation of pure immunoglobulin light and heavy chain gene sequences in cloned recombinant DNAs. Methods for the isolation of heavy and light chain polysomes have been developed and yield polysomes and polyA-bearing RNA which retain their biological activity in cell-free translation. Following fractionation on formamide gels, these RNAs can be eluted to provide partially purified messenger sequences. These partially purified fractions are being used for the preparation of cDNA transcripts from which recombinant molecules will be constructed. Construction of recombinant molecules and their cloning in the Curtiss host X1776 will be carried out at the Microbiological Research Establishment, Porton, England. A summary of progress on this project will be reported.

316 GENETIC CONTROL OF THE SUGAR-NEGATIVE PHENOTYPE IN YEAST, Bruce G. Adams, Univ. of Washington, Seattle, Wash., 98195.

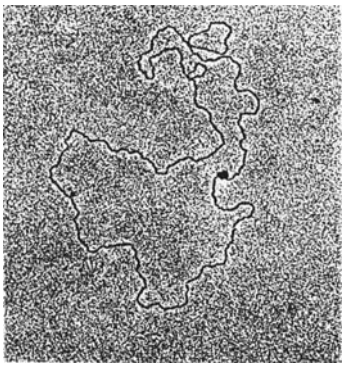
Different yeast strains have been observed to exhibit variable capacities to utilize galactose and maltose when in the respiratory-deficient (RD) state. Other investigators have postulated that the inability to utilize a given sugar when yeast are RD, termed the sugar-negative phenotype, is due to an impairment of requisite mitochondrial protein synthesis or to the presence of the mutant gal 3 locus. The nature of the sugar negative and positive phenotypes when yeast are RD is under investigation using yeast strains which have been characterized with respect to their content of mitochondrial DNA (mDNA), small cytoplasmic circular DNA (cir DNA), large double stranded RNA (LdsRNA), and to the presence of the gal 3 locus. The mutant gal 3 locus has been shown not to influence the sugar phenotype and tetrad analyses of crosses among different phenotypes indicates a complex nuclear segregation. The presence or absence of different combinations of non-mitochondrial genetic determinants has no direct affect on the sugar phenotype and strains lacking mDNA are observed to exhibit both the positive and negative phenotypes. The phenotypes of haploid strains obtained from cytoductant matings suggest the involvement of cytoplasmic factors other than mDNA, cirDNA, or LdsRNA in determining the sugar phenotype.

317 TEMPORAL INDUCTION OF DRUG RESISTANCE AND CELL INACTIVATION BY BUdR AND I-125 UdR IN SYNCHRONOUS HAMSTER CELLS, H. John Burki, Biology and Medicine Division, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720

Mutants resistant to 6 thioguanine and ouabain were induced by BUdR in synchronous Chinese hamster cell strains V79 and CHO. The induction of mutants was a function of the time of incorporation of BUdR into the DNA during the DNA synthesis period. The maximum induction of drug resistance occurred in the early periods of DNA replication before the peak in DNA synthesis. The peak for 6TG resistance occurs first, followed by the OUA resistance peak. The peak for 6TG resistance can be correlated with several bands on the X chromosome which replicate early.

BUdR toxicity is maximum in early replication DNA, while I-125 toxicity is less in the early replication regions, and it increases to a maximum in the later replication DNA, which appears to be centromeric.

318 ASSOCIATION OF HELA CELL MITOCHONDRIAL DNA WITH A MEMBRANE FRAGMENT NEAR ITS ORIGIN OF REPLICATION, Manfred Albring, Jack Griffith and Giuseppe Attardi, Division of Biology, California Institute of Technology, Pasadena, California 91125



Almost all (about 95%) of the mitochondrial DNA molecules released by Triton X-100 lysis of HeLa cell mitochondria in the presence of 0.15 M salt are associated with a single protein-containing structure varying in appearance between a 10-20 nm "knob" and a 0.1-0.5 µm membrane-like patch. Analysis by high resolution electron microscopy and by polyacrylamide gel electrophoresis after cleavage of mitochondrial DNA with the endonucleases Eco RI, Hind III and Hpa II has shown that the protein structure is attached to the DNA in the region of the D-loop, and probably near the origin of mitochondrial DNA replication. The data strongly suggest that HeLa cell mitochondrial DNA is attached in vivo to the inner mitochondrial membrane at or near the origin of replication, and that a membrane fragment of varying size remains associated with the DNA during the isolation. After sodium dodecyl sulfate extraction of mitochondrial DNA, a small 5-10 nm protein is found at the same site on a fraction of the mitochondrial DNA molecules.

319 Isolation and Characterization of a post-translational modification mutation affecting the developmentally regulated glycosidases of Dictyostelium discoideum. Stephen J. Free and Robert T. Schimke, Stanford University, Stanford, Calif. 94305

We have isolated a mutant of Dictyostelium discoideum, M31, which accumulates a reduced number of α-mannosidase-1 molecules per cell during the developmental program of this organism. We find that the responsible mutation, modA, affects the post-translational modification of α-mannosidase-1 and several other developmentally regulated lysosomal glycosidases. These enzymes do not share a common subunit but do share a common post-translational modification system (Dimond & Loomis, 1976). These enzymes are electrophoretically less negative in M31. The modA mutation affects the intracellular levels of these multimeric enzymes differently. The levels of α-glucosidase are unaffected by the presence of the modA mutation. We find only low levels of β-glucosidase-1 activity in M31 cells. The alteration in modification renders β-glucosidase-1 holoenzyme thermolabile and susceptible to degradation in vivo. α-Mannosidase-1 is found at 1/3rd of the wild-type level in the modA mutant. Degradation of holoenzyme does not appear to be responsible for the low level of activity. We propose that α-mannosidase-1 subunits are being degraded prior to subunit assembly. We conclude the modification bestows different properties upon the various glycosidases.

320 MACROMOLECULAR SYNTHESIS THROUGH THE CELL CYCLE OF THE YEAST SACCHAROMYCES CEREVISIAE, Steven G. Elliott and Calvin S. Mclaughlin, Dept. Mol. Biol. & Biochem. University of California, Irvine, Calif. 92715.
Centrifugal elutriation was used to separate the stages of the cell cycle in a culture of yeast in balanced exponential growth. Macromolecular synthesis was examined through the cell cycle. DNA synthesis is periodic but RNA and protein show an exponential increase in rate through the cell cycle. O'Farrell's two-dimensional electrophoretic technique was used to determine the rate of synthesis of individual proteins through the cell cycle. The rate of synthesis of each of 26 proteins examined in detail varies periodically through the cell cycle. Since 11 of the 26 proteins were selected at random from proteins scored as being variable according to visual inspection of autoradiograms, and the other 15 were randomly selected from proteins scored as having a constant rate of synthesis, we suggest that most of the proteins in this eucaryotic organism exhibit periodic changes in their rate of synthesis during the cell cycle.

321 DNA SEQUENCES AND ORGANIZATION OF SEA URCHIN (S. PURPURATUS) HISTONE GENE CODING AND SPACER REGIONS, Irmingard Sures, Ronald Cohn and Lawrence Kedes, Stanford Medical School and Veterans Administration Hospital, Palo Alto, Ca. 94305.
The histone genes of sea urchins are arranged in repeating segments containing one coding region for each of the five histone proteins interspersed with non-coding spacer DNA. The repeat segment in S. purpuratus is 6540 base pairs long and has been cloned in E. coli by plasmid technology. DNA sequences in both coding and spacer regions have been obtained by the methods of Maxam and Gilbert. A detailed map containing over 50 restriction endonuclease sensitive sites was prepared. Restriction fragments were labelled at their 5' ends with P^{32} and subjected to chemically controlled, base specific partial hydrolysis. The resulting fragments were analyzed by gel electrophoresis. Overlaps in several instances and colinearity of sequences in coding regions with the known amino acids of histone proteins confirm the validity of the sequencing technique and the stability of eukaryotic DNA cloned in E. coli. Spacer regions 300-500 base pairs long have been sequenced. Several areas of potential secondary structure have been detected. A polypyrimidine-polypurine $\binom{C-T}{G-A}_{26}$ complex has been identified in the spacer region between the H2A and H1 gene. Several small repeating elements have been detected but are infrequent. Available data on sequences surrounding mRNA initiation and termination sites will be presented.

211 ORGANIZATION OF CODING SEQUENCES IN Drosophila melanogaster, M.W. Young and D.S. Hogness, Department of Biochemistry, Stanford University School of Medicine, Stanford, California, 94305.

We have isolated a collection of cloned DNA segments that contain sequences homologous to the abundant messenger RNAs expressed in cultured Drosophila cells. This collection includes over 150 segments derived from different chromosomal regions. The lengths of these segments are generally much larger than the included structural genes; hence they also contain the DNA sequences that flank these genes. Both unique and repeated structural gene sequences have been found within the collection.

The most abundant species of mRNA produced in cultured cells is about 4,000 nucleotides long and is homologous to a repeated DNA sequence. The repeat is of a dispersed type; an entire messenger-coding sequence is present at each of about 50 widely separated chromosomal sites. Cloned DNA segments from 13 of these sites have been recovered and the mRNA-region in each mapped by hybridization of the mRNA to restriction endonuclease fragments obtained from the segments. Such restriction maps indicate that most sites contain a common mRNA region, although variants do occur and have been cloned. By contrast, the flanking sequences are generally different at the different sites. A similar dispersed topography has been observed for another multigene family that produces another abundant mRNA (Rubin, Finnegan, and Hogness --in preparation). An unusual feature of both families is the presence of like sequences at the 3'- and 5'-ends of the repeated gene.

A less abundant species of mRNA is derived from a non-reiterated segment of DNA. The RNA is 5,000 nucleotides long and its entire complement has been cloned as a part of a 16,000 base pair stretch of Drosophila DNA. All sequences in this segment are confined to a single position in the chromosomes. Hence the flanking sequences are also non-dispersed and may well be unique.

212 GENE ORGANIZATION IN DROSOPHILA, A. Chovnick, Genetics and Cell Biology Section, University of Connecticut, Storrs, CT 06268

Recently, several investigative directions have converged to focus attention upon the structural and functional organization of higher organism genes. Of interest are questions relating genes to chromomeres, dissection of genes into structural and control elements, their functional regulation during development, and their relative mutability. The present report summarizes our progress in the elaboration of one such genetic unit. The rosy locus (ry:3-52.0) in Drosophila melanogaster controls xanthine dehydrogenase (XDH) activity, and is located on the right arm of chromosome 3, within the polytene band region 87D8-12. Originally, the locus was defined by a series of non-complementing, brownish eye color mutants, which are enzymatically inactive. However, recent examination of an enlarged array of such mutants reveals a familiar pattern of allele complementation. In addition to these eye color mutants, rosy locus variants (both spontaneous and induced) include several classes with normal eye color and possessing XDH activity. These include: (a) electrophoretic variants, (b) thermal stability variants, (c) purine sensitives, and (d) variants in level of XDH activity. Pursuing the hypothesis that the rosy locus includes both structural and control elements, experiments will be described which (1) demonstrate that there is but one structural element at rosy, and (2) place genetic boundaries to that structural element in terms of a map of rosy locus variants. Finally, characterization and fine structure mapping of rosy locus variants differing in level of XDH activity will be described. These experiments serve to identify a control region contiguous to the XDH structural element, and characterize the first control element variants.

322 THREE LENGTHS OF THE HISTONE GENE REPEAT UNIT IN THE CHROMOSOMES OF THE SEA URCHIN L. PICTUS, Ronald H. Cohn and Laurence H. Kedes, Stanford Medical School and Veterans Administration Hospital, Palo Alto, California 94304.

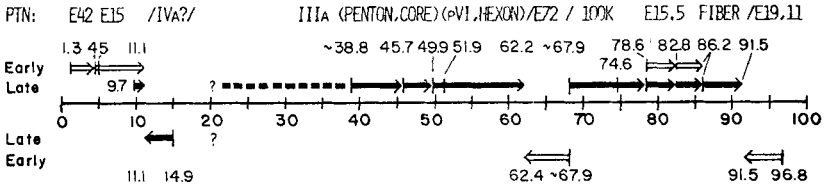
The histone genes of sea urchins which have been cloned in E. coli plasmids are arranged in contiguous repeating segments containing coding regions for each of the five histone proteins interspersed with non-coding spacer DNA. The organization of these repeat segments in the chromosomal DNA of L. pictus has been analyzed. Nick-translated P^{32} labeled DNA probes were made from five restriction fragments of the cloned S. purpuratus DNA which correspond to the individual histone proteins. Restriction endonuclease digested chromosomal DNA of L. pictus was separated by agarose gel electrophoresis and transferred to nitrocellulose filters by blotting. The nick-translated DNA probes were hybridized individually to the blots and revealed the presence of three sizes of repeat units 3.5, 4.7 and 7.4 kilo base pairs. Each repeat length contains sequences coding for all five histone proteins. The gene order, polarity and strandedness of the 7.4 kb repeat, which has been cloned in E. coli is identical to the previously analyzed urchin S. purpuratus which has only one major sized repeat unit. Data on the clustering of the various L. pictus repeats will be presented as well as the implications of these findings on the evolution of repetitive coding DNA sequences.

323 AN INVERTED TERMINAL REPEAT SEQUENCE IN THE GENE-SIZED PIECES OF OXYTRICHA MACRONUCLEAR DNA, Glenn A. Herrick, University of Colorado, Boulder, Colorado 80309

Oxytricha (a ciliated protozoan) has two types of nuclei -- micronuclei and macronuclei. The micronuclear genome is typical of most eukaryotes with a high sequence complexity and repetitive sequences as well; however, it supports no vegetative transcription, and, in fact, it is dispensable for vegetative growth. All vegetative transcription is from the macronuclear DNA; this DNA is derived by a post-conjugation processing of a micronuclear genome and has only about 10% of the sequences present in the micronucleus; it is present in gene- or oligo-gene-sized pieces (about 20,000 different pieces, each present 1,000 times per nucleus). These pieces all share in common the ability to yield single-strand circles, held together by a short duplex neck. This neck material has been isolated from total macronuclear DNA; its properties are 1) it is homogeneous in size, 23 BP, 2) it is present once per single-strand circle, and 3) it renatures extremely rapidly (1/2 Cot = 1.1×10^{-5} M-sec/l). Thus, it appears that all the 20,000 different macronuclear gene-sized pieces sport this same neck sequence (direct DNA sequencing of this material should be underway by February 1977). The possible implications of the existence of this sequence both for the vegetative function and replication of macronuclear DNA and for the sequence-diminution process which creates the macronuclear genome from the micronuclear genome will be considered. This work was supported by NIH grant #5 F32 GM05213 to Glenn A. Herrick and by NIH grant #5 R01 GM19199 to David M. Prescott.

324 AN EM R-LOOP MAP OF ADENOVIRUS 2 RNA, Thomas R. Broker, James M. Roberts, James B. Lewis, and Louise T. Chow, Cold Spring Harbor Lab, Cold Spring Harbor, New York 11724

Adenovirus 2 RNAs extracted from the cytoplasm of human KB cells at early and at late times during lytic infections were hybridized to partially denatured adenovirus 2 DNA or its EcoRI A (0-58.5%) or SalI A (45.9-100%) restriction fragments. "R-loops" of displaced DNA formed opposite the annealed RNA. The positions and lengths of about 1000 such loops were determined by electron microscopy. Most loops fell into reproducible classes, which allowed assignment of map coordinates to the transcripts with an accuracy of about ± 200 nucleotides (see the Figure). The positions of sense strand switches were located using convergent loops containing both early and late RNAs hybridized to opposite strands. Related transcripts from three regions (38.8-49.9; 49.9-62.2; and 67.9-86.2) have two or three alternative 5' and/or 3' termini, suggesting a limited variability in the processing of precursor RNA molecules.



325 SEQUENCE ANALYSIS OF THE HISTONE H4 GENE OF THE SEA URCHIN STRONGYLOCENTROTUS PURPURATUS. Michael Grunstein and Judith E. Grunstein, Molecular Biology Institute, UCLA, Los Angeles, Calif. 90024.

The histone H4 gene from the sea urchin Strongylocentrotus purpuratus has been sequenced by the use of dimethylsulfate and hydrazine using the procedures of Gilbert and Maxam (unpublished). Of 120 continuous nucleotides analyzed at the amino-coding terminus, all are colinear with the amino acid sequence of the highly conserved histone H4 protein. Approximately 100 continuous nucleotides directly "upstream" of the H4 gene have also been sequenced and the histone H4 ribosome binding site has been determined.

326 ISOLATION AND CHARACTERIZATION OF THREE ECORI-DNA FRAGMENTS FROM THE RIBOSOMAL RNA GENE CLUSTER OF DROSOPHILA MELANOGASTER. H. Biessmann, K.E. Diamond, A. Dugaiczyk, S. Wadsworth, H.L. Heyneker, H.W. Boyer & B.J. McCarthy. Departments of Biochemistry and Microbiology, University of California, San Francisco 94143

Three Drosophila DNA recombinant plasmids were isolated with sequences related to those in the ribosomal DNA containing plasmid pDm 103 characterized by Glover et al., Cell 5, 149 (1975) by screening with non-polyadenylated cytoplasmic RNA. Their average repetition frequency in the genome is about 200 copies. All three contain sequences homologous to ribosomal RNA as shown by solution hybridization. When hybridized to gel-fractionated EcoRI digested DNA, each reacted to a subset of the four major bands comprising the ribosomal gene cluster. Sequence homology between these three fragments and pDm 103 were defined by annealing in solution and heteroduplex mapping in the electron microscope. The existence of these different DNA fragments reflects heterogeneity in the spacer regions of the Drosophila ribosomal RNA genes.

327 A MEASUREMENT OF THE GENOME SIZE OF SV40 AND POLYOMA, Lauren Sompayrac and Kathleen Danna, MCD Biology Dept., University of Colorado, Boulder, CO 80302

The genomes of SV40 and Polyoma have been measured using gel electrophoresis and electron microscopy by comparing their sizes with the size of a sequenced restriction fragment of SV40. Results will be discussed with particular reference to possible undetected early gene products.

328 TISSUE-SPECIFIC PROTEIN PATTERNS OF DROSOPHILA AS AN INDICATION OF DIFFERENTIAL GENE ACTIVITY. Robert Arking and William E. Timberlake. Dept. Biol., Wayne State Univ., Detroit, Michigan 48202.

Using the newly developed technique of autofluorography, we have been able to rapidly visualize a large number of ^{35}S-labeled proteins from individual imaginal discs of developing Drosophila melanogaster larvae. In vivo labeling, performed by feeding ^{35}S-labeled yeast to developing larvae, has enabled us to attain high incorporation levels (i.e., 2 X 10^6 cpm/5λ TCA-precipitable material/larvae) with a minimum of effort. Pulse label experiments show that, during the third larval instar, each tissue investigated has its own distinct protein pattern. There are both qualitative and quantitative changes. We have concentrated our efforts on analyzing only the qualitative differences. Of the more than 40 different proteins visualized to date, there are 4 proteins which are common to all imaginal discs but which are absent from larval tissues. Larval tissues are characterized by at least 2 proteins not found in discs. In addition, there are at least 2 proteins found in larvae which are absent from prepupae, while the prepupal stage possesses at least one protein not found in larvae. Most striking of all is the fact that the wing disc, the first leg disc, and the eye-antennal disc each have at least one protein which is unique to that particular disc tissue. Recent technical improvements have allowed us to increase the resolving power of our technique and thus should enable us to visualize even more proteins. The experiments presently underway are designed to determine the stage at which these tissue-specific proteins first appear in wild type larvae as well as in individual larvae with a homeotic mutation.

329 METABOLISM OF NEWLY SYNTHESIZED HISTONE RNA IN DEVELOPING SEA URCHIN EMBRYOS, Geoffrey Childs, Shoshana Levy and Laurence Kedes, Stanford Medical School and Veterans Administration Hospital, Palo Alto, Ca. 94305.

Newly synthesized histone specific RNA in the developing sea urchin embryos (S. purpuratus) was studied by hybridization to histone DNA probes. These DNA probes obtained from the cloned histone plasmids pSp2 and pSp17 contain the coding regions of the five histone proteins as well as spacer non-coding regions. The plasmid DNA was digested with appropriate restriction enzymes and subfractions of the histone gene repeat unit corresponding to these individual coding and spacer regions were isolated. The fragments were covalently bound to cellulose and used for hybridization in DNA excess to probe histone RNA in pulse labeled embryos. Hybridization is completed in a short time period enabling individual histone mRNAs and intact nuclear histone RNA to be eluted and further analyzed. Using this hybridization method we have studied and will present data on: 1. The size of newly synthesized nuclear histone specific RNA. 2. The transport of histone specific RNA from the nucleus to the cytoplasm. 3. The differential inhibition of synthesis of the five histone mRNAs after treatment of embryos with ultraviolet light. 4. The onset of histone specific RNA transcription after fertilization.

330 Rapid Techniques for Secondary Structure Mapping of RNA, John Vournakis and Alan Maxam, Syracuse University, Syracuse, N.Y. 13210 and Harvard University, Cambridge, Ma. 02138.

A rapid technique for mapping helical and non-helical regions along the primary sequence of E-Coli 5S RNA and yeast phenylalanyl t-RNA will be discussed. The method is similar in principle to the Maxam and Gilbert rapid DNA sequencing procedure. RNA molecules are end-labeled specifically at their 5' terminus by the T4 polynucleotide kinase reaction, using highly radioactive γ-[^{32}P]-ATP. Partial digestion of 5'-[^{32}P]-RNAs with the single-strand specific nuclease S1 followed by electrophoresis on 20% acrylamide - 7 M Urea gels results in the resolution of oligonucleotide fragments that terminate in non-hydrogen bonded nucleotides. The exact position of each cleavage site is determined by comparing S1 digestion and partial KOH hydrolysis patterns. S1 digestions are performed at several temperatures and salt conditions. Secondary structural models are presented and compared to previously generated computer models. The applicability of this method for studying structure in the non-coding regions of eukaryotic mRNA is discussed.

331 UV-TRANSCRIPTIONAL UNIT MAPPING IN ADENOVIRUS TYPE 2, Sheldon Girvitz, A.J. Rainbow, Departments of Biology and Radiology, McMaster University Hamilton, Ontario, Canada L8S 4K1

Human KB cells, lytically infected with human adenovirus type 2 were pulse-labelled with ^{35}S-methionine during the late period of viral infection, and the resulting labelled polypeptides analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. A differential reduction of late adenovirus type 2 protein synthesis was observed following UV-irradiation of the infected cells. The UV-inactivation of specific viral proteins was assumed to correlate with physical distance between the genes encoding these proteins and their promotors. In this way a more detailed physical map of late adenovirus type 2 gene locations was determined; in addition the number of promotors for RNA synthesis on the light strand of the viral genome was determined.

332 ORGANIZATION OF RIBOSOMAL AND 5S RNA CODING REGIONS IN DICTYOSTELIUM DISCOIDEUM, William C. Taylor, Andrew F. Cockburn, Gary A. Frankel, Mary Jane Newkirk and Richard A. Firtel, Dept. of Biology, University of California, San Diego, La Jolla, Ca. 92093

The ribosomal DNA comprises 18 % of the nuclear DNA in Dictyostelium and is organized into repeating units 47 kb long. Only 8 kb codes for the 36S ribosomal precursor, the remainder is homogeneous spacer. The 5S coding region is contained within the repeating unit and is located several kb to the 3' side of the 26S coding region. The 47 kb repeating units occur as pallindromic dimers. Experiments are underway to determine if there is any linkage between the 94 kb dimers and if the ribosomal DNA is episomal. Further experiments will show whether the 5S RNA is transcribed from the same strand as the 17S and 26S RNAs.

We have isolated 5S containing clones by two methods: 1) insertion of a 7.2 kb Eco RI fragment into pSC101 by ligation; and 2) insertion of randomly sheared nuclear DNA into pMB9 by the homopolymer extension method. The pSC101 clone containing the 5S region was found to be unstable during subsequent replication. EM heteroduplex analysis of the resultant plasmids suggests that large portions of the original fragment were deleated but that the 5S region was retained.

333 ANALYSIS OF CLONES CONTAINING *DICTYOSTELIUM* DNA WHICH CODES FOR MESSENGER RNA, Karen L. Kindle, Richard A. Firtel, Mary Jane Newkirk and Michael McKeown, Department of Biology, University of California, San Diego, La Jolla, CA 92093

Using the homopolymer extension method to insert sheared *Dictyostelium* DNA into the tetracycline-colicin resistance plasmid pMB9, we have screened for clones which hybridize to [^{32}P]-labeled poly[A]$^+$-containing RNA and fail to hybridize to mitochondrial cRNA. Various properties of three clones which hybridize to a substantial proportion of this poly[A]$^+$ RNA have been studied.

One clone (M6) has been shown to code for a discrete size message. A second (KH10) has sequences complementary to a heterogeneous population of poly[A]$^+$ RNA, while a third (M4) is complementary to a small discrete message as well as a population similar to KH10.

Message complementary to M6 has been isolated using preparative hybridization to DNA cellulose. When this RNA is translated in a wheat germ *in vitro* protein synthetic system, it directs the synthesis of a protein which comigrates with *Dictyostelium* actin in polyacrylamide gels. These and additional studies suggest that plasmid M6 carries the coding sequence for actin.

Hybridization kinetics indicate that M6 contains both repeat and single-copy sequences. The reiterated sequences are repeated approximately 20 times per genome and have been mapped within the *Dictyostelium* genome by hybridizing M6 cRNA to Southern DNA blots of nuclear DNA digested with various restriction endonucleases.

The expression of the various clones has been followed throughout the developmental cycle of this organism. The plasmids are also being used to examine nuclear precursors to mRNA.

334 IMMUNE CHARACTERIZATION OF CHINESE HAMSTER HPRT MUTANTS. C.T. Caskey, T.H. Sawyer, G.D. Kruh, R.G. Fenwick, Jr. Baylor College of Medicine, Houston, Texas 77030.

Chinese hamster lung (CHL) hypoxanthine-guanine phosphoribosyl transferase (HPRT) is composed of three subunits ofidentical molecular weight. Monospecific antibody (Ab_n) to the native enzyme does not have detectable cross reactivity with HPRT which has been alkylated with NEM or denatured by heat or 8M urea. Since we have particular interest in nonsense mutations, efforts have been made to develop an antibody capable of recognizing HPRT subunit peptides (Ab_d) and their fragments. This was achieved by immunization of a sheep with urea-denatured HPRT. The Ab_d and Ab_n have been covalently linked to Sepharose 4B and employed as a simple means of purifying in vivo-labeled [^{35}S]-and [^{3}H]-labeled HPRT. The radioactive HPRT is identified by autoradiographic analysis following elution from the Sepharose-antibody and electrophoresis in SDS acrylamide gels. Using Ab_n ~90% of native HPRT can be absorbed to the Sepharose-antibody while urea-denatured HPRT is recognized less well (~7%). Using Ab_d ~90% of urea-denatured HPRT can be absorbed to the Sepharose-antibody while native HPRT is bound less well (~10%). Thus we now have the capacity to recognize immunologically both the native and subunit forms of HPRT. These techniques have been applied to the analysis of CHL HPRT mutants. We have now identified both CRM^- and CRM^+ HPRT mutants. Among those which are CRM^+ we have identified mutants with reduced and normal molecular weights. Determination of the type of mutation (deletion, frameshift, nonsense) responsible for reduced HPRT molecular weights requires additional study.

335 MUTATIONS AFFECTING NEUROTRANSMITTER-RELATED ENZYMES, Richard L. Russell, Carl D. Johnson, James B. Rand, Stewart Scherer, and Maurice S. Zwass, California Institute of Technology, Pasadena, CA 91125

Radiochemical assays based on the selective extraction of either substrate or product from an aqueous reaction volume into an organic scintillator have been developed for several enzymes of neurotransmitter metabolism. These rapid, convenient assays have made it possible to screen large numbers of mutant lines for potential enzymatic defects, and two mutants with acetylcholinesterase defects, plus two more with choline acetyltransferase defects have been identified. Two of these mutants have quite pronounced behavioral abnormalities which suggest potential roles for the enzymes they lack, and these roles will be discussed.

336 MOLECULAR CLONING OF RESTRICTION FRAGMENTS OF λh80dara DNA, Lawrence Greenfield, Donald A. Kaplan, and Gary Wilcox, University of California at Los Angeles, Los Angeles, California 90024.

λh80dara and pBR317 DNA were restricted with R. BamI endonuclease and ligated with T4-induced polynucleotide ligase. Transformation of E. coli K12 with the ligation mixture allowed the isolation of clones containing pBR317-λh80dara chimeras. The cloned DNA fragments were analyzed by digestion with pairs of restriction endonucleases. This method is rapid and allows one to determine the molecular weight and identity of the cloned DNA fragments.

R. BamI fragments of λh80dara were further restricted with R. EcoRI endonuclease under R. EcoRI* conditions. This second digestion yields 8-10 R. BamI-R. EcoRI* fragments which have been ligated to pBR317 DNA restricted with R. BamI and R. EcoRI. Transformation of E. coli K12 with this DNA has yielded clones which are now in the process of being identified. The simultaneous use of two enzymes allows cloning of defined regions of the arabinose operon starting at given restriction sites.

337 SEQUENCE COMPLEXITY OF YEAST RNAs, L.M. Hereford and M. Rosbash, The Rosenstiel Institute, Brandeis University, Waltham, Massachusetts.

The number and distribution of mRNA sequences has been examined using the technique of RNA-cDNA hybridization. This approach has led to the following conclusions: (1) Of the 4000 different mRNAs which are produced, only 20% are produced in any great abundance (i.e. greater than 1 copy 1 cell), (2) All m-RNA sequence complexity is present in poly A containing RNA, and (3) All sequence complexity is present in polysomal mRNA.

The nature of the abundant mRNA has been examined in more detail using a temperature-sensitive mutant, RNA_2. This mutant has previously been shown to selectively shut off the synthesis of ribosomal proteins. Hybridization studies have revealed that approximately 30% of the abundant mRNAs (i.e. 130) are not present at the restrictive temperature. This observation has given us an experimental system in which to look at the coordinate control of a large number of proteins.

338 HISTONE GENE ARRANGEMENT IN THE SEA URCHIN. David S. Holmes*, Norman Davidson*, R. Cohn+ and L. Kedes+. *Calif. Inst. of Tech. and +Stanford Medical School.

The ordering and spacing of five histone genes on 2 separate fragments of sea urchin (S. purpuratus) DNA cloned in the plasmid PSC101 has been determined by electron microscopy. The order of genes is H1, H4, H2b, H3 and H2a. Each gene is separated by spacer DNA of unknown function and the coding sequences are all on the same strand. Following hybridization of histone mRNA to either double or single stranded DNA genes were visualized in the electron microscope by R-loop formation or by complexing with gene 32-ethidium bromide respectively. A new modification of the R-loop procedure involving hybridization of histone mRNA to single stranded DNA followed by DNA-DNA reassociation was also used. In addition these techniques were employed to visualize histone genes on enriched sea urchin sperm DNA. Several contiguous repeats of the 5 histone genes were recognized.

213 CYCLIC AMP, REVERSE TRANSFORMATION, AND GENETIC REGULATION, Theodore T. Puck, Eleanor Roosevelt Institute for Cancer Research, University of Colorado Medical Center, B129, 4200 East Ninth Avenue, Denver, CO 80262

Agents which increase the cellular concentration of cyclic AMP cause transformed CHO-K1 cells to lose the in vitro characteristics associated with malignancy and assume the habitus of normal fibroblasts. The transformed cells lose their compact, pleomorphic character; the cells stretch out into typical fibroblastic forms; the rapidly oscillating knobs on the cell surface disappear or migrate to the two ends of the spindle-shaped cell which results, causing extensive surface tranquilization; the cells orient together in parallel to their long dimension; and while growth on plastic surfaces remains at 100% plating efficiency, growth in agar suspension falls to zero. This change, called Reverse Transformation, is synergized by testosterone, testololactone, or various prostaglandins.

A variety of experiments utilizing electron microscopy (1), immunofluorescence with antibodies to tubulin (2) and actin (3), and kinetics studies with colcemide and cytochalasin B, demonstrate that the transformed state is accompanied by a randomized arrangement of microtubules and microfibrils. Increase in cAMP levels causes organization of a highly ordered arrangement of both microtubules and microfibrils which traverse the entire length of the cell.

Increase in cyclic AMP levels causes cells of a hybrid between CHO and hamster brain, which in normal growth medium resembles the transformed CHO parent, to extend dendritic processes which link up among the cells of a colony to form a network.

A theoretical formulation has been proposed in which normal cells are committed to a given pattern of microtubular and microfibrillar organization; cyclic AMP is required for this organized pattern to form; microtubular processes are connected to specific chromosomal sites (presumably on the reiterated DNA regions) even in interphase; this organized structure is important in the control of genomic functions involved in reproductive regulation; disruption of this organization yields a cell which exhibits transformed characteristics in vitro and may be malignant in vivo.

1) In collaboration with Dr. Keith Porter.
2) In collaboration with Dr. Bill R. Brinkley.
3) In collaboration with Dr. Elias Lazarides

214 THE USE OF AMINO ACID RESISTANT MUTANTS IN A STUDY OF AMINO ACID TRANSPORT IN MAMMALIAN CELLS IN CULTURE AND SV40 TRANSFORMANTS, Ellis Englesberg, William Heiser, and Richard Bass, Biochemistry, Molecular Biology Section, Department of Biological Sciences, University of California, Santa Barbara, California 93106

Raising the concentration of each of the amino acids in MEM inhibits the growth of mammalian cells in culture (A9, CHO, Babl 3T3, SV40 transformed Balb 3T3 (SVT2), W138) (1). There are some similarities in the inhibitory patterns shown by each of the above cell lines but there are some unique differences. Mutants resistant to 15 and 20 mM L-phenylalanine have been isolated in the mouse cell line A9 (1) and to 20 and 30 mM in the hamster cell lines V79 and CTK$^-$. These mutants were isolated by a single selection step. Phenylalanine resistant mutants (Phe^r) in A9 were shown to be stable, and to occur spontaneously at a rate of 4.9×10^{-5} per cell per generation. The frequency of occurrence of these mutants can be increased several fold by chemical mutagens such as EMS and NG. A Lineweaver-Burk plot of initial rates of phenylalanine uptake by parent and mutants showed a biphasic curve suggesting two transport systems. Each Phe^r mutant had altered properties of both transport systems. It is suggested that the selection of clones resistant to high concentrations of several of the natural amino acids may be used as a general method for the isolation of mutants affecting the various amino acid transport systems in mammalian cells. Transport mutants have also been isolated in a single step procedure by selecting for variants resistant to 5-fluorotryptophan (2).

We studied the pattern of amino acid inhibition of growth of 3T3 and SVT2. Since SV40 transformed cells have been previously shown to be more efficient in transporting certain amino acids than 3T3 (3), we thought it might be possible that transformed cells would be more severely inhibited by some amino acids as a result of an amino acid antagonism at the transport or amino acid activation level. There were several differences shown in the amino acid inhibition pattern between 3T3 and SVT2. Of particular interest was the finding that methionine killed SVT2 under conditions in which growth of 3T3 is only temporarily delayed. Methionine resistant variants of SVT2 have been isolated. These mutants resemble more closely 3T3 than SVT2 with respect to cellular morphology, growth pattern, and maximum density of growth. One mutant had a higher efficiency of plating in soft agar than SVT2 while another resembled 3T3 in this respect. Maximum growth in low serum was not affected. The association between methionine sensitivity and transformation characteristics provides a means of analyzing the genetic basis of reversion of SV40 transformants.

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215 A SEARCH FOR NONSENSE MUTANTS AND NONSENSE SUPPRESSORS IN MAMMALIAN CELLS, Mario R. Capecchi, Department of Biology, University of Utah, Salt Lake City, Utah 84112.

The isolation and characterization of nonsense mutants and their suppressors in mammalian cell lines would greatly aid the genetic analysis of mammalian cells and their viruses. For this purpose we have isolated 500 independent mouse fibroblast cell lines lacking hypoxanthine-guanine phosphoribosyl transferase (HGPRT) activity. These cell lines have been screened for immunological cross reacting material (CRM). About 40% of these $HGPRT^-$ cell lines were CRM^+ (1,2). Included in this pool of $HGPRT^-$ CRM^+ cell lines, are mutants which contain altered HGPRT molecules whose subunits migrate faster and slower than wild type HGPRT subunits on SDS polyacrylamide gel electrophoresis. The reason for the altered electrophoretic mobility is being investigated by tryptic peptide analysis. Obviously, the mutants whose subunits migrate faster than the wild type enzyme subunits are candidates for nonsense mutants. Alternative methods of identifying nonsense mutants are also being investigated. Included in these studies are experiments designed to test phenotypic suppression of HGPRT nonsense mutants by microinjection of *E. coli* and yeast suppressor tRNAs into the mammalian cells.

Identification of mammalian cell lines containing nonsense suppressors is being pursued by screening revertants of the $HGPRT^-$ cell lines *in vitro* for suppressor tRNAs. These assays utilize a cell-free protein synthesizing system derived from the parental mouse cell line programmed with RNA from a derivative of Q_β containing an amber mutation in the viral coat protein gene. The *in vitro* system responds to suppressor tRNA isolated from *E. coli* and yeast (3). A comparable system which responds to ochre suppressor tRNAs has also been developed.

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