MOLECULAR BIOLOGY OF DEVELOPMENT

Eric H. Davidson and Richard A. Firtel, Organizers

March 31 — April 7, 1984

Plenary Sessions

April 1:
Cytoplasmic Localization in Early Embryos 3
Pattern Formation and Gene Expression 5

April 2:
Molecular Aspects of Oogenesis 6
Molecular Approaches to Nerve Cell Differentiation 8

April 3:
Developmental Expression of Gene Families 11
Gene Expression in Hematopoietic Cell Lineages 13

April 4:
Molecular Aspects of Plant Development 15
Transcription and Gene Expression in Embryos I 17

April 5:
Transcription and Gene Expression in Embryos II 19

April 6:
Whole Organism Transformation I 22
Whole Organism Transformation II 24

Poster Sessions

April 1:
Gene Structure I
Poster Abstracts 0892 — 0908 26

Gene Expression I
Poster Abstracts 0909 — 0938 31

April 2:
Gene Structure II
Poster Abstracts 0939 — 0954 41

Gene Expression II
Poster Abstracts 0955 — 0979 47

April 3:
Developmental Genetics
Poster Abstracts 0980 — 0994 55

Plant Molecular Biology
Poster Abstracts 0995 — 1016 60

April 4:
Embryogenesis and Oogenesis
Poster Abstracts 1017 — 1041 67

Neurobiology
Poster Abstracts 1042 — 1049 76

April 6:
Hematopoietic Gene Expression
Poster Abstracts 1050 — 1062 78

Transformation
Poster Abstracts 1063 — 1084 83

Cytoplasmic Localization In Early Embryos

0841 LOCALIZATION AND DETERMINATION IN EARLY EMBRYOS OF C. ELEGANS, William B. Wood, Einhard Schierenberg, and Susan Strome, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

We are studying the mechanisms by which the developmental fates of early blastomeres become determined in C. elegans embryos. Previous evidence from our laboratory (1) indicated that at least one of these fates, that of the gut lineage, is governed by internally segregating, cell-autonomous determinants (1). We have asked whether the gut determinants are nuclear or cytoplasmic in experiments using laser microsurgery to expose the nucleus of one cell to cytoplasm of another. The results show that cytoplasm from a gut precursor cell, when introduced into a non-gut precursor cell, will cause the progeny of the resulting hybrid cell to express gut-specific differentiation markers, supporting the view that the determinants are cytoplasmic.

A possible candidate for another determinant is the P granules, cytoplasmic components that are segregated specifically to cells of the germ line (P lineage) in early cleavages, and are apparently present in all germ line cells throughout the life cycle (2). We have obtained anti-P-granule monoclonal antibodies and used them to follow segregation of the granules to the posterior pole of the zygote prior to first cleavage, during migration and meeting of the two pronuclei. Observations on mutant embryos defective in various early events and wild-type embryos treated with specific inhibitors of microtubule and microfilament function indicate the following: Fertilization is required for P-granule segregation. Pronuclear migration is blocked by microtubule inhibitors but not by microfilament inhibitors. Conversely, P-granule segregation as well as other early manifestations of embryonic asymmetry are blocked by microfilament inhibitors and not by microtubule inhibitors (3). These results suggest that P-granule segregation does not require either the spindle or cytoplasmic microtubules, but that this process as well as generation of other asymmetries do require cytoskeletal functions that depend on actin microfilaments. The nature of the P-granule antigens, now being investigated, and their possible role in development will be discussed.

(1) Laufer, J.S., Bazzicalupo, P., and Wood, W.B. (1980). Segregation of developmental potential in early embryos of Caenorhabditis elegans. Cell 19, 569-577.
(2) Strome, S. and Wood, W.B. (1982). Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of Caenorhabditis elegans. Proc. Nat. Acad. Sci. U.S. 79, 1558-1562.
(3) Strome, S. and Wood, W.B. (1983). Generation of asymmetry and segregation of germ-line granules in early C. elegans embryos. Cell 35, 15-25.

0842 MESSENGER RNA LOCALIZATION IN THE MUSCLE-FORMING CYTOPLASM OF ASCIDIAN EMBRYOS, William R. Jeffery, Richard D. Brodeur, and Craig R. Tomlinson, Center for Developmental Biology, University of Texas, Austin, TX 78712.

The tail muscle and mesenchyme cell lineages of ascidian embryos arise from the myoplasm, a specialized cytoplasmic region which is localized in the egg (1). Cytoplasmic shift experiments suggest that the myoplasm contains determinants that cause muscle-cell features to form in non-muscle cell lineages (2,3). The myoplasm is originally localized in the egg cortex, participates in ooplasmic segregation after fertilization, and is concentrated into a crescent-shaped localization in the vegetal region of the zygote where it is progressively segregated into the muscle and mesenchyme cell lineages during cleavage (1). The myoplasm contains yellow pigment granules that are histologically distinct in the eggs of the ascidian, Styela plicata. We exploited this attribute to map the distribution of mRNAs in this region during early development and to probe the mechanism of mRNA localization by in situ hybridization with poly(U) and cloned DNA (4). The myoplasm was enriched in actin mRNA relative to histone mRNA and poly(A)+RNA at each stage of early development. Actin mRNA was concentrated in the egg cortex, remained associated with the myoplasm during its migration into the vegetal region of the egg, was localized in the myoplasmic crescent, and was preferentially distributed to the muscle and mesenchyme cell lineages during cleavage. The myoplasm is estimated to contain about 45% of the total actin mRNA although it represents only about 10% of the egg by volume. Isolation of egg mRNA and analysis of its translation products by 2D gel electrophoresis indicated that eggs contain translatable messages for only the cytoplasmic actin isoforms. The muscle actin isoform is first detected at gastrulation and its translation is directed by zygotic mRNA. The results suggest that cytoplasmic-type actin mRNA is localized in the myoplasm. The localization of actin mRNA in the myoplasm during ooplasmic segregation suggests that these sequences may be associated with a structural framework present in this cytoplasmic region. To test this possibility we subjected Triton X-100-extracted eggs and embryos to in situ hybridization. The myoplasm of Triton X-100-extracted eggs contains a cytoskeletal domain composed of F-actin and other cytoplasmic filaments (5). Actin mRNA was shown to exhibit the same spatial distribution in Triton X-100-extracted eggs as it did in intact eggs suggesting that this mRNA localization is mediated by an association with the myoplasmic cytoskeletal framework.

1. Conklin, E.G. (1905). J. Acad. Nat. Sci. Phila. 13, 1-119.
2. Whittaker, J.R. (1980). J. Embryol. Exp. Morphol. 55, 343-354.
3. Whittaker, J.R. (1982). Dev. Biol. 93, 463-470.
4. Jeffery, W.R., Tomlinson, C.R., and Brodeur, R.D. (1983). Dev. Biol. 99, 408-417.
5. Jeffery, W.R., and Meier, S. (1983). Dev. Biol. 96, 125-143.

0843 MATERNAL mRNA AND DETERMINATION IN SEA URCHIN AND XENOPUS EMBRYOS, Fred H. Wilt, Carey Phillips, and J. Akif Uzman, Dept. of Zoology, University of California, Berkeley, CA 94720. The egg of X. laevis is highly organized. In addition to the obviously arranged pigment granules and nucleus, yolk platelets come in several size classes, and particular regions of the egg may be characterized by the size of yolk platelets contained there and the density of their packing. These areas of yolk platelets remain coherent during early development, but shift relative to one another, indicating extensive cytoplasmic rearrangement. The redistribution patterns of yolk platelets also serve as reliable guide-posts for following changes in the localization of RNA during development. In situ hybridization and autoradiography have been used to determine changes in the localization of poly A+ RNA and other RNA molecules during development. Poly A is especially concentrated in the cortex of the animal pole prior to cleavage; as cleavage begins this localization disappears and a region of the vegetal cytoplasm becomes more heavily adenylated. There is a conspicuous increase in the amount of poly A present in one specific cytoplasmic region on the dorsal side of the blastocoel at stage 8; this is probably due to changes in the poly A tract length rather than formation of new transcripts. The very extensive changes in poly A concentration that occur in this defined area indicate that there is a post-transcriptional, cytoplasmic control of poly A content that is region specific. Manipulations of the egg which change the orientation of the forming dorsal-ventral axis also cause changes in the distribution of various RNAs.

The embryos of the sea urchin, Strongylocentrotus purpuratus, show an increase in transcription at the 4th cell division, and this is also true of the early histone genes. Run-off transcription experiments show that the increase in transcription is probably due to loading of more polymerase. Cessation of early histone gene transcription after cleavage is accompanied by loss of initiation complexes from these genes. The timing of the increase of RNA synthesis in cleavage is apparently under control of molecules present in the egg. If the volume of cytoplasm is reduced before fertilization, the resultant nucleated merogons divide and develop precisely on schedule, but the time course of increase in RNA synthesis is accelerated by one or two cell divisions.

0844 THE ROLE OF AXIAL PROPERTIES DURING EMBRYOGENESIS, Gary Freeman, Department of Zoology, University of Texas at Austin 78712

Most animals have a well defined set of symmetry properties. These symmetry properties can usually be described in terms of one or more axes. These axes arise at an early period during development such as oogenesis or just after fertilization. An axis is not just a descriptive term, it is a physical entity with the property of polarity. In oocytes and early embryos it serves as a source of positional information that plays a role in establishing where cytoplasmic components will be localized and/or provides cells at different positions along the axis with information which they can use to differentiate in an appropriate manner. In this talk the role of axial properties during early embryogenesis will be examined in two groups of animals: hydrozoans and echinoids.

In hydrozoans the egg generates a radially symmetrical planula larva with an anterior and posterior end. Prior to gastrulation any part of a hydrozoan embryo can regulate to form a normal planula. Blastula stage hydrozoan embryos can be dissociated into single cells and the cells can be reaggregated. There is no sorting out of cells in the reaggregates. All of these reaggregates develop into normal planulae. Polarity can be entrained in an aggregate by grafting a sheet of marked cells from an intact embryo to the aggregate. The position taken up by the marked sheet of cells corresponds to the position the marked cells had in the donor embryo. If the sheet of cells from an intact embryo is dissociated and reaggregated prior to grafting it will not entrain polarity (1).

In echinoids the egg generates a bilaterally symmetrical pluteus larva. This embryo can also regulate, however the ability to regulate is constrained as a consequence of localizations of developmental potential which are distributed along the animal-vegetal axis of the embryo (2). It is possible to dissociate echinoid embryos into single cells and the cells can be reaggregated, however only a small proportion of these reaggregates form normal larvae (3). If a small graft of an intact sheet of marked cells from a normal blastula or gastrula is grafted to a reaggregate there is a dramatic increase in the proportion of normal larvae and the position the graft in the larva corresponds to the position the graft would have had in the donor embryo. Grafts from the animal and lateral regions of donor embryos must be intact cell sheet in order to entrain polarity, but grafts from the vegetal region will entrain polarity after the cells have been dissociated and reaggregated.

Supported by NIH grant GM20024.

(1) Freeman, G. 1981. W. Roux's Arch. 190:168-184.
(2) Hörstadius, S. 1973. Experimental Embryology of Echinoderms. Oxford University Press.
(3) Giudice, G. and Mutolo, V. 1970. Adv. in Morphog. 8:115-158.

0845 DORSOVENTRAL AXIS DETERMINATION IN THE XENOPUS EGG, Stanley R. Scharf, Jean-Paul Vincent, and John C. Gerhart, Dept. of Molecular Biology, University of California, Berkeley, CA 94720. In the period between fertilization and first cleavage the Xenopus egg undergoes a complex and developmentally critical cytoplasmic reorganization. In the course of the first cell cycle, the egg cytoplasm becomes regionally differentiated such that material initially opposite the point of sperm entry will give rise to the future dorsal structures of the embryo. We have investigated this early dorsalization process and find that it is comprised of at least three distinct phases, as distiguished by changes in cortical and endoplasmic organization and differential senstivity to treatments inducing abnormal axis formation. These phases are: (1) The sperm aster phase. From 25 to 50 min post-fertilization (p.f., first cleavage equals 100 min at 19° C) the sperm aster microtubule array grows to fill the animal (upper) hemisphere. The interaction of the aster with the animal hemisphere cortex is marked by a change in cortical organization, such that pigment granules become aligned toward the sperm entry point, a process completed by 50 min p.f. In this interval, but not at any other time, treatment with the microtubule stabilizing agent D_2O (70%, 6 min) induces the formation of secondary and tertiary axes. In D_2O-treated eggs, numerous cytasters are seen and the sperm aster is reduced in size. (2) The translocation phase. We have found that vegetal (lower) hemisphere pigment, as well as cortical and endoplasmic vital dye marks, abruptly begin to translocate with respect to the egg surface at 50 min p.f., in a direction which accurately predicts the location of future dorsal structures. These movements are likely to be equivalent to the classically observed grey crescent formation. The extent of movement is reduced in a dose-dependent manner by UV irradiation. From 50 to 75 min p.f., but not at any other time, the egg is sensitive to cold and hydrostatic pressure, treatments known to depolymerize microtubules (and in the case of pressure, microfilaments). Exposure results in a dose-dependent reduction and loss of dorsal axial structures [1]. Since vegetal hemisphere UV irradiation during or prior to this period also induces such axis deficiency, it is likely that all of these treatments affect a translocation event required for axis determination. (3) The mitotic phase. At approximately 75 min p.f., as mitosis begins, both pigment granule and dye spot translocation end. Simultaneously, granules aggregate into clumps which seem tightly connected to the egg surface. Coincident with this transition, the egg becomes insensitive to axis impairment by cold, pressure and UV. Thus in these three phases, (1), the egg cortex is vectorially organized with respect to the sperm aster; (2), following this directional cue, the egg cortex generates force in a unified manner and the endoplasm is shifted; and, (3), the cortex and endoplasm are immobilized, stabilizing the new egg organization. The likely consequence of this complex process is the localization or regional activation of presumed "axis determining agents", later utilized in embryonic axis formation. [1] Scharf, S. R. and Gerhart, J. C., Dev. Biol. 99, 75-87 (1983).

Pattern Formation and Gene Expression

0846 ANALYSIS OF CELL SURFACE AND EXTRACELLULAR MATRIX ANTIGENS IN D.DISCOIDEUM PATTERN FORMATION, Keith L.Williams[1],Warwick N.Grant[2], Marianne Krefft[3],Ludwig Voet and Dennis L.Welker, Max Planck Institut für Biochemie,D-8033 Martinsried bei München,Fed.Rep.Germany. Present addresses: 1,Sch.Biol.Sci.,Macquarie Uni.,North Ryde,NSW 2113,Australia; 2, ICRF, London, UK; 3, Biochem.Dept.,Uni.Wuppertal,5600 Wuppertal 1, FRG

The migratory "slug" stage of the simple eukaryote Dictyostelium discoideum is a cylinder of ~10^5 cells enclosed in an extracellular matrix,the slime sheath. The slug is formed in ~15h by aggregation of starving vegetative cells and it exhibits a pattern of prestalk cells at the front and prespore cells in the rear. Components of the patterning system probably include morphogenetic signalling, cell surface interactions and cell-matrix (sheath) interactions. To investigate the roles of the cell surface and matrix,we are characterizing cell surface and slime sheath antigens using monoclonal antibodies. Monoclonal antibodies MUD1,MUD50,MUD51,MUD52,which recognize four different antigenic determinants on slime sheath, have been characterized (1, Grant et al.,In prep.).Three of these determinants are also found on the surface of prespore (but not prestalk or vegetative) cells.One monoclonal antibody,MUD1, recognizes a single 32kd glycoprotein on prespores and in the slime sheath. Two size polymorphisms of this protein have been identified and the gene mapped to linkage group I (Grant et al.,In prep.).The other monoclonal antibodies recognize antigenic determinants that are present on more than one protein, and different proteins carry the antigenic determinants on prespore cells and slime sheath. A further monoclonal antibody,MUD9, recognizes a ~40kd protein on the surface of vegetative and prestalk cells. This determinant is progressively lost from developing prespore cells. These results emphasize that differentiated cells have characteristic surfaces and some relationship between slime sheath and prespore cells is apparent.
A quantitative pattern assay has been established using monoclonal antibody MUD1 and a flow cytometer (2,3).This makes possible studies on environmental and genetic factors which affect the pattern in D.discoideum slugs.

1.Grant,W.N.& Williams,K.L.(1983)EMBO J.2,935-40.
2.Krefft,M.,Voet,L.,Mairhofer,H.& Williams,K.L.(1983)Exp.Cell Res.147,235-9.
3.Voet,L.,Krefft,M.,Mairhofer,H.& Williams,K.L.(1984)Cytometry In Press.

0847 FUNCTIONAL AND PHYSICAL CORRELATIONS AT THE ANTENNAPEDIA LOCUS OF DROSOPHILA MELANOGASTER, Thomas C. Kaufman and Michael K. Abbott, Department of Biology, Indiana University, Bloomington, IN 47405.--Results of experimentation in this (1) and other laboratories (2, 3) have revealed that the function of the Antennapedia (Antp) locus is required to specify proper segmental identity in the thorax of the embryo and adult of Drosophila melanogaster. This function is similar to that found for the Ultrabithorax (Ubx) locus of the bithorax complex (4). However, unlike Ubx mutations, the structures into which thoracic elements are homoeotically transformed in $Antp^-$ animals are different in the embryo as compared to the adult. Embryos lacking a functional Antp locus have three thoracic segments, all of which express a phenotype more characteristic of only the first thoracic segment. In adult flies, $Antp^-$ cells develop normally in the head and abdomen but cause transformation of legs into antennae (1, 5). Additional defects are produced in the anterior portions of the dorsal mesothorax. We have now identified Antp mutations which apparently affect these two transformations separately. Thus, alleles exist which only cause the leg-to-antenna transformation but exhibit normal embryonic development. Other alleles produce the embryonic transformation previously described but have no effect upon leg development. Both types of lesions fail to complement presumed null alleles and deletions of Antp but complement one another to produce normal adult flies.--The recent molecular characterization of the DNA from the Antp locus (6) has allowed us to begin an analysis of these lesions at the macromolecular level. Mutations of the Antp locus are distributed over somewhat more than 100 kb of DNA as are those regions of the locus which are transcribed. The lesions associated solely with the leg-to-antenna transformation, however, map to a discrete 1 kb region within the locus, while those lesions associated with the dominant antenna-to-leg transformations serve to dissociate this segment from the normal 5' end of the Antp transcription unit. The correlation of specific functional defects with certain subregions of the DNA of the locus may indicate the positions of differing functional domains within the gene. One interpretation of the complementation behavior of the various Antp mutations is that the function of each of these units is, to some extent, autonomous.

1) Kaufman, T.C. and M.K. Abbott, 1983. In "Molecular Aspects of Early Development," Plenum (in press). 2) Struhl, G., 1982. Proc. Natl. Acad. Sci. USA 79:7380-7384.
3) Duncan, I., 1982. Genetics 100:s20. 4) Lewis, E.B., 1978. Nature 276-565-570.
5) Strul, G., 1981. Nature 292:635-638. 6) Scott, M., et al., 1983. Cell (in press).
Supported by PHS-GM 24299 and PHS-GM 29709.

0848 REGULATION IN CIS AND TRANS OF THE BITHORAX GENE COMPLEX IN DROSOPHILA, E. B. Lewis, Division of Biology, California Institute of Technology, Pasadena, CA 91125

The bithorax gene complex in Drosophila (BX-C) exhibits many unusual regulatory features: (1) The direction of polar position effects seems to be opposite to the direction of transcription (as determined in unpublished work of Hogness and collaborators) suggesting that polarity is not of the usual translational type but involves another mechanism perhaps that controlling puffing or gene amplification. (2) A recessive loss-of-function (LOF) mutant, such as bx^3, shows a slight cis-dominant gain of function (GOF) for the wild-type allele of a locus immediately proximal (namely: abx, in the case of bx^3). Dominant GOF mutants (such as Cbx) tend to act as weak LOF changes in genes located cis and distal thereto. The genes of the complex evidently produced substances which determine whether certain types of structures controlled by still other genes will be present or absent in a given segment. In which segment those structures first appear is assumed to be controlled by the activity of trans-regulatory loci acting upon cis-regulatory regions of the BX-C genes. A short film will use animation to present a model for the cis- and trans-regulation of the complex.

Molecular Aspects of Oogenesis

0849 THE CYTOPLASMIC REGULATION OF 5S, OAX, AND ACTIN GENE TRANSCRIPTION IN XENOPUS OOCYTES AND EMBRYOS, J.B. Gurdon, E.J. Ackerman, Larissa Wakefield, S. Brennan, AND T.J. MOHUN.

The rate of transcription of the oocyte- and somatic-types of 5S genes has been determined for full-sized oocytes and for developing embryos. Another class of genes (OAX, for oocyte-activated genes of Xenopus), like the oocyte-type 5S genes, are very abundant, and are transcribed by polymerase III primarily in oocytes. In sequence, however, they differ substantially from the oocyte-type 5S genes. Comparison between the characteristics of these two classes of genes makes it possible to determine the extent to which their activation by oocyte cytoplasm depends on factors specific to each class (such as TFIIIA) or common to all genes transcribed by polymerase III.

Another class of genes subject to cytoplasmic regulation is that coding for actin in Xenopus laevis. The use of cloned cDNAs for α-cardiac and γ-cytoskeletal actins has permitted a description of the changing content of mRNAs coded by these genes in oocytes and embryos. The α-actin gene shows temporal regulation and the α-cardiac gene is regulated both temporally and cell-type specifically at an early stage in development. Transcription of the α-cardiac gene is subject to cytoplasmic regulation, as shown both by nuclear transplantation experiments and by the culture of early embryo fragments.

0850 REGULATION OF TRANSLATION DURING OOGENESIS. L. Dennis Smith+, J. D. Richter‡, and M. A. Taylor.+ +Department of Biological Sciences, Purdue University, W. Lafayette, IN 47907 ‡Department of Biochemistry, University of Tennessee, Knoxville, Tenn. 37996-0840.

Growing oocytes characteristically synthesize and accumulate mRNA far in excess of their immediate needs. For example, the full grown Xenopus laevis oocyte contains as much as 90 ng of poly(A) RNA, but less than 3 ng is located on polysomes. This same mass of poly (A) RNA is present as early in oogenesis as the onset of vitellogenesis (¼ size oocytes), but the amount of poly(A) RNA on polysomes in these oocytes is estimated to be an order of magnitude lower. The mechanism(s) which limits the ability of oocytes to translate all of the putative mRNA remains unknown, but three possibilities have been suggested: A) components of the translational apparatus other than mRNA are limiting, B) the structural organization of the mRNA prevents translation, and C) proteins associated with the mRNA prevent translaltion. These possibilities are not mutually exclusive. Thus, recent studies involving the injection of heterologous mRNAs into oocytes have demonstrated that translation in full grown oocytes is limited by availability of components of the translational apparatus (1). Furthermore, as much as 70% of the mass of poly(A) RNA in full grown oocytes displays an interspered sequence organization in which regions transcribed from single copy and repetitive sequences are covalently associated (2). This maternal poly(A) RNA is not translatable within the oocyte (3). Finally, we present evidence that a certain class of oocyte-specific proteins extractable from native RNPs may be reconstituted with mRNAs in vitro to form particles which resemble native mRNPs. These proteins, which decrease in content during oogenesis (4), repress the translation of the mRNA with which they are reconstituted when tested both in vivo and in vitro. We suggest that in small oocytes in which the rate of protein synthesis is low, excess mRNA may be associated with proteins which prevent translation. As oogenesis progresses and protein synthesis increases, the need for such proteins decreases and limitations in the protein synthetic machinery regulate quantitative aspects of protein synthesis. At all stages, however, a significant fraction of the so-called maternal stockpile is not translatable without some structural modifications. Presumably such changes, if they occur, would take place after fertilization.

1. Richter, J. D. and Smith, L. D. (1981) Cell 27, 183.
2. Anderson, D. M., Richter, J. D., Chamberlin, M. E., Price, D. H., Britten, R. J., Smith, L. D., and Davidson, E. H. (1982) J. Mol. Biol. 155, 281.
3. Richter, J. D., Anderson, D. M., Davidson, E. H., and Smith, L. D. (1984) J. Mol. Biol., In press.
4. Richter, J. D. and Smith, L. D. (1983) J. Biol. Chem. 258, 4864.

0851 **REGULATION OF XENOPUS RIBOSOMAL GENE TRANSCRIPTION BY THE SPACER, Ronald H. Reeder, Sharon Busby, Marietta Dunaway, Paul Labhart, Judith Roan, Garry Morgan* and Aimee Bakken*, Basic Sciences Division, Hutchinson Cancer Center, Seattle, WA 98104 and *Zoology Department, University of Washington, Seattle, WA 98195**

Transcription of the ribosomal genes of Xenopus laevis is controlled by a promoter located between nucleotides -142 to +6 relative to the site of transcription initiation (1). The frequency of activation of this promoter is influenced by repetitive sequence elements that are either 60 or 81 bp long and which are present in 20 to 50 copies further out in the non-transcribed spacer. The 60/81 bp elements share sequence homology with a 42 bp region of the promoter which is essential for promoter function. The 60 81 bp elements appear to act by attracting a factor which is essential for activating the promoter. Thus, a promoter attached to many of the 60/81 bp elements is transcriptionally dominant over a promoter attached to few of these elements when both are co-injected into oocyte nuclei (2). The 60/81 bp elements can influence a promoter in either orientation, from the 5' or the 3' side, and their influence can be transmitted through at least 1 kb of pBR322 sequence.

We have also observed that when cloned ribosomal genes are injected into fertilized eggs before first cleavage, they come under developmental control and do not begin transcription until the mid-blastula transition. The level of transcription obtained is dependant on the amount of spacer present upstream of the promoter (3). From this result we conclude that the spacer probably has a significant regulatory effect on ribosomal gene transcription throughout development.

References

1. Sollner-Webb, B., Wilkinson, J.A.K., Roan, J. and Reeder, R.H. (1983). "Nested Control Regions Promoter Xenopus Ribosomal RNA Synthesis by RNA Polymerase I. Cell, in press, November.

2. Reeder, R.H., Roan, J.G. and Dunaway, M. (1983). "Spacer Regulation of Xenopus Ribosomal Gene Transcription: Competition on Oocytes." Cell, in press, December.

3. Busby, S.J. and Reeder, R.H. (1983). "Spacer Sequences Regulate Transcription of Ribosomal Gene Plasmids Injected into Xenopus Embryos." Cell, in press, October.

Molecular Biology of Development

0852 MULTIPLE GENETIC CONTROL OF DEVELOPMENTAL PATTERNS. Anthony P. Mahowald,
L. Engstrom and N. Perrimon. Department of Developmental Genetics and Anatomy. Case Western Reserve University, Cleveland, OH 44106

A series of genetic loci produce a distinctive lethal embryonic phenotype as a result of a strict maternal effect[1]. The initial defect occurs at the blastoderm stage when a small number (about 10) of cells fail to form at the posterior blastoderm, below the pole cells. During gastrulation both the anterior and posterior endodermal primordia fail to form. At segmentation the region of the forming larva posterior to the seventh abdominal segment remains undifferentiated, and eventually these cells become resorbed such that the last segment of the mature embryos is the seventh abdominal. In addition to finding up to ten female sterility loci producing this phenotype, we have discovered a pupal lethal which produces this sequence of defects when embryos are produced by homozygous germ line clones[2]. Finally, we have found an allele at a collapsed egg locus which also produces this phenotype. Molecular analysis of one locus is underway.

References.

1. Konrad, K.D. and Mahowald, A.P. (1983) Genetic and developmental approaches to understanding determination in early development. In: Molecular Aspects of Early Development (Eds. G.M. Malacinski and W.H. Klein). Plenum Press (in press).

2. Perrimon, N., Engstrom, L., and Mahowald, A.P. (1984) Analysis of the effects of zygotic lethal mutations on germ line functions in Drosophila. (submitted).

0853 RNA PROCESSING IN MICROINJECTED XENOPUS OOCYTES AND EGGS, D.A. Melton,
Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Ave., Cambridge, MA 02138

To analyze the signals important for RNA processing during development unprocessed pre-mRNAs are synthesized in vitro using a bacteriophage (SP6) promoter. The SP6 promoter has been fused to several eucaryotic genes and these DNA templates are used to direct the synthesis of large amounts of pre-mRNAs in vitro. The activity of the pre-mRNAs and mutants thereof is analyzed by microinjection into Xenopus oocytes and eggs. Experiments on the regulation of RNA splicing, 3' processing, and localization demonstrate that these in vitro transcripts are biologically active in injected oocytes and eggs. The ability to synthesize RNAs of virtually any structure allows for a direct analysis of RNA processing events during development.

Molecular Approaches to Nerve Cell Differentiation

0854 GENE EXPRESSION IN THE CENTRAL NERVOUS SYSTEM OF APLYSIA CALIFORNICA. Richard H.
Scheller, Anne C. Mahon, John R. Nambu, and Ronald Taussig, Department of Biological Sciences, Stanford University, Stanford CA 94305.

The abdominal ganglion of the marine mollusc, Aplysia, governs a number of reflex and fixed action patterns. These simple behaviors include withdrawal of the gill and mantle organs, inking and egg laying. This collection of 2,000 neurons also maintains the internal homeostatis of the animal by regulating a variety of visceral functions such as excretion, respiration and cardiac output. The large size and reproducible locations of the neurons has made it possible to isolate cDNA and genomic clones specifically expressed in the bag cells as well as neurons R3-14, L11, and R15. These genes have been shown to encode the precursors for peptides used as extracellular messengers by these cells. The precursor proteins are proteolytically cleaved into a number of components that act independently upon release. These multiple neuroactive substances released from a single neuron are thought to coordinate activities on functionally related yet distinct targets. In addition these molecules may modulate independent inputs to a single target. ELH like peptides are encoded by a small family of genes that are differentially expressed in the CNS and an exocrine gland. 20-30 KB transcription units give rise to a variety of mRNAs ranging in size from 1.0-1.4 KB. The tissue specific expression and the patterns of RNA splicing are being investigated.

0855 CELL RECOGNITION DURING NEURONAL DEVELOPMENT, Corey S. Goodman, Michael J. Bastiani, John B. Thomas, Department of Biological Sciences, Stanford University, Stanford, CA 94305

We have been studying the development of the insect nervous system in order to understand the cellular and molecular mechanisms underlying neuronal specificity, i.e., how individual neurons find and synapse with their appropriate targets. The important recognition events occur largely at the growing tips of embryonic neurons; we have focused on the cell surface interactions that mediate the specific choices made by the individual growth cones and their filopodia. Our results in the grasshopper embryo suggest that cell recognition during neuronal development is likely to involve many different molecular specificities that mediate the differential adhesion of growth cones to particular cell surfaces. Early in development, growth cones are confronted with a scaffold of axon fascicles. Although numerous axon bundles are within filopodial grasp, each growth cone, according to its lineage and previous interactions, selectively fasciculates with a particular bundle of axons. Extensive observations of individual growth cones, and ablations of particular axons, has confirmed the notion that individual neurons have a high affinity for small subsets of axons within their filopodial environment. Furthermore, monoclonal antibodies have revealed cell surface antigens whose temporal and spatial distribution in the embryo correlate with the predictions of the cellular studies, namely, neurons whose axons fasciculate together share common surface antigens. In order to isolate and characterize the surface molecules implicated by our cellular and immunological studies using the grasshopper embryo, we have begun studying cell recognition in the CNS of the Drosophila embryo. Fortunately, our recent cellular studies show that the embryonic development of the fly CNS is identical, albeit in a miniature form, to the hopper CNS in terms of the identified neurons and their selective fasciculation. The early cell recognition events, previously so well characterized in the hopper, occur between hours 10-13 of fly development. We can now isolate 100,000s of fly CNS's from this 3 hour period. We have begun using a variety of molecular genetic and immunological approaches in the fly in an attempt to isolate and study genes expressed in small subsets of embryonic neurons during this 3 hour period.

0856 GENERATION OF DIVERSITY IN EXPRESSION OF GENES THAT CODE FOR NEUROENDOCRINE PEPTIDES, Edward Herbert, James Douglass, Gerard Martens, Lloyd Fricker, Michael Comb, Haim Rosen, and Olivier Civelli, Department of Chemistry, University of Oregon Eugene OR 97403

In the past two decades a great variety of small peptides have been discovered that mediate specific behavioral responses in animals. Many of these peptides are synthesized from large precursor proteins that contain more than one kind of neuroactive peptide. The peptides must be cleaved out of these precursors (called polyproteins) and undergo post-translational modifications to acquire activity. Use of the polyprotein mechanism for generating diverse bioactive substances is particularly evident in the expression of the opioid family of peptides. More than 16 opioid peptides have been shown to be derived from 3 polyproteins; pro-opiomelanocortin (POMC), pro-enkephalin and pro-dynorphin. These polyproteins are very similar in length, arrangement of domains of bioactive peptides and sequences flanking the bioactive domains (potential proteolytic cleavage sites). Sequencing of the genes that code for these precursors reveals a very similar arrangement of exons and introns. Sequencing of pro-enkephalin genes from human, rat and Xenopus laevis reveals a very similar organization of exons and introns in these genes. The major exon in the genes from each species codes for 7 copies of enkephalin. However, there is a very important difference. The amphibian pro-enkephalin genes (a closely related family of 3 genes), code for 7 copies of met-enkephalin whereas the human and rat genes code for 6 copies of met-enkephalin and one copy of leu-enkephalin.

Production of bioactive peptides from polyproteins involves endoproteolytic cleavage at pairs of basic amino acid residues flanking the peptides followed by trimming of basic amino acids from the peptides with carboxypeptidases. Very little is known about these enzymes. We have attempted to identify mRNA species that code for processing enzymes by using recombinant DNA techniques. We have used genomic clones that code for a family of proteolytic enzymes known as kallikreins (kindly provided by John Shine). The kallikrein enzymes are known to be involved in processing of growth factors. We have been able to identify a cDNA clone (a cDNA library) that codes for a kallikrein enzyme in AtT-20-D_{16v} cells (mouse pituitary tumor cells that produce large quantitites of pro-opiomelanocortin). Northern blot analysis reveals that the mRNA that codes for the species of kallikrein is present at very high levels in these cells (20% the level of POMC mRNA). Furthermore, sequencing of a number of cDNA clones in the library indicates that there is only one species of kallikrein enzyme in these cells. A carboxypeptidase enzyme has also been identified in AtT-20-D_{16v} cells and its relationship to processing of POMC peptides is being studied.

0857 THE GENETICS AND MOLECULAR BIOLOGY OF POTASSIUM CHANNEL GENES IN DROSOPHILA, Mark A. Tanouye, C. A. Kamb, and Linda E. Iverson, Division of Biology, Calif. Inst. Tech., Pasadena, CA 91125

Recent studies have shown that in the nervous system, different classes of K^+ channels play a decisive role in the differentiation of membrane excitability. As a model for examining membrane excitability, we study K^+ channel genes in Drosophila. Our initial focus is on the Shaker (Sh) gene complex. Electrophysiology experiments have suggested that Sh mutants encode abnormal K^+ channels or channel subunits. For example, we have recorded abnormal action potentials in Sh mutant axons using intracellular microelectrodes (1). The K^+ channel blocker, 4-aminopyridine, mimicked the mutant defect when applied to normal animals but had little effect when applied to mutants. These results are in good agreement with other physiological studies (2,3). Experiments using other drugs have eliminated other likely explanations (eg. Na^+, Ca^{++} channel) for the Sh defect (1,2). Voltage clamp analyses have identified a fast, transient voltage-sensitive K^+ current specifically altered by Sh mutations(3,4).

Genetic studies (1) have shown that Sh is a complex locus located on the X-chromosome between salivary gland chromosome bands 16F1-6. The region contains about 100 Kb of DNA. Sh appears to be organized into three regions. Mutations in two flanking regions give rise to animals with Sh abnormalities. A central region, when deleted, results in haplolethality. The left flanking region contains about 50 Kb of DNA and has been characterized in the greatest detail genetically (Ferrus & Tanouye, unpublished). The limits of the region are clearly defined by bracketing chromosomal breakpoints. Five putative Sh genes, defined by mutation, have been identified and mapped by recombination.

Sh genetics provides the basis for its molecular analysis. A cDNA clone has been mapped to the left flanking region of Sh by Southern and in situ hybridization analysis of intact and broken Sh chromosomes. The cDNA clone was used as an hybridization probe to screen libraries of cloned Drosophila DNA segments. About 30 Kb of DNA in the Sh region have been cloned and restriction mapped. Several Sh mutations have been mapped in the cloned region.

1. Tanouye, et al. (1981) PNAS 78:6548-6552.
2. Jan, et al. (1977) Proc. R. Soc. Lond. B. 198:87-108.
3. Salkoff and Wyman (1981) Nature 293:228-230.
4. Wu, et al. (1983) Science 220:1076-1078.

0858 **INDUCTION OF ACETYLCHOLINE RECEPTOR CLUSTERS AT NEWLY FORMED NERVE-MUSCLE SYNAPSES** **G.D. Fischbach, L. Role, R. O'Brien, T. Usdin and D. Roufa. Washington University School of Medicine, St. Louis, Missouri 63110**

Dissociated chick ciliary ganglion neurons rapidly form synapses with chick myotubes grown in cell culture. In fact growth cones of these neurons can release ACh even before they contact the target myotubes. This was demonstrated using "patch-clamp" microelectrodes sealed with out-side out patches of ACh receptor rich myotube membrane. Within 2-3 hours after contact, the neurites induce several, high density, clusters of ACh receptors. By locating the receptor patches with a monoclonal antibody that does not block receptor function, we have determined that more than 50% of the receptor patches are associated with sites of transmitter release. Quantitative fluorescence measurements, in which receptors were "prelabeled" (before synapse formation) with rhodamine-bungarotoxin and then "end-labeled" with fluorescein-antireceptor antibody, indicate that more than 80% of the receptors at a newly formed receptor patch have been synthesized and inserted into the membrane within the preceding six hours.

Using two different protocols, we have purified trypsin-sensitive material from chick brain tissue that is capable of increasing the synthesis of myotube ACh receptors by two to four fold. Our assumption is that the same material is present in embryonic motoneurons and that it is responsible for the induction of receptor synthesis at newly formed synapses. In the first approach, the brains are extracted in trifluoroacetic acid (TFA) and purified by ion exchange chromatography, semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) and sequential analytical RP-HPLC using a Vydac C_4 column developed with a gradient of acetonitrile in 0.1% TFA. We have obtained a single, sharp, symmetrical OD_{210} peak that is biologically active. This peak yields a single N-terminal amino acid sequence. It appears to be between 10 and 14 kilodaltons in size. In the other approach, the brains are extracted in saline at neutral pH and activity is precipitated with ammonium sulfate, further purified by preparative isoelectric focusing in a sucrose gradient and then by preparative SDS-PAGE gel electrophoresis. Activity can be recovered from SDS and it appears to be associated with molecules less than 10,000 k daltons in size. Experiments are underway to determine the relation between the acid and saline extracted material and to document the relevance of both in the intact nervous system.

Developmental Expression of Gene Families

0859 The Structure and Expression of Human Actin and Muscle Specific Genes.

Larry Kedes*, William Bains*,#, Choy-Pik Chiu#, James Garrison*, Peter Gunning*, Adrien Minty*, Sun-Yu Ng*, Phyllis Ponte* and Helen Blau,# Departments of Medicine* and Pharmacology#, Stanford Medical School, Stanford CA 94305; Robert Hickey and Arthur Skoultchi, Department of Cell Biology, Albert Einstein College of Medicine, Bronx NY 10461; Roger Eddy and Thomas Shows, Department of Human Genetics, Roswell Park Memorial Institute, Buffalo, NY 10461; John Leavitt, Linus Pauling Institute of Biology and Medicine, Palo Alto, CA.

Southern blot analysis of restriction digests of human genomic DNA reveals that the α- and cardiac-actin genes are single copy genes, on chromosomes 1 and 15 respectively, whereas the cytoskeletal actin sequences are multicopy genes. Most of the members of the cytoskeletal multicopy family appear to be pseudogenes. We have cloned expressed β and γ actin genes from human DNA using recombination vectors carrying the isotype specific 3' non-translated segments of the corresponding cDNAs. I will discuss the evidence that identifies these clones as *bone fide* actin genes.

Expression During Myogenesis. We have examined the expression of actin genes in human myogenesis using pure populations of human muscle cells cloned from biopsies. Myoblasts synthesize large amounts of β- and γ-actins but only trace amounts of α-actin. Sarcomeric actin synthesis was induced about 30-fold upon differentiation. The synthesis of β- and γ-actins, on the other hand, decreased substantially. β and γ actin mRNA levels also decrease steadily after differentiation. Both α and cardiac actin mRNA begin to accumulate when differentiation is initiated and they reach a maximal level by 48 hours. An elevated mRNA level is maintained at least 7 days in culture and is at least 20 fold greater than the initial values. Surprisingly, the cardiac actin mRNA is present at each time point in excess over the α-actin mRNA. We performed identical studies with the established mouse myoblast cell line, C2, and observed that the only the cardiac actin gene is induced during differentiation, not the skeletal muscle α-actin gene. Thus cardiac actin may be activated in the proliferative phase of mammalian muscle growth or repair. Results obtained from human biopsies demonstrates that the genes encoding skeletal α-actin and cardiac α-actin are co-expressed in both human skeletal muscle and heart.

Human Sarcomeric Actin Deposits in Mouse Fibroblast Cytoskeletons. The human cardiac actin gene has been introduced into mouse L-cells which do not normally express sarcomeric actins. We detect in these murine cells the accumulation of mRNA sequences that co-migrate with authentic sarcomeric actin mRNA and the production of human cardiac actin protein. The amount of cardiac actin synthesized is about one-third the synthesis of endogenous mouse β-actin but five fold more than the synthesis of mouse γ-actin. The human cardiac actin partitioned to the mouse cytoskeletons to the same degree (~50%) as did the endogenous mouse β actin. Thus it appears that a heterologous, sarcomeric actin can participate with non-sarcomeric actins in the formation of cytoskeletal microfilaments.

Rat Myoblasts Correctly Express the Human Cardiac Actin Gene During Differentiation. The human cardiac actin gene has also been introduced into the rat myoblast cell line, L_6E_9. A significant number of transfectant clones accumulate the human cardiac actin gene mRNA only after the cells are switched to growth conditions that permits their differentiation into myotubes. In no myoblast cell line carrying human cardiac actin genes have we yet detected its constitutive expression. We estimate that none of the stably transformed cell lines examined to date carry fewer than 30-50 copies of the human cardiac actin gene.

Isolation of Additional Muscle Specific cDNAs. We have used the plasmid library linearization method to isolate more than 60 of the most abundant cDNA clones from human skeletal muscle. In each case, the clones represented mRNAs that were present in muscle at about $\geq$0.1% the amount of actin mRNA. About half the clones are specific to skeletal and heart muscle or are greatly upregulated compared to fibroblast RNA. In several cases the RNA is only detected in skeletal muscle and not in heart muscle. In most cases the muscle specific clones were represented in one or only a few copies per genome. Several of the muscle specific genes appear to be on the X chromosome.

0860 ACTIN AND MYOSIN GENES, AND THEIR EXPRESSION DURING MYOGENESIS IN THE MOUSE.

Margaret E. Buckingham, Serge Alonso, Paul Barton, Gabriele Bugaisky, Arlette Cohen, Philippe Daubas, Ian Garner, Adrian Minty, Benoît Robert, André Weydert.
Department of Molecular Biology, Pasteur Institute, 25, rue du Dr. Roux, 75015 Paris, France.

During the initial formation of skeletal muscle fibres and their subsequent maturation, different isoforms of the contractile proteins accumulate. Using recombinant probes, the transcripts coding for actins, myosin heavy chains and myosin alkali light chains have been characterized both at different stages of foetal muscle development in the mouse in vivo, and at the onset of muscle fibre formation in a differentiating mouse muscle cell line. In the case of the actin and myosin light chain multigene families transcripts of a gene expressed as a major species in adult cardiac tissue accumulate during skeletal muscle development, although not with the same kinetics. Transcripts coding for adult cardiac actin co-accumulate with skeletal actin transcripts in late foetal mouse muscle, with subsequent down modulation of the cardiac mRNA after birth. Foetal light chain transcripts, also present in adult heart atria, accumulate during early skeletal muscle formation and are replaced by mRNAs encoding the adult skeletal light chains in late foetal muscle. In contrast, the myosin heavy chain family has developmental isoforms specific to skeletal muscle ; at birth in the mouse, a foetal mRNA is rapidly replaced by one encoding adult myosin heavy chains. The strategy of gene expression during development is thus clearly different for each of these multigene families.

The organization of the genes concerned has been investigated using a genetic approach in the mouse. Genes expressed in the same muscle phenotype are not linked. Genes within the actin or myosin light chain families are also not linked. The two alkali light chains of adult skeletal muscle are a special case, since they are encoded by a common 3' genomic region with two different contiguous 5' termini. Cardiac and skeletal muscle myosin heavy chain genes map on different chromosomes, but there is evidence that the genes expressed within these tissues during development may be linked. These results indicate that regulation is trans acting, with possible sequential activation of myosin heavy chain genes in cis, within a developing muscle. The 5' upstream sequences of the actin and myosin genes and their differential response to developmental signals are under investigation.

Buckingham, M. & Minty, A. (1983) "Contractile protein genes" in Eukaryotic Protein Genes (MacLean, N., Gergory, S. & Flavell, R. Eds.) Butterworths, London, In press
Minty, A.J., Alonso,S., Guénet,J.L. & Buckingham,M. (1983) J. Mol. Biol. 167, 77-103
Weydert,A., Daubas,P., Caravatti,M., Minty,A., Bugaisky,G., Cohen,A., Robert,B. & Buckingham,M (1983) J.B.C. (Nov)(In press).

0861 THE REGULATION OF CELL-TYPE-SPECIFIC GENES IN DICTYOSTELIUM, Mona C. Mehdy, Department of Biology, University of California, San Diego, La Jolla, CA 92093

We are using cloned probes of genes which are preferentially expressed in prestalk or prespore cells to examine regulation of cell differentiation in Dictyostelium (1). Members of each cell-type-specific group of genes that we are studying show coordinate regulation. The prestalk specific mRNAs are low or absent in vegetatively growing cells, accumulate during aggregation, and require cAMP and a diffusible factor for their accumulation in culture. The induction of a cell-type-nonspecific gene having similar developmental kinetics as the prestalk genes requires only the diffusible factor. In contrast to the prestalk specific mRNAs, the prespore specific mRNAs accumulate much later in development and their accumulation may require cell surface interactions in addition to cAMP and the diffusible factor. The diffusible factor has different effects on gene expression than the previously reported regulatory molecules, DIF (Differentiation Inducing Factor) and NH_3 (2). Its effect is selective for these developmental genes; no effect is observed on the mRNA levels of actin and another gene transcribed in vegetative cells and throughout development. The cell-type-specific genes are regulated differently than genes whose expression is induced very early in development and then shut off. In culture, the levels of early mRNAs are increased by nanomolar cAMP pulses similar to in vivo cAMP pulsing during aggregation. Much higher levels of exogenous cAMP inhibit the accumulation of early mRNAs while precociously inducing the cell-type-specific genes. Later in development, both groups of cell-type-specific genes require only cAMP for their continued expression although the mechanisms of regulation are different. Dissociation of aggregates results in the rapid loss of both sets of mRNAs but not the cell-type nonspecific gene in the absence of exogenous cAMP. Addition of cAMP to disaggregated cell deprived of cAMP for several hours results in the rapid reaccumulation of the cell-type-specific mRNAs. Dissociation does not decrease these mRNAs in several rapidly developing mutants. Maintenance of mRNA levels may be correlated with the altered cAMP metabolism displayed by these mutants. The structures of the coordinately regulated prespore and prestalk genes are presently being examined. It is hoped that the complementary studies of gene structure and regulation by physiological inducers will clarify the structural features of genes involved in their differential regulation during development.

(1) Mehdy, M. C., Ratner, D., and Firtel, R. A. Cell 32, 763-771 (1983)
(2) Gross, J. D., Bradbury, J., Kay, R.R., and Peacey, M. J. Nature 303, 244-245 (1983)

0862 CONTROL OF CELL TYPE BY THE YEAST MATING TYPE LOCUS, Robert Jensen, Katherine Wilson & Ira Herskowitz, Department of Biochemistry & Biophysics, University of California, San Francisco, San Francisco, CA 94143

The mating type locus (MAT) determines the three yeast cell types, a, @ and a/@. Alleles of this locus, MATa and MAT@, encode regulators that control the expression of unlinked genes required for mating, sporulation and mating type interconversion (for review, see Herskowitz, 1983). MAT@ codes for two regulators, @1 and @2. @1 is a positive regulator required for the expression of "@-specific" genes (for example, the @-factor structural gene, MF@1). @2 negatively regulates the expression of "a-specific" genes (for example, a gene required for a cells to mate, STE6). MATa codes for the a1 protein which has no known role in a cells. However, in MATa/MAT@ diploid cells, the @2 product of MAT@ and the a1 product of MATa specify a third regulator— a1-@2. a1 and @2 together in some way negatively regulate a family of "haploid-specific" functions that include a gene that promotes mating type interconversion (HO).

We are focussing on the mechanism by which the @2 protein controls the expression of two genes— STE6, representative of a class of genes that are negatively regulated by @2 acting alone, and HO, representative of a class of genes negatively regulated by @2 acting in combination with the a1 protein (a1-@2). We have identified DNA sequences required for normal expression of STE6 and HO and find that these sequences are located more than 100 base pairs upstream of the STE6 RNA start site and more than 250 base pairs upstream of the HO RNA start site. Experiments to determine whether these sequences are sufficient to confer cell-type specific control over heterologous genes are in progress.

In addition, we are determining whether there are proteins that specifically interact with the STE6 or HO genes. For example, in DNA protection experiments, do extracts from @ cells contain proteins (perhaps the @2 product itself) that bind to STE6 DNA?

0863 CYTOCHROME P-450 GENES AND THEIR REGULATION, Daniel W. Nebert, Frank J. Gonzalez, Shioko Kimura and Howard J. Eisen, NICHD, NIH, Bethesda MD 20205.

Dozens of adverse chemicals in the environment induce one or more forms of P-450 protein, the terminal oxidase in a membrane-bound multicomponent system. This system is responsible for the metabolism of thousands of drugs, carcinogens, and other foreign chemicals, as well as steroids, fatty acids, prostaglandins and numerous other endogenous substrates. How much diversity exists in each P-450 family, and how many P-450 gene families there are, is presently not known. Phenobarbital-inducible genes appear to span 11 to 15 kb, have at least nine exons, and are clustered on mouse chromosome 7. P_1-450 and P_3-450 represent polycyclic-aromatic-inducible genes in another family: the genes appear to span 4 to 7 kb, have at least five exons, and are closely linked on mouse chromosome 9. Extensive homology over ca. 1000 bp in the 5' portion exists between P_1-450 and P_3-450 cDNA. During P_1-450 induction, polycyclic aromatic compounds bind avidly to the cytosolic Ah receptor ($K_d \sim$ 1nM), the inducer-receptor complex undergoes temperature-dependent translocation into the nucleus, the transcription rates of the P_1-450 and P_3-450 genes are markedly enhanced, a rise in intra-nuclear pre-mRNA can be found, cytoplasmic mRNA levels are dramatically increased, and the newly synthesized P_1-450 and P_3-450 proteins can be immunoquantitated in the endoplasmic reticulum, concomitant with dramatic rises in benzo(a)pyrene and acetanilide metabolism, respectively. Mutant clones of mouse hepatoma Hepa-1 cultures have been characterized as: lacking normal amounts of Ah receptor (r-), nuclear translocation-impaired (nt-), and defective P_1-450 mRNA (nRNA-). New insight should be provided for this interesting system of gene expression by transfecting the appropriate DNA into a particular Hepa-1 mutant cell line.

Gene Expression In Hematopoietic Cell Lineages

0864 TOWARD A MOLECULAR BASIS FOR GROWTH CONTROL IN T LYMPHOCYTE DEVELOPMENT. Ellen Rothenberg, Barry I. Caplan, James P. Lugo, and Rochelle D. Sailor, Division of Biology, California Institute of Technology, Pasadena, CA 91125

A central aspect of the immune responsiveness of T lymphocytes is their proliferation when and only when they encounter a specific antigen. The mature T cell thus needs not only an antigen-specific receptor but also the molecular apparatus that transduces antigen binding as a mitogenic signal. Recently, two key components of this apparatus have been identified. One is the T-cell-specific growth hormone, interleukin-2 (IL-2). The other is the IL-2 receptor. Both molecules are expressed only by antigen-activated T cells and not by resting cells. The IL-2 receptor is expressed by all activated T cells, whereas IL-2 is secreted by a particular subpopulation called "helper" T cells. As long as IL-2 receptor expression is maintained, T cells need only IL-2 to sustain their proliferation (1).

We have been studying the role of this mitogenic mechanism in the thymus, the organ in which the rate-limiting steps in T cell differentiation take place. Thymic processing of the lymphocyte precursors includes a phase of very rapid cell division followed by the destruction of most of the newly-generated cells. These events may have immunological significance, possibly eliminating autoreactive T cell clones and/or selecting for T cells with particular antigen-binding specificities. We have investigated the differences between dividing thymic lymphoblasts, postmitotic thymocytes, and peripheral T cells for two reasons. First, the thymic lymphoblasts are precursors that may still lack certain mature functions. It is of interest, for example, whether or not the lymphoblasts in the helper lineage can be triggered to secrete IL-2. Second, we want to determine whether intrathymic proliferation could be driven by antigen recognition or whether it involves a mechanism that overrides normal growth control.

We have been able to enrich extensively for the proliferating thymic lymphoblasts and to resolve them into subpopulations of different lineages (2,3). Using a sensitive bioassay for IL-2 production, we have found that the competence to produce IL-2 is already present in a particular subset of these lymphoblasts. If these cells are triggered to secrete IL-2 in response to self-antigens *in vivo*, they could stimulate both their own growth and that of neighboring cells. To look for evidence of IL-2 production in the thymus, we have been working to clone the mouse IL-2 gene from a thymoma cDNA library. Current RNA expression results using this probe will be presented.

1. Smith, K. (1980) Immunol. Rev. 51: 337-357.
2. Rothenberg, E. (1982) J. Exp. Med. 155: 140-154.
3. Caplan, B. I. and Rothenberg, E. (1983) submitted for publication.

0865 LYMPHOCYTE SPECIFIC CELL SURFACE RECEPTORS. Irving Weissman, Roger Reichert, Michael Gallatin, Georg Kraal, Pamela Fink, Sophie Ezine, Robert Rouse, and Eugene Butcher. Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305.

The immune system reliably responds to foreign antigens utilizing clonally distributed monospecific antigen receptors on the surface of lymphocytes. The antigen specific receptors on B cells are immunoglobulins, whereas on T cells they represent a new class of molecules whose structure and function is still somewhat unclear. In addition to antigen specific receptors, lymphocytes also bear cell-interaction receptors necessary for cell-cooperation in immune responses, and other cell interaction receptors necessary for the morphogenesis and function of lymphoid organs. Lymphocytes are mobile elements which normally recirculate from the bloodstream into lymphoid tissues and back to the blood. They normally carry on their surface cell membrane receptors that enable them to home to lymphoid organs via recognition and binding to specialized endothelial cells (HEV) lining the postcapillary venules in these organs. We have demonstrated that lymphocytes possess at least two types of independent homing receptors, one to gain entry into the gut-associated lymphoid tissues (such as Peyer's patches), and the other to gain access to peripheral lymph nodes. We have prepared a monoclonal antibody (MEL-14) to the lymph node homing receptor which identifies a 80 kd glycoprotein found only on normal lymphocytes and lymphoma cells capable of binding peripheral node HEV. The MEL-14 antibody blocks in vivo or in vitro binding and homing of cells to peripheral node HEV, but does not affect their binding or homing to Peyer's patch HEV. Following antigen activation, both B and T lymphocytes rapidly lose MEL-14 antigenic determinants. In vivo or in vitro activated antigen-specific T and B cells are therefore unable to home to peripheral lymphoid organs upon release into the bloodstream. This seriously limits the analysis of the in vivo function of antigen-activated T and B lymphoid populations or clones; and may prevent the use of clonal T lymphocyte populations for therapy of antigen bearing virus-infected cells or tumor cells. Nevertheless, in vivo, progeny of these activated lymphocyte cells must regain expression of these receptors, as long-lived memory cells do have the MEL-14 antigen and are able to home.

T cells develop from rare bone marrow cells which seed to the thymus. The expression of the lymph node homing receptor is a late event in thymus cell maturation, and is normally limited to a subset of cells in the thymic cortex. All precursors of killer T cells present in the thymus are contained in the 1-3% MEL-14 positive thymic cells. We have found that the bone marrow has two types of cells capable of clonogenic seeding of the thymus: A population which gives rise to all cortical and a subset of medullary lymphocytes; and another subset which appears to be medulla-limited. The development of medullary cells is somewhat complex, and it is not known if they contribute another subset of antigen-specific functional T cells. We shall describe the appearance of these different thymic lymphocyte subsets during fetal development.

0866 DEVELOPMENTAL CONTROL OF GLOBIN GENE EXPRESSION, Harold Weintraub and Jonathan G. Izant, Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, WA 98104

We have found a supranucleosomal particle that is responsible for packaging inactive genes. This structure may be responsible, in part, for the massive suppression (10^{-7}-10^{-9} or greater) of tissue-specific gene expression in non-expressing tissues (e.g., refs. 1 & 2). In order to try to understand the genetic circuits that regulate whether a given gene is expressed or not and whether it is or is not packaged into these supranucleosomes, we have begun an analysis that attempts to turn-off the expression of a given gene -- in the ideal case, a "regulatory" gene — given the possibility that such a gene will be included within a pool of recombinant DNA clones derived by "plus-minus" screens of cDNA libraries from different tissues. The method depends on microinjection of a recombinant DNA plasmid where the anti-sense RNA is transcribed by a suitably placed promoter. As a model system, we have shown that in the appropriate conditions an anti-sense Herpes T.K. construction prevents the expression of normal Herpes T.K. activity, possibly due to the formation of RNA-RNA hybrids.

1. Groudine, M. and Weintraub, H. (1975) Rous Sarcoma Virus Activates Embryonic Globin Genes in Chicken Fibroblasts. Proc. Natl. Acad. Sci. USA 72, 4464-4468.
2. Ivarie, R.D., Schacter, B.S., and O'Farrell, P.H. (1983) The level of expression of the rat growth hormone gene in liver tumor cells is at least eight orders of magnitude less than that in anterior pituitary cells. Molec. Cell. Biol. 3, 1460-1476.

0867 TRANSCRIPTION OF CLONED HUMAN α- AND β-GLOBIN GENES IN ERYTHROID AND NONERYTHROID CELLS, Patrick Charnay, Pamela Mellon, Moses Chao, Richard Axel and Tom Maniatis, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge, MA 02138 and Institute of Cancer Research, Columbia University, College of Physicians and Surgeons, 701 West 168th Street, New York, New York 10032.

We have investigated the regulation of cloned human α- and β-globin genes introduced into mouse erythroleukemia (MEL) cells by DNA mediated gene transfer. The human β-globin gene, as well as a hybrid mouse-human β-globin gene, is appropriately regulated during MEL cells differentiation in culture (1,2). In approximately 80% of the lines examined, the addition of dimethylsulfoxide (DMSO) results in a 5 to 50 fold increase in the level of mRNA transcribed from the exogenous genes. In contrast, the transcription of cloned α-globin genes introduced into MEL cells was not correctly regulated in 35 out of 36 cloned lines examined as well as in pools of independent colonies. Typically, the level of human α-globin mRNA remains constant upon DMSO induction. When both human α- and β-globin genes are introduced on the same plasmid into MEL cells, the constitutive level of α-globin mRNA is greater than the induced level of β-globin mRNA. Based on these data and on the differential behavior of human α- and β-globin genes in nonerythroid cells (3,4), we postulate a model involving a fundamentally different regulation of α- and β-globin gene expression in erythroid cells. To define the sequences required for transcription and transcriptional regulation of the mouse β-major globin gene in MEL cells, we have constructed a series of 5' deletions in the 5' flanking region of the gene and of linker substitutions in the 110 bp region immediately upstream to the mRNA capping site. To date, we have shown that sequences upstream to -78 are not required for regulation in MEL cells. The sequences required for efficient transcription appear to be the same in MEL and HELA cells and consist of the TATA and CAAT box regions and an upstream region located between -94 and -84.

1. Chao, M.V., Mellon, P., Charnay, P., Maniatis, T. and Axel, R. (1983) Cell 32, 483-493.
2. Wright, S., DeBoer, E., Grosveld, F.G. and Flavell, R.A. (1983) Nature 305, 333-336.
3. Humphries, R.K., Ley, T., Turner, P., Moulton, A.D. and Nienhuis, A.W. (1982) Cell 30, 173-183.
4. Treisman, R., Green, M.R. and Maniatis, T. Proc. Nat. Acad. Sci. USA, in press.

Molecular Aspects of Plant Development

0868 ORGANIZATION AND EXPRESSION OF DEVELOPMENTALLY REGULATED GENES IN SOYBEAN, Robert B. Goldberg, Department of Biology, University of California, Los Angeles, CA 90024.
Soybean seed protein genes represent an excellent model for the study of gene regulation during plant development. Mutant lines exist which are unable to synthesize specific seed proteins. Experiments will be discussed which provide insight into the molecular basis of several seed protein gene mutations. In addition, comparative DNA sequencing studies will be presented which indicate that there is a short consensus sequence in the 5' upstream region of several different seed protein genes.

0869 CONTROLLING ELEMENTS AND GENE EXPRESSION IN MAIZE, Peacock, W.J., Dennis, E.S., Ellis, J., Finnegan, E.J., Gerlach, W.L., Llewellyn, D. and Sachs, M.M.

The alcohol dehydrogenase genes of maize have provided us with a system to study plant gene structure and expression. The two genes have common ancestry, are unlinked, have tissue-specific expression, and are induced by anaerobic conditions. Sequence analysis has shown that both loci have nine intervening sequences, the introns being in identical positions but differing in length and sequence.

We have identified sequences in the 5' and 3' flanking regions which may be important for the environmental and developmental control of gene expression, and which may influence the probability of recombination at the Adh1 locus. Differences in the polyA addition signal of animals and plants will be discussed.

The Adh1 locus has also provided us with the opportunity of studying a Ds controlling element. The Ds element in the Adh1-Fm335 mutant is inserted between the transcription and translation start points and markedly modifies transcription levels and RNA processing. We have defined sequence events at Ac-induced insertion and excision of the Ds element and examined the effects of sequence perturbation on transcription of the Adh1 gene.

We will also describe other Ds elements isolated from the maize genome and will comment on the sequences of terminal and internal segments. Genetic and developmental characteristics of the Ac/Ds system will be discussed in relation to the molecular data.

0870 HORMONAL REGULATION OF EMBRYO-SPECIFIC STORAGE PROTEIN GENES IN BRASSICA NAPUS.
Martha L. Crouch and Karen M. Tenbarge, Department of Biology. Indiana University. Bloomington, IN 47405

Two major storage proteins, cruciferin and napin, accumulate during embryo development in Brassica napus (oil seed rape). Using antibodies, the proteins can first be detected in embryos about 24 days after fertilization (60 days = mature). They accumulate for about 20 d until the embryo desiccates and becomes quiescent. Both proteins disappear rapidly upon germination (1).

If embryos are removed from the seed at the beginning of storage protein synthesis and cultured on medium containing the growth regulator abscisic acid (ABA), they will show the normal increase in storage protein accumulation observed in the seed. Without exogenous ABA, storage protein synthesis declines to a low level and the embryos germinate precociously (1). Therefore, we can experimentally modulate the level of storage protein synthesis and dissect the process at the molecular level.

We have made and characterized cloned cDNAs for B. napus storage proteins (2) and used them as probes to show that exogenous ABA acts at the level of mRNA accumulation. By densitometric scanning of Northern blots, we measured levels of both cruciferin and napin mRNAs relative to ribosomal RNA in embryos cultured for different lengths of time with or without ABA. The embryos were put into culture at 26 d after fertilization, at which time the level of napin mRNA is already high and cruciferin mRNA is still increasing. In the presence of 5×10^{-6} M ABA, napin mRNA levels remain at the same high level while cruciferin mRNA levels increase at least three-fold over 3 d in culture. In contrast, on the basal medium there is a dramatic and rapid decrease in levels of both mRNAs relative to ribosomal RNA. After just 2 h of culture, cruciferin mRNA levels have decreased five-fold, reaching a level 50-100 times lower than in the starting embryos by 6 h of culture. Cruciferin mRNA levels stay at this low level for at least several weeks if the embryos are kept on basal medium. Napin mRNA levels respond in a similar fashion. However, if the embryos growing on basal medium are transferred to medium with exogenous ABA, the low storage protein mRNA levels are restored to high levels within a day. Therefore, even though these embryos are showing morphological signs of germination, they are expressing embryo-specific genes at low levels, and the genes are still capable of responding to hormonal regulation. We are in the process of determining the relative contributions of synthesis and turnover rates to the changes in mRNA levels, and studying cloned genes.

1) Crouch, M.L. and I.M. Sussex. Planta 153:64-74 (1981). 2) Crouch, M.L., K.M. Tenbarge. A.E. Simon, and R. Ferl. J. Mol. Appl. Gen. (1983), in press.

0871 EXPRESSION OF STORAGE PROTEIN GENES DURING MAIZE ENDOSPERM DEVELOPMENT.
B.A. Larkins, M.D. Marks, L.Z. Morand, and K. Pedersen, Dept of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.

During development of a maize seed large amounts of protein are synthesized and deposited in the endosperm. About half of this protein is composed of a group of alcohol-soluble polypeptides that are known as zeins. When separated by 2-dimensional gel electrophoresis zeins are resolved into several groups of polypeptides with apparent mol wts of 27,000, 22,000, 19,000, 15,000 and 10,000. These proteins are synthesized by polysomes bound to rough endoplasmic reticulum (RER), and are transported inside the lumen of the RER where they associate and form dense insoluble masses called protein bodies. An analysis of zeins synthesized at different stages of development indicates slight differences in the initiation of their synthesis; however, the expression of these genes is tightly coordinated throughout the period of seed formation.

We have used cDNA clones corresponding to different families of zein mRNAs to study the organization of these genes in the maize genome and their expression during seed development. Southern hybridization analysis indicates that zeins are encoded by several families of genes. There appear to be 20-25 genes encoding the Mr 22,000 zeins, and there are at least two families of Mr 19,000 zein genes with 20 members each. On the other hand, the Mr 27,000, Mr 15,000 and Mr 10,000 proteins appear to be encoded by genes present in one or two copies. From genetic analyses it has been shown that genes encoding proteins of similar mol wt are clustered on the same chromosome. In support of this data, we have isolated several genomic clones containing multiple copies of Mr 19,000 zein genes. These genes are arranged tandemly 5' to 3'. DNA sequence analysis indicates that the genes are closely related, and one appears to be a pseudo gene since it has a premature stop codon in the coding sequence.

In certain mutant genotypes, such as opaque-2, zein synthesis is delayed several days, and it ceases midway through endosperm development. This results in a 50% reduction in total zein protein. Although all classes of zein polypeptides are reduced, the Mr 22,000 proteins are nearly absent. The reduced levels of these proteins appears to result from alterations in gene transcription or post-transcriptional processing.

0872 GENE SETS ACTIVE IN COTTONSEED EMBRYOGENESIS. Leon Dure III, Caryl A. Chlan, Jean C. Baker and Glenn A. Galau, Department of Biochemistry, University of Georgia, Athens, GA 30602.

The embryogenic development of cottonseed is characterized by the rise/fall in abundance of seven subsets of mRNA as shown by the examination of the 2D electrophoretic patterns of proteins synthesized in vivo and in vitro from extracted RNA, both at time points during embryogenesis. The expression of each set appears to be regulated independantly in time. cDNA copies of several representatives of each set have been cloned, identified by hybrid selected and arrested translation, and used to follow more precisely the changes in levels of the mRNAs during development by dot and solution hybridization.

Several of these sets appear to be the products of developmental genes in that they are expressed in an all-or-nothing fashion during a specific phase of embryogenesis. e.g. The genes of the cottonseed storage proteins. Other sets appear to emanate from genes that are constitutively "on", but whose expression is up/down modulated during development.

Genomic DNA containing genes represented in several of the sets has been isolated from a gene bank and sequenced in order to search for sequences involved in their developmental expression or in the up/down modulation of their constitutive expression.

Transcription and Gene Expression in Embryos I

0873 SEX DETERMINING IN DROSOPHILA, B. Baker, M. Wolfner, J. Belote, Biology Department, University of California, San Diego, La Jolla, California 92093

Sex determination in eukaryotes provides one of the most striking examples of the differential control of gene expression during development. Although sex determination in D. melanogaster was one of the first major developmental processes demonstrated to be under genetic control (Bridges, 1921), only recently have we begun to appreciate and piece together the complex regulatory hiearchy controlling sexual devdlopment. The functions that are responsible for sex determination can be divided into three categories; 1) the X:autosome (X:A) ratio, the primary determinant of sex and dosage compensation, 2) a set of regulatory loci (Sxl, dsx, tra, tra-2, ix) whose expression is controlled by the X:ratio, which in turn control the expression of 3) the batteries of genes encoding sex-specific differentiation functions. The X:A ratio appears to act early in development to determine both sex and dosage compensation irreversibly (Baker, in preparation).

By defining the phenotypes of null mutations at four of the regulatory loci controlling sex determination and characterizing the phenotypes of double mutant combinations, we have developed a model for how these gene products interact to determine sex (Baker & Ridge, 1980). dsx is a bifunctional locus which can be expressed in either of two ways: in male individuals, dsx^+ functions to repress female differentiation, whereas in female flies, dsx^+ represses male sexual differentiation. The tra^+ and $tra\text{-}2^+$ gene products are thought to act in chromosomally female individuals to maintain the dsx^+ gene in the female mode of expression. The product of the ix^+ gene is required in females for the suppression of male sexual differentiation. In males the tra^+ and $tra\text{-}2^+$ loci are not expressed; as a consequence dsx^+ is expressed in the male mode.

To extend our knowledge of this regulatory hiearchy to the molecular level, we are cloning and characterizing the regulatory genes. Our initial efforts focused on the dsx locus with the initial aim of understanding the molecular basis for the active but opposite roles it plays in controlling sex determination in males and females (Baker & Wolfner, in prep.). The dsx locus has been cloned and the analysis of mutants at dsx show it is at least 27kb. The DNA organization of dsx appears to be the same in both sexes. However Northern analysis shows that different size transcripts homologous to dsx DNA are found in males and females.

The analysis of temperature-sensitive tra-2 alleles has shown that this locus is expressed throughout the larval, pupal and adult periods and acts, even within single cell lineages, to determine different aspects of sex at different times (Belote & Baker, 1982). The ts tra-2 mutants have also been used to show that this locus regulates the expression of three female-specific sexual differentiation functions (the yolk protein (YP) genes) at the transcriptional level: functional tra-2 product is needed in the adult both to turn on and to maintain transcription of the YP genes (Belote et al., in preparation).

0874 A GENETIC, DEVELOPMENTAL, AND MOLECULAR ANALYSIS OF THE ENGRAILED LOCUS,
Thomas Kornberg, Zehra Ali, Barry Drees, Jerry Kuner, Patrick O'Farrell, and M. Nakanishi, Department of Biochemistry & Biophysics, University of California, San Francisco, San Francisco, California 94143

The best evidence indicating how genes control development is provided by the homeotic mutants of Drosophila. These mutations transform one body part into another, normal structures appearing in abnormal locations. It is believed that these genes have evolved to control the developmental pathway of the individual segment, each segment an independent experiment in evolution. There also exist genetic functions that initially set aside the cells that will construct each of the segments, the cells in which the homeotic gene functions will be expressed. We call these merismatic genes, from the Greek merismos, meaning to subdivide the whole into parts. We have subjected one such genetic locus, engrailed, to intensive genetic, developmental, and molecular analysis.

Mutations in the engrailed locus define a single complementation group in a region in excess of 60 kb of DNA. Animals deficient for engrailed function die during embryogenesis, defective in segmentation. Developmental and molecular analysis indicate a time dependent and position specific requirement for engrailed locus expression.

0875 HOW MOUSE EGGS PUT ON AND TAKE OFF THEIR EXTRACELLULAR COAT,
Paul M. Wassarman, Jeffrey M. Greve, Rosario M. Perona, Richard J. Roller, and George S. Salzmann, Department of Biological Chemistry, Harvard Medical School, Boston, MA 02115

During a two to three week period, as mouse oocytes grow from 12 to 85 μm in diameter, a relatively thick (7 μm) extracellular coat appears that completely surrounds the oocyte's plasma membrane. This extracellular coat, called the zona pellucida, serves important functions during fertilization and early development, and is not shed by embryos until just prior to implantation (day-5 of embryonic development). In mice, the zona pellucida is composed of three different glycoproteins, designated ZP1 (200 kd), ZP2 (120 kd), and ZP3 (80 kd) (1). Since synthesis of these relatively abundant glycoproteins is stringently regulated with respect to both cell type and stage of development (2-5), they are attractive candidates for studies of the regulation of gene expression during early mammalian development. Furthermore, since hatching of mouse embryos from the zona pellucida involves a specific differentiative event at a particular time during early embryogenesis, this too represents an attractive system for study. I will discuss various aspects of these topics.

1. Bleil, J.D., and Wassarman, P.M., Develop. Biol. 76: 185 (1980)
2. Bleil, J.D., and Wassarman, P.M., Proc. Natl. Acad. Sci., USA 77: 1029 (1980)
3. Greve, J.M., Salzmann, G.S., Roller, R.J., and Wassarman, P.M., Cell 31: 749 (1982)
4. Salzmann, G.S., Greve, J.M., Roller, R.J., and Wassarman, P.M., EMBO J. 2: 1451 (1983)
5. Roller, R.J., and Wassarman, P.M., J. Biol. Chem. 258: in press (1983)

Supported by the National Institute of Child Health and Human Development

0876 GENE ORGANIZATION AND INTERACTION IN MOUSE t-HAPLOTYPES, Dorothea Bennett, Karen Artzt, Hee-Sup Shin, Laboratory of Developmental Genetics, Sloan-Kettering Institute, New York, NY 10021.

Seven different, but functionally related [1], t-lethal mutations have been mapped and found to occure in 3 clusters distributed over 14 cM of chromosome 17. The largest cluster closely flanks the H-2 complex, which is rearranged and inverted in t-haplotypes [2,3]. In the two cases examined, mutations belonging to the same complmentation group map to the same location. The data suggest that the t-lethal factors of the t^{12} complementation group represent dual genetic lesions, rather than a single mutant site, and that there is significant interaction in cis among the mutant sites of different lethal factors that determine the ultimate lethal phenotype.

[1] Shin, H-S., McCormick, P., Artzt, K., and Bennett, D. (1983) Cis-trans test shows a functional relationship between non-allelic lethal mutations in the T/t-complex. Cell 33: 925-929.
[2] Artzt, K., Shin, H-S., and Bennett, D. (1982) Gene mapping with the T/t complex of the mouse. II. Anomalous position of the H-2 complex in t-haplotypes. Cell 28: 471-476.
[3] Shin, H-S., Flaherty, L., Artzt, K., Bennett, D., and Ravetch J. DNA rearrangement of the H-2 complex in t-haplotypes of mice. Nature, in press.

Transcription and Gene Expression in Embryos II

0877 GENE EXPRESSION DURING EMBRYOGENESIS IN XENOPUS LAEVIS,
Igor B. Dawid, Milan Jamrich, Erszebet Jonas, Brian K. Kay, Seiji Miyatani, Thomas D. Sargent and Jeffrey A. Winkles, Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20205

The unfertilized egg of Xenopus laevis contains a supply of $polyA^+$ RNA which includes sufficient mRNA to support protein synthesis at least through cleavage stages (1). However, gastrulation appears to require transcription of the embryonic genome, suggesting the existence of a set of gastrula RNA molecules that are not included in maternal RNA. Because egg and gastrula $polyA^+$ RNA populations are very similar, we utilized a selective cDNA cloning procedure to prepare a library highly enriched in sequences that are present in gastrulae but absent in eggs, which we call DG RNAs (2). We are engaged in detailed analysis of several cloned sequences from this DG library.

DG 42 represents an mRNA that becomes quite abundant at gastrulation (about 0.2% of $polyA^+$ RNA), and disappears thereafter. A fragment of this cDNA clone has been inserted into a bacterial expression vector (MR100, ref. 3). The fusion protein produced in this way is being used to raise antiserum that is expected to react specifically with the natural DG 42 protein. DG 42 genomic clones have also been isolated and are being analyzed. Other cloned DG RNA molecules range from undetectable to quite abundant in tadpole RNA, and the spatial distribution of several of these sequences is being studied by in situ hybridization.

1. Dawid, I.B., Kay, B.K., and Sargent, T.D. (1983). Symp. Soc. Dev. Biol. 41, 171-182.
2. Sargent, T.D. and Dawid, I.B. (1983). Science 222, 135-139.
3. Gray, M.R., Colot, H.V., Guarente, L., and Rosbash, M. (1982). Proc. Nat. Acad. Sci. 79, 6598-6602.

0878 NUCLEAR ACTIVITY AND CELL CYCLE REGULATION IN THE XENOPUS EMBRYO,
Marc W. Kirschner, John W. Newport*, and Douglass J. Forbes*, University of California at San Francisco, San Francisco, California, 94143, and University of California at San Diego*, La Jolla, CA, 92093.

Early embryogenesis in Xenopus is divided into two stages with respect to transcription. The first stage, which lasts from fertilization through 12 synchronous cleavages, lacks detectable RNA synthesis. In the second stage, transcription is abruptly activated, cells become motile, and cell division slows and becomes asynchronous (1). Among the major transcripts at this transition (termed the mid-blastula transition) are tRNAs and the small nuclear RNAs, U1, U2, U4, U5, and U6 (2). It appears a specific factor is stored in the cytoplasm and inhibits transcription, until at the twelfth cleavage, titration of the factor by the replicating DNA allows the inhibition to be relieved and transcription to be activated (3). Although we originally thought that this factor might be binding directly to the DNA, recent experiments have caused us to reevaluate this conclusion. We have found that when bacteriophage lambda DNA is injected into Xenopus eggs, the DNA is assembled into synthetic "nuclei". These nuclei are structurally identical by a number of criteria to normal eukaryotic nuclei: they are bounded by a double bilayer membrane separated by a perinuclear space and interrupted by nuclear pores. The inner nuclear membrane is lined by a nuclear lamina composed of one or more of the lamin proteins, as judged by immunofluorescence. The synthetic nuclei respond to several cell cycle modulators in a way identical to that in which normal nuclei respond (4). We conclude that the Xenopus embryo contains a store of all the protein components necessary to form the nucleus and simple addition of DNA is sufficient to seed the assembly of these components into nuclei. Thus the factor that the DNA is titrating could be any molecule involved in the formation of the nucleus, from a DNA binding protein to a structural nuclear protein. To attempt to investigate the turn-on of transcription and the assembly of the nucleus at a more molecular level, we have begun to use the small nuclear RNA, U1 (a major product at the onset of transcription), both as a probe for transcriptional control and as a model molecule for examining nuclear functions such as transport and binding of nuclear molecules.

1&3. Newport and Kirschner, Cell 30, 675-686 and 687-696 (1982).
2. Forbes, Kornberg, and Kirschner. J.Cell Biol. 97, 62-72 (1983).
3. Forbes, Kirschner, and Newport. Cell 34, 13-23 (1983).

0879 GENE EXPRESSION IN THE SEA URCHIN EMBRYO, Eric H. Davidson, Rosemary J. Shott, Samuel J. Rose, III, James J. Lee, Carlos V. Cabrera, Frank J. Calzone, Roy J. Britten, Division of Biology, California Institute of Technology, Pasadena, CA 91125.
Patterns of gene expression in sea urchin embryos are described, as revealed by recent investigations on a number of clones representing poly(A) RNAs. About 90% of the non-mitochondrial transcript species in the late embryo cytoplasm, at all prevalence levels, are also represented in the RNA of the unfertilized egg, and about 10% appear anew during development. Measurements of synthesis and turnover rates have indicated the following: 1) cytoplasmic turnover is a major factor in determining embryo transcript prevalence; 2) rate of transcription varies from several initiations per min for highly active genes to < 1 per hr for genes coding for rare cytoplasmic sequences; 3) there is a different point in development when maternal sequences are replaced by new transcripts for each transcript studied. Contrary to previous assumptions, maternal transcripts of some species persist to the end of embryogenesis. Expression of the sea urchin actin genes has been studied using probes specific for each of the genes. There are 8 actin genes in the _S. purpuratus_ genome, of which at least 6 are active. This genome contains one muscle actin gene. The remainder code for cytoskeletal actins. Each of the actin genes displays a unique pattern, being expressed in specific extents as particular cell types, and sometimes in specific stages of the life cycle.

0880 NOVEL PROTEINS BELONGING TO THE TROPONIN C SUPERFAMILY ARE ENCODED BY A SET OF MRNAS IN SEA URCHIN EMBRYOS, William Klein, Department of Biology, Indiana University, Bloomington, IN 47405.

The properties of several cDNA clones representing a family of mRNAs found in the embryonic ectoderm of _Strongylocentrotus purpuratus_ are described. These mRNAs, termed Spec for _Strongylocentrotus purpuratus_ ectoderm, accumulate in the presumptive dorsal ectoderm of post-cleavage stage embryos and code for a group of 10 to 12 low molecular weight acidic proteins (1, 2). Using antibodies raised against the major Spec protein we demonstrate that the protein is localized in the cytoplasm of dorsal ectoderm cells. Hybridization analysis and DNA sequencing show that the mRNAs coding for the Spec proteins, although all related, can be divided into two subfamilies: Spec 1 type mRNAs are 1.5 kb in length, begin to accumulate at the blastula stage, and code for the major Spec protein; Spec 2 type mRNAs are 2.2 kb in length, begin to accumulate at the gastrula stage and code for several of the minor Spec proteins (3). Comparison of the translational reading frames of the Spec mRNAs with known protein sequences shows a significant homology with troponin C-related proteins, especially in the calcium-binding domains. Based on structural considerations, the major Spec protein is more troponin C-like and the minor Spec proteins are more calmodulin-like, although it is clear none of the Spec proteins are troponin C or calmodulin. We suggest that the Spec proteins are previously uncharacterized members of the troponin C superfamily and that they are involved in the changes in shape of dorsal ectoderm cells which occur during larval development and metamorphosis.

(1) Bruskin, A.M., Bedard, P.A., Tyner, A.L., Showman, R.M., Brandhorst, B.P., and Klein, W.H. (1982). Develop. Biol. 91:317-324.
(2) Lynn, D.A., Angerer, L.M., Bruskin, A.M., Klein, W.H., and Angerer, R.C. (1983). Proc. Natl. Acad. Sci. USA 80:2656-2660.
(3) Klein, W.H., Spain, L.M., Tyner, A.L., Anstrom, J., Showman, R.M., Carpenter, C.D., Eldon, E.D., and Bruskin, A.M. (1984). The Molecular Aspects of Early Development. G.M. Malacinski and W.H. Klein, eds. Plenum Press, Inc. New York.

0881 SPATIAL AND TEMPORAL SPECIFICITY IN THE EXPRESSION OF CELL SURFACE ANTIGENS DURING DEVELOPMENT. David R McClay and Gary M Wessel, Dept. of Zoology, Duke University, Durham, NC. 27706.

Ectoderm, mesoderm, and endoderm cell surfaces of the sea urchin embryo are shown to differ in a number of antigens as early as the gastrula stage. More than 100 monoclonal antibodies were found in a screen that selected for germ layer-specific expression at the cell surface. These antibodies were studied to establish the variety of patterns by which antigens reached the cell surface and became specific or selective to one germ layer. Many of the antigens were found in the egg. These were compartmentalized during development by a variety of patterns and at timetables that were unique for many of the antigens. Some of the antigens became localized to parts of a germ layer, or were distributed in an area between two germ layers. Western blot analysis of the antigens indicated that for those tested, the antibodies identified molecules with the same molecular weight throughout development. There were some apparent shifts, however, in antigen location by subcellular fractionation studies.

Some antigens were detected *de novo* during development. One family was detected at the mesenchyme *blastula* stage. These appeared on the surface of primary mesenchyme cells at a very precise time for each of the +/- 30 primary mesenchyme cells. About 30 min. later an endoderm-specific antigen appeared. This antigen ultimately became confined to the midgut and hindgut of the embryo. Again, Western blots showed these antigens to be the same throughout development.

Other examples of antigens detected included an antigen with a gradient-like distribution, antigens that were localized to highly restricted areas of the embryo, appearance and later the loss of an antigen, and temporal differences in appearance of the same antigen in two species. These data demonstrate a dramatic change in cell surfaces at gastrulation and show that these changes can originate by a number of pathways. Supported by USPHS HD 14483.

0882 TRANSLATIONAL CONTROL OF GENE EXPRESSION IN EARLY DEVELOPMENT, Joan V. Ruderman, Nancy M. Standart, Eric T. Rosenthal, Department of Anatomy, Harvard Medical School, Boston MA 02115, and Tim Hunt, Department of Biochemistry, University of Cambridge, Cambridge, England CB2 1QW.

The mature oocytes of most animals, including the marine clam Spisula, contain a large pool of mRNA. Before fertilization, Spisula oocytes translate a small subset of mRNAs; within 10 minutes after fertilization, those mRNAs are released from polysomes and another, completely different subset of pre-existing mRNAs are translationally activated and recruited onto polysomes (1, 2). This translational switch leads to dramatic changes in the kinds of proteins that are made at fertilization. The activation of certain mRNAs is essential for development to proceed: it has long been known that, despite the possession of a large maternal stockpile of structural proteins and enzymes, ongoing protein synthesis is absolutely required for the embryo to proceed through first cleavage and beyond. What is responsible for the translation repression of one group of mRNAs and the activation of another group right after fertilization? What roles do these translationally regulated proteins play? Why is it so important for certain mRNAs to be kept inactive until fertilization? Using cDNA clones complementary to maternal mRNA sequences, we have found that the mRNAs active in the oocyte are poly(A)+ and lose their poly(A) tails when they are released from polysomes after fertilization, whereas mRNAs that are stored in the oocyte in an inactive form are poly(A)-deficient and gain a poly(A) tail when they are activated in the embryo. No other gross structural changes are detectable. The two most abundant stored mRNAs, those encoding proteins called A and C, have been sequenced. Comparisons for potential translational regulatory sequences are underway. As to the functions of these proteins some hints are beginning to surface. Protein A (53 kd), also known as cyclin, is synthesized continuously after fertilization and is destroyed at each cleavage division (3). Protein C (40 kd) is homologous to a Herpes virus 39 kd early protein that is closely associated with ribonucleotide reductase, a key enzyme in DNA synthesis.

1. Rosenthal, Hunt and Ruderman (1980) Cell 20, 487.
2. Rosenthal, Tansey and Ruderman (1983) J. Mol. Biol. 166, 309.
3. Evans, Rosenthal, Youngblom, Distel and Hunt (1983) Cell 33, 389.

Whole Organism Transformation I

0883 AMPLIFICATION AND EXPRESSION OF DROSOPHILA CHORION GENES, Allan C. Spradling, Diane V. de Cicco, Laura J. Kalfayan, Joseph F. Levine, Suki Parks, and Barbara T. Wakimoto, Department of Embryology, The Carnegie Institution of Washington, 115 W. University Pkwy., Baltimore, MD 21210.

During oogenesis, Drosophila ovarian follicle cells specifically amplify and selectively express chorion (eggshell) genes contained in two separate gene clusters. The amplification of these genes and 80-100 kb of flanking sequences occurs by a chromosomal mechanism; repeated rounds of local replication are initiated at a site or sites within each cluster (1,2). Individual chorion genes are then expressed at specific times during the process of eggshell synthesis. We have used P element-mediated gene transfer (3) to investigate the location and structure of presumptive amplification origins in the vicinity of the chorion genes, and to define cis-acting sequences necessary for developmentally regulated chorion gene expression. In the case of both gene clusters, only transposons containing a specific central 4-5 kb region induced amplification. Although the tissue-specificity of induced amplification was unchanged, the behavior of origin-containing transposons was subject to position effects, and fewer rounds of replication usually occurred than normal. Chorion genes introduced into germ line chromosomes within transposons containing varying amounts of flanking sequences were also analyzed for their ability to function with the appropriate tissue and temporal specificity. Regardless of whether amplification occurred, mRNA was produced from the transformed gene according to its normal developmental program. As little as 1.3 kb of DNA containing the 5' end of the gene was sufficient for transcription to occur with its characteristic time and tissue specificity. The ability of defined sequences to program normal gene expression allows the construction of fusion genes which can simplify the analysis of gene regulation and function.

1. Spradling, A. (1981) Cell 27, 193.
2. Osheim, E. and Miller, O.J. (1983) Cell 33, 543.
3. Spradling, A. and Rubin, G. (1983) Cell 34, 47.

0884 white LOCUS DNA TRANSFORMATION IN DROSOPHILA: DOSAGE COMPENSATION, zeste INTERACTION AND POSITION EFFECTS, Tulle Hazelrigg, Robert Levis, and Gerald M. Rubin, Department of Biochemistry, University of California, Berkeley, Berkeley, CA 94720

The expression of the white locus in Drosophila is regulated in several ways. First, it is located on the X chromosome and is dosage compensated. Second, the white locus is subject to transvection effects in an interaction which occurs with the zeste locus. Third, the expression of the white locus is sensitive to genomic position effects. We are using P-element mediated DNA transformation to define the DNA sequences of white which mediate these regulatory events. Transduced copies of an 11.7 kb segment of DNA, inserted at many different chromosomal sites, rescue the white phenotype, and are properly dosage-compensated. These same transduced copies of white DNA show variable repression by $zeste^1$. Two transformants have novel patterns of eye pigmentation which appear to be due to position effects on the expression of the transduced white DNA. Recent results of transformation with constructs containing deletions of putative regulatory sequences of the white locus will be reported.

0885 REGULATION OF EXPRESSION OF THE DROSOPHILA 68C GLUE GENE CLUSTER, Elliot M. Meyerowitz, Madeline A. Crosby, Thomas E. Crowley, Mark D. Garfinkel, Christopher H. Martin and Peter H. Mathers, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

The polytene chromosome puff found in the 68C region of the third chromosome in Drosophila melanogaster salivary glands contains the genes for three coordinately expressed salivary gland secretion proteins, sgs-3, sgs-7 and sgs-8. These proteins are part of the "glue" that is secreted by third instar larvae just prior to pupariation, and is used to affix the puparial case to a solid surface for the pupal period (1). The mRNAs for the three proteins are coded in a region of less than 5,000 base pairs, and are coordinately expressed in both tissue and time (2). While the signals that initiate the expression of these three RNAs are not known, accumulation of newly synthesized RNA from each of the glue genes is rapidly and directly repressed by the steroid hormone ecdysterone (3). One question raised by this system is that of the coordination of expression of the three RNAs: is each RNA controlled independently of the others, each with its own set of regulatory sequences, or is there a single control region responsible for puffing or expression of all three genes? The DNA sequence of the three genes and of their flanking regions shows no common sequence elements upstream or downstream of the genes (4), indicating that a simple model in which each gene has identical controlling sequences is untrue. The reintroduction of cloned 68C sequences to the fly genome by P-factor mediated transformation has shown that the Sgs-3 gene acts autonomously; it expresses in the normal tissue, at its normal time, and in normal amounts when integrated at locations other than 68C without the Sgs-7 and Sgs-8 genes adjacent. So far, though, the Sgs-7 and Sgs-8 genes have not been found to express when reintroduced to the fly genome without an adjacent Sgs-3 gene. In these transformation experiments it is possible to distinguish between the expression of introduced and resident Sgs-3 genes, because the two genes code for different wild-type length variants of the Sgs-3 mRNA. No such variants are known for Sgs-7 or Sgs-8, thus the introduced genes have been altered to change the length of the RNAs they will express. It is possible that these alterations have affected the stability of the RNAs. Experiments now in progress will test if the altered Sgs-7 and Sgs-8 genes will express when reintroduced with the Sgs-3 gene adjacent.

(1) Crowley, T. E., Bond, M. W., and Meyerowitz, E. M. (1983) Mol. Cell. Biol. 3:623-634.
(2) Meyerowitz, E. M., and Hogness, D. S. (1982) Cell 28:165-176.
(3) Crowley, T. E. and Meyerowitz, E. M. (1983) Devel. Biol., in press.
(4) Garfinkel, M. D., Pruitt, R. E., and Meyerowitz, E. M. (1983) J. Mol. Biol. 168:765-789.

0886 TRANSFORMATION OF ASPERGILLUS NIDULANS USING A TRPC PLASMID, William E. Timberlake, M. Melanie Yelton, John E. Hamer and Edward J. Mullaney, Department of Plant Pathology, University of California, Davis, CA 92037

We have constructed a chimeric plasmid carrying a complete wild-type copy of the trifunctional trpC gene from the Ascomycete fungus Aspergillus nidulans. This plasmid, designated pHY201, replicates in E. coli, where if confers resistance to ampicillin and chloramphenicol and complements trpC mutants lacking phosphoribosylanthranilate isomerase activity. We have used pHY201 to transform an A. nidulans $trpC^-$ strain to $trpC^+$ at frequencies of greater than 20 stable transformants per ug of DNA. Southern blot analysis of DNA from transformants showed that pHY201 DNA had integrated into the A. nidulans chromosomes in a majority of cases. Most of the integration events appeared to occur at the site of the $trpC^-$ allele of the recipient strain. In several instances, we have succeeded in recovering pHY201, or derivatives thereof, from A. nidulans transformants by restriction digestion of chromosomal DNA, ligation and transformation of E. coli. Thus, pHY201 has properties which suggest that it may serve as a valuable prototype for the development of more sophisticated Aspergillus cloning vectors. Preliminary data indicate that the trpC gene is appropriately regulated in transformants regardless of its position in the genome.

0887 TRANSFER AND REGULATED EXPRESSION OF GENES IN PLANTS, Jeff Schell*[+], Marc Van Montagu*, Patricia Zambryski*, Luis Herrera-Estrella*, Lothar Willmitzer[+], Fritz Kreuzaler[+], Ann Depicker*, *Laboratorium voor Genetika, Rijksuniversiteit Gent, B-9000 Gent, [+]Max-Planck-Institut für Züchtungsforschung, D-5000 Köln 30
Large plasmids in Agrobacterium tumefaciens (Ti) and A.rhizogenes (Ri) are known to endow these bacteria with the capacity to transfer a defined DNA fragment (T-DNA) into the plant cell nucleus and to covalently integrate this T-DNA segment in chromosomal DNA, thus creating a new locus at a number of possible sites. Under normal circumstances only the T-region of the Ti-plasmid is inserted in the chromosomal DNA and thus stably maintained. No functions located within the T-region are required for either transfer or integration. The plasmid derived T-DNA was shown to consist of a number of well defined transcriptional units transcribed by the host polymerase II and coding for a number of different functions, i.e. enzymes involved in opine synthesis and functions involved in the inhibition of plant differentiation Thus separate shoot suppressing and root suppressing functions have been identified. By a combination of in vivo and in vitro recombinant DNA techniques it was possible to eliminate by deletion mutations each of the differentiation controlling onc genes, either singly or in various combinations. - These studies indicated that the genes that code for shoot inhibition simultaneously code for a stimulation of root formation and that reciprocally the gene coding for root inhibition simultaneously stimulates shoot formation. Removal of all these tumor controlling genes does not affect DNA transfer or integration. Thus it was possible to design modified Ti-plasmids that can insert foreign genes in plant cells from which normal plants can be regenerated that express the foreign genes and transmit them sexually with normal mendelian segregation ratios. - Several foreign genes thus introduced in plants failed to be expressed. To test whether or not 5' upstream sequences of genes that are expressed in plants can be used to promote the expression of coding sequences of foreign genes, chimeric genes were constructed and introduced in plants. Two promotor regions were used
1° the 5' promotor region of the nopaline synthase gene
2° the 5' promotor region of the small subunit of RuBisCo.
Different coding sequences were fused to these promotor sequences. Thus it was shown that the nopaline synthase promotor allowed the expression of neomycinephosphotransferase, of chloramphenicol transacetlyase and of a Mtx Resistant dihydrofolate-reductase of E.coli.
The RuBisCo promotor was shown to allow the light inducible expression of a chloramphenicol transacetylase coding sequence. These experiments open the way for a very detailed analysis of the regulation of gene expression in plants. - Similar experiments involving the use to chimeric genes constructed in vitro and introduced back in plants are being conducted to study the light regulation of the Chalcone synthase gene and to study organ specific expression in Potatoes.

Whole Organism Transformation II

0888 DNA TRANSFORMATION IN C. ELEGANS, David Hirsh, Dan Stinchcomb, Jocelyn Shaw, Richard Jefferson, Stephen Carr and William B. Wood, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309

We are developing DNA transformation to study gene activity during embryogenesis in C. elegans. We have injected exogenous DNA into the syncytial ovary of C. elegans and three generations later detected the foreign DNA by hybridization to the plasmid originally used in the injection. By restriction enzyme analyses, we found the foreign DNA to be present in high copy number, tandem arrays. One plasmid containing C. elegans DNA sequences was found to have recombined with the host genome by homologous recombination. The stability of transformed DNA not containing worm sequences was measured and it was found that 50% of the DNA was lost per generation; all transformed worms contained the same concatameric array of DNA when analyzed with restriction enzymes indicating that loss of exogenous DNA was all-or-none. We do not know whether these transformants carry the exogenous DNAs integrated into the genome or as extrachromosomal elements.

Chimeric vectors have been constructed to assay for expression of the transformed DNA. These vectors contain the 5'- and 3'-sequences of C. elegans collagen and actin genes flanking the Tn5 neomycin phosphotransferase gene. Transformants have been found and are being assayed for expression based on resistance to the neomycin analogue, G418. Similar chimeric vectors containing nematode collagen and actin flanking sequences and the E. coli β-glucuronidase gene have been constructed and used to transform mutant nematodes lacking β-glucuronidase. Transformants are being screened visually for β-glucuronidase enzyme activity which can be detected histochemically and fluorometrically. This vector will also be injected into specific blastomeres to measure lineage-specific activation of nematode collagen and actin gene control sequences. We are also attempting complementation of nematode developmental mutants by transformation with purified DNA.

0889 DNA TRANSFORMATION IN THE SEA URCHIN, C. N. Flytzanis, A. P. McMahon, K. S. Katula, B. R. Hough-Evans, E. M. Nolan, F. Teng, E. H. Davidson, California Institute of Technology, Pasadena, CA 91125.

DNA is microinjected into the cytoplasm of unfertilized Strongylocentrotus purpuratus eggs. Following fertilization the embryos grow in sea water up to the late pluteus stage (four days postfertilization) and then are fed single cell algae (Rhodomonas sp.) for the next five weeks. Metamorphosis occurs about six weeks after fertilization and the juvenile sea urchins are transferred onto algae covered rocks. 50% of the injected eggs survive without undergoing activation, and when fertilized, half of these complete development normally and become juvenile sea urchins. After this stage the sea urchins can be maintained in the lab for years with few if any losses.

The fate of the injected DNA is followed by DNA dot-blots and genomic blots at different stages throughout development. We have shown that when linearized plasmid DNAs are injected, the molecules are ligated into high molecular weight concatimers. The amount of DNA that we detect indicates that the injected DNA undergoes as much as 100X replication during early embryogenesis. Five weeks after fertilization, about 50% of the larvae retain the injected DNA, some at very high copy number and others at about one copy per cell. Genomic DNA blots from two-month old postmetamorphic juveniles revealed that some of the animals contain injected DNA sequences that migrate on gels along with high molecular weight genomic DNA. Microinjected supercoiled plasmid DNAs are not found to be ligated or amplified at the early embryo stages and the DNA is not detected at all in five-week old larvae.

0890 EXPRESSION OF GENES INTRODUCED INTO MICE BY MICROINJECTION

Richard D. Palmiter* and Ralph L. Brinster#
*Department of Biochemistry, University of Washington, Seattle, WA 98195
#Department of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19103

Introduction of foreign DNA into the genome of mice by microinjection of DNA into fertilized eggs provides new approaches for studying many fundamental problems of developmental biology. Several different foreign genes have now been successfully introduced into mice. In some cases the foreign genes are expressed in the transgenic animals. I will summarize some of the salient applications of this new technique.

1. Analysis of DNA sequence requirements for tissue specific gene expression and regulation by exogenous agents.
2. Analysis of mechanisms of DNA integration, modification, and inheritance.
3. Analysis of the consequences of perturbing developmental, endocrine or metabolic pathways.
4. Isolation of new genes involved in developmental pathways.

In addition to these fundamental areas of investigation, there are some practical applications such as correcting or mimicing genetic disease and gene farming.

0891 INSERTION OF RETROVIRUS INTO FIRST INTRON OF α1(I) COLLAGEN GENE LEADS TO RECESSIVE LETHAL MUTATION IN MICE, Rudolf Jaenisch, Klaus Harbers, Michael Kuehn, Angelika Schnieke and Jürgen Löhler, Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg, Martinistrasse 52, 2000 Hamburg 20, Federal Republic of Germany

Experimental insertion of the Moloney leukemia virus into the germ line has resulted in an embryonic recessive lethal mutation in Mov-13 mice (1). Integration of the proviral genome occurred at the 5' end of the α1(I) collagen gene blocking formation of stable mRNA (2). Sequence and SI mapping analyses were performed to characterize the position of the proviral genome in relation to the transcriptional map of the mutated gene. The results indicated that the virus has inserted into the first intron 19 bp 3' of the intron-exon boundary. Sequence comparison showed a striking homology of exon sequences and sequences up to 215 bp 5' of the mRNA start between the mouse and the human α1(I) collagen gene. This indicates that the sequences 5' of the mRNA start are highly conserved during evolution suggesting that this region has an important role in the control of tissue specific collagen expression.

Embryos homozygous at the Mov-13 locus are arrested in development between days 11 and 12 of gestation (1). Histological examination of day-12 embryos revealed a general cell necrosis without obvious malformation. In vitro organ explantations are being performed for functionally testing the role of collagen in embryonic development.

1. Jaenisch, R., Harbers, K., Schnieke, A., Löhler, J., Chumakov, I., Jähner, D., Grotkopp, D. and Hoffmann, E. (1983). Cell 32, 209-216.
2. Schnieke, A., Harbers, K. and Jaenisch, R. (1983). Nature 304, 315-320.

Gene Structure I

0892 SINGLE-STRANDED REGIONS IN THE 5' FLANKING DNA OF THE DROSOPHILA HSP 70 GENE. Ronald L. Seale, Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The hsp 70 gene of Drosophila nuclei was cleaved at two positions by single-strand endonucleases. The cleavage sites were in the 5' flanking region and were not identical to either the DNase I or micrococcal nuclease hypersensitive sites. Plasmids containing the entire coding region plus 1.5 kb of 5' flanking DNA were not cleaved at the positions recognized in chromatin; this was true of supercoiled plasmids, linear plasmids, and DNA incubated under conditions that induce Z-DNA formation. When nuclei were digested in progressive increments of ionic strength, the cleavage sites disappeared between 200 and 300 mM NaCl. Thus, presence of single-strand regions in the control region of hsp 70 is attributed to chromatin structure, possibly due to non-histone proteins, or to higher order configurations.

0893 Analysis of the Glucocorticoid Receptor Binding Site in the LTR of Mouse Mammary Tumor Proviral DNA. Magnus Pfahl, The Salk Institute for Biological Studies, Regulatory Biology Laboratory, P.O. Box 85800, San Diego, CA 92138.

Knowledge of the exact DNA sequence or sequences recognized by a steroid-receptor complex is necessary for a better understanding of the regulatory mechanism by which steroid hormones act. We have therefore undertaken a detailed analysis of the DNA sequences to which glucocorticoid receptor when complexed with a steroid (agonist) binds on the LTR of MMTV and have also investigated the possibility of altered DNA structure in or near those sites. A 500 bp LTR fragment previously shown to contain a specific receptor binding site or sites was cloned into the PsTI site of PBR322 and deletions were constructed extending from the EcoRI site on PBR322 to various degrees into the cloned LTR fragment. Binding characteristics of the 500 bp fragment and deletions extending to -139, -100 and +26 with respect to the RNA cap site were analyzed in the DNA-cellulose competition assay. DNA footprinting studies using purified glucocorticoid receptor and cloned DNA fragments were also carried out. Results of these and previous studies suggest that the glucocorticoid receptor recognizes DNA sequences 200 to 70 bp 5' of the RNA cap site and that this binding region is composed out of subsites. To investigate the possibility that an altered DNA structure, sensitive to S1 nuclease is present in or near the receptor binding site, a supercoiled plasmid carrying a LTR insert was digested with S1 nuclease. An S1 sensitive site in the receptor binding region could be detected by blot hybridization.

0894 A POSSIBLE FUNCTION FOR THE CHICKEN CR1 REPETITIVE DNA SEQUENCE FAMILY, William E. Stumph, San Diego State University, San Diego, CA 92182; Ming-Jer Tsai and Bert W. O'Malley, Baylor College of Medicine, Houston, TX 77030.

Members of the chicken CR1 repetitive DNA sequence family are dispersed throughout the chicken genome and share homology with the human Alu and mouse B1 repetitive DNA sequence families. We have analyzed in detail seven different CR1 family members that are located in the proximity of three different structural gene loci in the chicken genome. Three of these family members flank the X, Y, ovalbumin gene cluster. One member exists upstream from a U1 gene, and the remaining three CR1 sequences are present downstream from a calmodulin-like gene. In all cases examined, those CR1 sequences that exist on the 5' flanking side of nearby structural genes have been found to exist in the same orientation as those genes. In contrast, those CR1 sequences that exist 3' of nearby structural genes have been found to be directed in the opposite orientation. It is also known that the CR1 sequences that flank the ovalbumin cluster are found in regions of the DNA where there is a transition from an active chromatin structure to an inactive chromatin structure in oviduct cell nuclei. These correlations (location, orientation, and position with respect to a change in chromatin structure) could be indicating that CR1 sequences may play a role in the establishment of "active" chromosomal domains around genes that are to be expressed in any given cell type.

0895 ISOLATION OF cDNA CLONES FROM A DICTYOSTELIUM TRANSPOSABLE ELEMENT. Stephen M. Cohen, Joe Cappello, Charles Zuker and Harvey F. Lodish, M.I.T., Cambridge, MA 02139

Dictyostelium intermediate repeat sequence 1 (DIRS-1) is present in about 40 copies in the genome and appears to be a transposable element. The intact element is 4.5 kb in length and has 375 bp inverted repeats. Each repeat contains a heat shock promotor and TATA boxes. There are about 160 additional DIRS-1 related sequences in the genome that probably represent deleted copies of the intact element. DIRS-1 related RNAs are not found in vegetative cells except under conditions of stress, such as heat shock or high cell density. These RNAs are transcribed during normal development. The RNAs produced by DIRS-1 and related sequences are heterogeneous in size, with a major transcript of 4 kb (the size of the intact element). We have isolated several DIRS-1 related cDNA clones from a library in the λgt11 expression vector prepared using RNA from 8 hour developing cells. One of these cDNAs appears to be full length 4 kb transcript of DIRS-1. The cDNAs contain some restriction site polymorphisms with respect to the eight cloned genomic copies of DIRS-1. DIRS-1 related RNAs are polyadenylated and are associated with polysomes. None of the cDNAs that we have isolated produce a hybrid protein in the expression vector. However, examination of the DNA sequence of the 5' end of DIRS-1 suggests that translation of this region would not be expected. There are at least three long open reading frames in DIRS-1. Constructions to generate recombinants in which the open reading frames are in phase with the lacZ gene are in progress. Antibodies raised against these hybrid proteins may help elucidate the functions of the DIRS-1 derived RNAs.

0896 SEQUENCES OF EPIDERMAL KERATINS CORRELATES WITH THEIR DEVELOPMENTAL EXPRESSION, Peter M. Steinert and Dennis R. Roop, National Cancer Institute, N.I.H., Bethesda, MD 20205. Keratin filaments form the class of intermediate filaments in epithelial cells. At least 20 different subunits have been identified, but any given tissue usually expresses only 2-6 subunits. In a complex epithelium such as the epidermis, the subunits expressed change during normal development and terminal differentiation. To explore these changes, cDNA libraries were prepared to mRNAs of terminally differentiating mouse epidermis (E) and undifferentiated mouse epidermal basal cells (BC) grown in cell culture. Clones to subunits of MW 67K and 59K in the E library, and subunits of MW 60K, 59K, 55K and 50K in the BC library were sequenced to deduce their amino acid sequences. All subunits contained a central 300-residue domain that forms a coiled-coil α-helical structure, of two distinct types: Type I, E 59K, BC 55K and 50K; Type II, E 67K, BC 60K and 59K. Thus irrespective of their degree of differentiation, epidermal cells express at least 1 or 2 members of each type; that is, assembly of keratin filaments in vivo requires one member of each type, an observation that is consistent with earlier in vitro keratin filament assembly experiments (JMB 108, 547, 1976). However, the non-α-helical amino- and carboxyl-terminal domains of the subunits varied widely in both size and sequence. The E 67K and 59K subunits contain numerous repeat sequences enriched in glycines, which may reflect their interaction in terminally differentiated cells with filaggrin, an interfilamentous matrix protein also containing glycine-rich sequences. The BC subunits contain conserved sequences enriched in serines. The data suggests that the properties and functions of the different sets of keratin subunits expressed during differentiation in the epidermis are mediated by different terminal amino acid sequences.

0897 POSSIBLE ROLE OF ENHANCER-LIKE SEQUENCES IN DIFFERENTIATION-DEPENDENT CHROMATIN ALTERATIONS ALONG IgL GENES, Stephen M. Rose and William T. Garrard, The University of Texas Health Science Center, Dallas, Texas 75235.

We have studied the nature of chromatin alterations along immunoglobulin light chain (IgL) genes during B cell development. Employing a chromatin fractionation procedure on micrococcal nuclease-treated nuclei, we demonstrate that transcriptionally active κ IgL chromatin lacks a canonical nucleosomal repeat and exhibits a pronounced association with insoluble nuclear material, but is processed by nuclease to a soluble nucleosomal component that apparently lacks histone H1 and in enriched in HMG proteins. Furthermore, utilizing a variant plasmacytoma cell line that has transcriptionally inactivated one κ allele via a promoter deletion, we demonstrate that transcription per se is not responsible for these novel alterations. Finally, we show that the chromatin encompassing germline and transcriptionally silent λ IgL alleles in κ producing plasmacytomas exhibits some of the same alterations. These findings led us to propose a model that predicts B cell stage-specific alterations in IgL chromatin prior to gene rearrangement and transcription and that the IgL enhancer-like sequences may be responsible for these chromatin alterations. We are testing this hypothesis by reintroducing IgL genes with and without the enhancer-like sequences into lymphocytes at different stages of B cell development and examining the chromatin structure along these reintroduced sequences. (Supported in part by Grants from NIH, Amer. Heart Assoc., Welch Foundation and the Leukemia Society of America).

0898 ORGANIZATION OF THE BETA GLOBIN COMPLEX OF THE DEERMOUSE, PEROMYSCUS MANICULATUS,
Richard W. Padgett, Daniel D. Loeb, Lee R.G. Snyder, Clyde A. Hutchison III and Marshall H. Edgell, University of North Carolina, Chapel Hill, NC 27514

Recombinant clones containing 80 kb of the beta globin complex from the deer mouse, Peromyscus maniculatus, have been isolated. Unlike other mammals studied, this rodent has three adult genes, two of which appear to be a recent duplication of 8.5 kb or less. Partial sequence analysis reveals no structural abnormalities in these genes. All three adult genes exhibit characteristics that are beta-like and not delta-like in sequence.

Genomic probings verify the presence of βh1 and βh3 which have not been isolated in clones. Comparisons of this complex with the laboratory mouse (65 MY separation) indicate the 5' end of the complex containing the embryonic genes (y and βh0) is more stable than the 3' end containing the adult genes. Weak homology with βh3 indicates this pseudogene is evolving more rapidly than the functional genes, and at least parts of its sequence were present early in rodent speciation. However, βh0, whose role in the ontogeny of mice is unknown, hybridizes strongly, suggesting its presence predates rodent speciation and the gene is functional. Studies are underway to determine if this gene produces a protein product.

By examining the evolutionary history of sequences in the complex, we hope to increase our understanding of their regulation and function.

0899 CHARACTERIZATION OF DNA SEQUENCES WHICH ARE REREPLICATED IN A SINGLE CELL CYCLE AFTER TREATMENT WITH AGENTS WHICH FACILITATE GENE AMPLIFICATION
T.D.Tlsty and R.T.Schimke

Our laboratory has been studying the molecular events which lead to the amplification of the dihydrofolate reductase (DHFR) gene in response to methotrexate (MTX) selection We nave found that agents ,such as UV radiation and hydroxyurea, which transiently inhibit DNA synthesis facilitate the emergence of MTX resistant colonies and that a large proportion of these colonies were found to have an increased gene copy number of the DHFR genes (i.e. gene amplification). Using the dendity label, bromodeoxyuridine, and the Fluorescence Activated Cell Sorter, recent work from this laboratory has identified the initial event of this process as a multiple replication of a set of DNA sequences within a single cell cycle. Analysis of the rereplicated sequences shows that the DHFR gene is not the only sequence to increase in gene copy number. In a synchronized population of CHO K1 cells the majority of sequences which are replicated in a semi-conservative manner early in the S phase, before the initiation of the DNA synthetic block, are rereplicated when the block is released. We are now in the process of characterizing the subset of DNA sequences which are rereplicated under these conditions in an effort to determine the criteria necessary to target gene amplification.

0900 GENOMIC CHICKEN MYOGLOBIN GENES, David A. Konkel, Gregory Alsip, and Amanda McWatters, University of Texas Medical Branch, Galveston, TX 77550

Myoglobin (mb) is a heme-binding globin superfamily member synthesized in slow-twitch (red), but not white muscle. It is of interest from an evolutionary standpoint, and as a marker to study regulation of gene expression during neuromuscular interaction. The genomic myoglobin gene(s) must be isolated and characterized to address both these issues. A heterologous probe containing the highly conserved middle exon of the genomic seal mb gene was used to screen a genomic chicken phage library at low stringency. One strongly hybridizing (λCM 13.1) and four partially overlapping, weakly hybridizing clones were isolated (Alsip et al., 1983. J. Cell Biol. 97, 133a). Further analysis has focused on the strongly hybridizing λCM 13.1 clone, while study of the putative pseudogenes has been deferred.

Restriction mapping, Southern blotting, and preliminary DNA sequence analysis confirm isolation of mb-specific sequences, and suggest that there are at least two homologous genes in the clone. Whether these are two expressed genes, an expressed gene and a pseudogene, or even two pseudogenes awaits completion of the DNA sequence analysis. This question will be examined further by northern dot blot hybridization analysis of RNA isolated from different types and developmental stages of muscle and non-muscle tissue.

We have used homologous probes derived from the λCM 13.1 clone to isolate additional overlapping clones. These are being analyzed to identify additional mb family genes, and in an attempt to establish linkage of the entire mb gene cluster. This work is supported by NIH Grant NS17026.

0901 THREE DIFFERENT FIBRONECTIN mRNAs ARISE BY ALTERNATIVE SPLICING, John W. Tamkun, Jean E. Schwarzbauer and Richard O. Hynes, M.I.T., Cambridge, MA 02139

We have isolated a number of cDNA clones for fibronectin from a rat liver library constructed in the expression vector λ gt11. The DNA sequence of these clones establish the sequence of the C-terminal 35% of rat fibronectin, covering the cell-, heparin-, and fibrin-binding domains. The amino acid sequence of the cell- and heparin-binding regions suggest interesting potential relationships between the two activities. The nucleotide sequence of overlapping clones, together with S1 nuclease mapping, demonstrate the existence of at least three different fibronectin mRNAs in rat liver which differ in coding potential. Furthermore, the sequences of the different clones suggest that they arise from a single gene and are products of alternative splicing events, in which one of three different splice acceptor sites is utilized by a single splice donor site, allowing for the inclusion of an additional 95 or 120 amino acid residues within a fibronectin subunit. This model has been confirmed by the isolation and partial sequencing of fibronectin clones from a rat genomic library constructed in the vector EMBL 3B. Continuing sequence analysis of genomic clones is yielding substantial information concerning the organization of the fibronectin gene. Therefore, different forms of fibronectin(probably cellular and plasma) arise by differential splicing, suggesting that cell-type specific regulation of splicing plays a role in generating different forms of this developmentally important protein.

0902 BACTERIAL EXPRESSION OF THE NATURAL AND PROCESSED GENES FOR CHICKEN CALMODULIN, J.A. Putkey & A.R. Means, Dept. Cell Biol., Baylor Coll. Med., Houston, TX 77030

We have reported the isolation of two genomic clones for chicken calmodulin (CaM). One clone (CL-1) contains multiple introns and encodes authentic chicken CaM with 148 amino acids (JBC 258, 11869-11870). The second gene (CM-1) also encodes a protein with 148 amino acids, 19 of which are different than the corresponding amino acids in chicken CaM (PNAS 80, 6985-6989). CM-1 has no introns, is in open reading frame and seems to be expressed in a tissue specific manner. To investigate the characteristics of the protein product of CM-1 (CM-1 protein) we have constructed bacterial expression plasmids which harbour either the CaM cDNA or CM-1. The properties of bacterially synthesized CM-1 protein were compared to bacterially synthesized chicken CaM and purified rat testis CaM. CM-1 protein comigrates with CaM on SDS gels in the absence of Ca^{++} and is recognized by a monospecific CaM antibody. In the presence of Ca^{++}, the electrophoretic mobility of CM-1 protein in an SDS gel is increased. However, this increased mobility is only half that observed for CaM in the presence of Ca^{++}. Similar to CaM, the CM-1 protein will bind to phenothiozine-sepharose in the presence of Ca^{++} and can be eluted with EGTA. The presence of two cys residues in CM-1 allow it to bind to thiol-sepharose while CaM will not. These results predict the existence of a novel CaM-like protein which, although similar to CaM, exhibits altered Ca^{++}-binding characteristics and therefore may have limited CaM-like regulatory functions. The physicochemical properties of the bacterially-produced CM-1 protein are currently being exploited to demonstrate the presence of this protein in chicken tissues.

0903 STRUCTURAL ANALYSIS OF AN EXPRESSED CALMODULIN PSEUDOGENE, J.P. Stein, Univ. of Texas Health Science Center at Houston, Houston, Tx.

We have recently reported the isolation of a "pseudo" calmodulin gene (CM1) from a chicken gene library (PNAS (1983) 80:6485). Even though CM1 appears to be a processed copy of the authenic calmodulin gene (CL1) (JBC (1983) 258:11864), it is expressed in chickens in a tissue-specific manner. We have undertaken a structural analysis of CM1 to determine the basis for its tissue-specific expression. CM1 contains no introns, has a 74% sequence homology to the exons of CL1, and is flanked on either side by a 9 bp direct repeat, GTGCTTCCT. It contains at least one TATA box sequence in the 5' flanking region, an intact coding sequence, and a poly (A) addition signal near the 3' direct repeat. The 3' flanking region also contains 2 copies of CR1, a middle repetitive sequence possibly involved in maintenance of open chromatin structure around expressed chicken genes; a 5' flanking analysis is now underway. Northern gel analysis demonstrates a CM1 transcript, smaller than the multiple CL1 mRNAs, in cardiac and skeletal muscle. The pseudo calmodulin (CaM) coded for by CM1 has 19 aa differences from other vertebrate CaMs, located mostly in the center of the 1st and 4th Ca^{++}-binding subdomains, indicating that pseudo CaM probably has only 2 functional Ca^{++}-binding domains. Expression plasmid constructs of both genes have been prepared to examine the basis of the tissue-specific differences in expression of CL1 and CM1, as well as the role these two genes play in the differentiation of embryonic chick cardiomyoblasts.

0904 CONTROLS OF SARCOMERIC MYOSIN HEAVY CHAIN DURING DEVELOPMENT IN PRIMARY CHICK CELL CULTURE, JEFFREY ROBBINS, UNIVERSITY OF MISSOURI, COLLEGE OF MEDICINE, COLUMBIA, MO 65212

An analysis of the mechanisms of control of gene expression of the myosin heavy chain family has been carried out using primary cultures derived from embryonic chick breast muscle. Control of the process at the level of transcription has been analyzed in terms of possible genomic rearrangements during terminal differentiation, promoter structure and flanking sequences, as well as identification of the particular members of the myosin gene family which are expressed during the process.

Possible points of post-transcriptional controls have also been studied, in terms of correlating myosin heavy chain mRNA levels with the rate of synthesis of the protein. We confirm that in this culture system, there exists a transient pool of myosin mRNA which is under utilized in protein synthesis. This pool of mRNA exists in the early stages of myotube development.

0905 MYOSIN LIGHT CHAIN 1 & 3 PROTEINS ARE ENCODED BY A SINGLE GENE. M. Periasamy, E.E. Strehler, N. Ruiz-Opazo, B. Nadal-Ginard, Dept. of Pediatrics, Harvard Medical School, Dept. of Cardiology, Children's Hospital, Boston, MA 02115.

Myosin light chain (LC) 1 & 3 are proteins associated with the thick filament in vertebrate adult fast muscle fibers. LC1 & 3 have complete sequence homology for the first 141 aminoacids (aa) from their COO^-end, but they differ in length and aminoacid sequence at their NH3+ ends (LC1=49aa, LC3=8aa), and they are translated from different mRNAs. During rat muscle development LC1 appears before LC3. It has been our interest to know whether LC1 & LC3 are the products of a single gene and, if so, how the differential expression of the two mRNAs is regulated. To approach this question, we have isolated cDNA clones corresponding to LC1 & 3. DNA sequence analysis revealed that LC1 & 3 mRNAs are identical for the entire common coding and 3' untranslated regions but differ in their specific coding and 5' untranslated regions. Using the cDNAs as probes we have isolated the gene for LC1 & 3 in 4 overlapping genomic clones spanning over 25kb. The sequences coding for the common body (141aa) and 3' untranslated region are split into at least 3 exons and clustered in a 4.5kb region. The LC3-specific exon(s) are 5kb apart from the common body, while the LC1-specific exon(s) lie about 10kb further upstream. Thus, the ratio of coding to non-coding sequences in this gene is roughly 1:20, one of the highest reported so far. Alternative splicing must play a role in the production of the two mature messenger RNAs. In addition, the presence of two different 5' untranslated regions for LC1 & 3 suggests the existence of two different promotors.

0906 PURIFIED GLUCOCORTICOID RECEPTOR BINDS TO A HUMAN GROWTH HORMONE GENE INTRON David D. Moore, Andrew R. Marks, Doug I. Buckley, Howard M. Goodman Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

We have investigated the mechanism of glucocorticoid hormone regulation of gene expression by examining the interaction of purified glucocorticoid receptor protein with a variety of DNA sequences. We have shown that the hormone receptor complex binds tightly and specifically to a unique site in the first intron of the human growth hormone gene, approximately 100 base pairs downstream from the start of transcription. Chemical probes indicate that the receptor interacts symmetrically with two halves of a short inverted repeat the centers of which are 10 base pairs apart. This complex is strikingly similar in general anatomy to the complexes of procaryotic transcriptional regulatory proteins with their cognate sites.

0907 ISOLATION OF THE CHICKEN CARBONIC ANHYDRASE II GENE, Corinne M. Yoshihara, Mark Federspiel and Jerry B. Dodgson, Michigan State University, E. Lansing, MI 48824-1101
We have isolated and begun characterizing the chicken carbonic anhydrase II (CA II) gene. The recombinant plasmid containing the chicken CA II cDNA clone was isolated by screening the non-globin clones of a chicken red blood cell cDNA library with a 300 bp 5'-end fragment of a mouse CA II cDNA clone (Curtis (1983), JBC 258, 4459). The identity of the chicken clone was confirmed by DNA sequence analysis. The amino acid sequence predicted from the nucleotide sequence shows extensive homology with the known amino acid sequences of human and rabbit CA II. The chicken cDNA clone contains sequence from the coding region at the 5'-end of the CA II mRNA from amino acids 7 to 86. The chicken cDNA clone has been used to isolate phage from a λ Charon 4A chicken genomic library. The phage which hybridized to the cDNA clone presumably contain the chicken CA II gene. These recombinant clones are presently being characterized by restriction enzyme analysis. Further experiments include subcloning and DNA sequence analysis of the gene-containing region and flanking regions to try to understand the relationship between the structure and organization of carbonic anhydrase genes and carbonic anhydrase gene expression.

0908 CHANGES IN CHROMATIN STRUCTURE ACCOMPANY MODULATION OF 5S GENE TRANSCRIPTION RATES IN TETRAHYMENA. David S. Pederson and Martin A. Gorovsky, Department of Biology, Univ. of Rochester, Rochester, N.Y. 14627
The nuclease sensitive regions of a cluster of 5S ribosomal genes in Tetrahymena thermophila have been mapped by indirect end labeling. DNAse I cuts the actively transcribed macronuclear genes 5' to and within the coding region. When the rate of 5S gene transcription is reduced through starvation, the coding region sensitivity to DNAse I is lost, while the 5' site remains. DNAse I can therefore distinguish changes in chromatin which occur in response to changes in physiological as well as developmental states. In contrast, Staphylococcal nuclease cuts 5S gene chromatin from growing and starved cells similarly.

Gene Expression I

0909 EXPRESSION AND MODULATION OF THE HUMAN CALCITONIN GENE, Barry D. Nelkin,[1] Andree deBustros,[1] Charles L. Berger,[1] Bernie A. Roos,[2] Susan S. Leong,[3] and Stephen B. Baylin,[1] Johns Hopkins Oncology Center, Baltimore, MD 21205;[1] Case Western Reserve University, Cleveland, OH 44106;[2] Roswell Park Memorial Institution, Buffalo, NY 14263.[3]

The RNA transcript for calcitonin (CT), a polypeptide hormone produced by the C-cells of the thyroid, has been shown to be subject to alternative processing in the rat (Amara et al, Nature 298:240-244, 1982). Little is known about regulation of human CT gene transcription or RNA processing. Human C-cell tumors (medullary thyroid carcinoma, MTC) usually contain high CT concentrations; however, some human MTC tumors, usually rapidly growing with high metastatic potential, have low CT content and a heterogeneous cellular distribution for CT. In order to study human CT gene expression and its modulation in MTC, we are using a cell line derived from human MTC. This cell line maintains a high level of CT production, and the rate of CT production is inversely related to growth rate. From mRNA from these cells, we have isolated three cDNA clones for calcitonin. We have treated these MTC cells with various agents which modulate differentiation or expression of other genes. We have been able to increase concentrations of CT 2- to 4-fold by treatment with 12-0-tetradecanoyl phorbol 13-acetate (TPA). Using our cDNA clones, we have found that this increased CT concentration is accompanied by a similar increase in CT-specific mRNA. The effect is evident at the mRNA level within 6 hrs and is maximal at TPA concentrations of 10^{-10}M to 10^{-8}M. No increase in CT mRNA is seen when the MTC cells are treated with phorbol esters which are biologically inactive as tumor promoters. Studies are in progress to examine the basis of these and other possible regulatory events in human CT production.

0910 INVARIANT TEMPORAL ORDER OF REPLICATION OF THE FOUR ACTIN GENE LOCI DURING S-PHASE OF MITOTIC CYCLES OF PHYSARUM POLYCEPHALUM, Gerard Pierron and Helmut W. Sauer, Texas A&M University, College Station, TX 77843

The chronology of replication of the four unlinked actin loci ard A,B,C,D has been established. Utilizing (1) BUdR incorporation, preparative separation of HL newly replicated from LL late replicated DNA, Hind III digestion, gelelectrophoresis, Southern blotting and hybridization with a nicktranslated specific actin probe from sea urchin or (2) "gene dosage" determination from hybridization intensities of actin restriction fragments, it is shown that loci C and D replicate before 8 min, locus B at 8-10 min and locus A at 80-90 min of S-phase, i.e. at times when 5, 5-6 and 73-75% of the genome has replicated. The chronological order of actin gene replication remains constant in 2 consecutive mitotic cycles in a single plasmodium, and during at least six months in continuous culture.
EM spreads from early S-phase (at 10% genome replication) reveal activated transcription units (TUs) predominantly in newly replicated chromatin. Typically, both segments of replicated chromatin are symmetrically transcribed and the replication origin is located within the TUs.
Invariant chronological order of replication and evidence for replication-transcription-coupling in Physarum may reflect an endogenous cell cycle program and shed new light on quantal cell cycles and cellular determination

0911 DICTYOSTELIUM DIFFERENTIATION: SEQUENTIAL INDUCTION OF CELL-TYPE SPECIFIC mRNAS IS MEDIATED BY DEVELOPMENTALLY REGULATED CELL SURFACE PROTEINS, Rex L. Chisholm, Eric Barklis and Harvey F. Lodish, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139

We have characterized the induction of a large number of developmentally regulated mRNAs, most of which are expressed in a specific cell type (prestalk or prespore cells) and the induction of developmentally regulated cell surface components. This analysis suggests that the Dictyostelium developmental program can be explained by a small number of dependent sequences, each mediated by specific surface molecules. Progression through at least four dependent stages is required for Dictyostelium amoebae to complete the aggregation stage of their developmental cycle. Two factors are involved in initiating the developmental program: starvation for amino acids and placing the cells at a high cell density. Starvation induces one class of genes specific for prestalk cells (prestalk I) and also the cell surface cAMP receptor. The appearance of the cAMP receptor enables the cells to undergo chemotaxis, a process mediated by cell-cell signalling of extracellular cAMP. Binding of cAMP to cell surface receptors induces synthesis of the second class of prestalk cell-specific mRNA (prestalk II) and one class of prespore cell-specific mRNA (prespore I). Cyclic AMP also induces the expression of cell surfact molecules necessary for the formation of cohesive cell contacts. The step in the developmental cascase following chemotaxis is the formation of multicellular aggregates. Cell-cell contact induces the expression of a large class of genes, the second class of prespore mRNAs (prespore II).

0912 Drosophila RNA Polymerase I Transcription. Bruce D. Kohorn and Peter M.M. Rae. Dept. Biology, Yale University, New Haven, CT 05401
We have developed an extract of Drosophila tissue culture cells that gives specific and accurate transcription of cloned Drosophila ribosomal DNA (rDNA). The cell extract has been used to transcribe rDNA templates mutated in regions that surround the site at which transcription initiates. We have found that rRNA synthesis is regulated by a ca. 50bp region that includes sequences both upstream and immediately downstream of the site of initiation. One identifiable component of a promoter lies within the sequence -43 to -27, and another is contained within the first four nucleotides of the transcription unit. These two components may define the limits of a single promoter, or they may constitute segments of a dual promoter separated by 30bp. The intervening region of 30bp between the two components may contain a phasing signal that directs polymerase to initiate properly.
We have also studied the structure of the DNA spacer that separates individual Drosophila rDNA transcription units. This region, the nontranscribed spacer (NTS), is comprised in part of a 240bp tandem repeat of an imperfect copy of the sequence that surrounds and includes the site of transcription initiation. These NTS repeats contain sequences able to promote polymerase I activity, and serve as templates for accurate in vitro transcription. The data indicate that the NTS repeats have the potential to act as a loading area for polymerase molecules, and this may influence the rate at which rRNA is synthesized.

0913 ORGANIZATION OF X-LAEVIS GLOBIN GENES AND EXPRESSION IN OOCYTES,R.K.Patient,T.Enver, D.R.Greaves,M.E.Walmsley,King's College,London University,LONDON WC2R 5LB and M.M. Bendig & J.G.Williams,Imperial Cancer Research Fund,Mill Hill,LONDON NW5.

In X.laevis the α- and β- globin genes are linked. During development a switch in expression occurs from tadpole globins (αT,βT) to adult globins (α,β). The tadpole globin genes are linked to the adult genes in the order αT-α-β-βT. Two globin loci have been detected which are expressed at different levels and therefore referred to as major and minor. The two loci are thought to have arisen as a result of duplication by tetraploidization. The distances between the αT and α genes differ at the major and minor loci (5kb and 11.5kb respectively). We show that this is due to at least two separate insertion/deletion events. In addition, the distances between the αT and α genes seem to differ in two alleles of the major locus, again as a result of insertion/deletion events. The nature of these inserted/deleted segments will be presented. Sequences from the αT1 and αT2 genes will also be presented. J.Herbert Taylor's group have isolated a fragment of X.laevis DNA which exhibits enhanced efficiency of replication when injected into the Xenopus egg. We show that there are regions of homology to this fragment in between each of the globin genes in the major locus. In the major locus we are mapping templates for transcription by RNA polymerase III. We are also studying transcription of the various globin genes by microinjection into Xenopus oocytes. α1 and β1 are transcribed very inefficiently compared to the Herpes tk gene or relative to their own efficiency during erythropoiesis. There is much more non-specific initiation than specific and the inefficiency does not appear to be due to rapid methylation by the oocyte. We shall discuss our attempts to stimulate specific transcription by DNA titration and coinjection of red cell extracts.

0914 β-LIKE GLOBIN GENES EXPRESSION DURING EARLY DEVELOPMENT IN MOUSE, M.G.Farace,B.A.Brown G. Raschellà,J.Alexander,A.Fantoni,S.C.Hardies,C.A.Hutchison III, and M.H.Edgell,Università di Roma,Italy,University of North Carolina,Chapel Hill and Laboratorio di Biofisica, ENEA,Italy.

The mouse globin locus Hbb contains seven β-like genes closely linked within 65 Kb pairs.β-like globin sequences are arranged in this order:5' y-βh0-βh1-βh2-βh3-β1-β2 3' and they have all been sequenced. β1 and β2 are expressed in the adult mouse, whereas y, βh0 and βh1 are expressed during embryonic development.Our recent results (M.G.Farace et al.,manuscript in preparation) show that βh1 codes for the embryonic β-like globin z. Dot-blot hybridization experiments are reported here which demonstrate that βh1 is transcribed mostly during early stages of yolk sac erythroid cell differentiation, that is in the course of the early primitive erythropoiesis of 10 and 11 day embryos. Conversely, the concentration of y globin gene transcripts increases with mouse fetal development up to day 14. βh0 is also transcribed in 10 day embryos, as shown by S1 analysis, with kinetics of expression similar to βh1. βh0 transcripts are at least five fold less abundant than βh1 transcripts as estimated from band intensities of S1 nuclease protected fragments and specific activities of the probes. We were unable to demonstrate translatability of the βh0 mRNA due to its low abundance, but we feel that the most likely role for the gene is to code for a minor globin with kinetics of expression similar to βh1.

0915 TRANSIENT PARALYSIS BY HEAT-SHOCK OF HORMONAL REGULATION OF GENE EXPRESSION
Alan P. Wolffe, Andrew J. Perlman and Jamshed R. Tata
M.R.C., Nat. Inst. Med. Res., Mill Hill, London, NW7 IAA. U.K.

We have used heat-shock to induce a transient and reversible paralysis of the hormonally regulated expression of the vitellogenin genes in Xenopus laevis primary hepatocyte cultures.

In estrogen-stimulated cells, heat-shock at temperatures greater than or equal to 34°C causes a cessation of vitellogenin gene transcription and a destabilisation of vitellogenin mRNA. On return to normal incubation temperatures in the presence of estrogen, vitellogenin mRNA accumulation rapidly recovers.

In naive male cells, having had no prior exposure to estrogen, heat-shock leads to a protracted lag-period before vitellogenin mRNA begins to accumulate, on return to normal temperatures in the presence of estrogen.

We correlate this paralysis of estrogen responsiveness to heat-shock induced inactivation of estrogen receptor, in both naive and estrogen-stimulated hepatocytes.

These studies present a new aspect of the role of thermal stress or heat-shock proteins in the regulation of transcription and translation. They represent the first evidence that heat-shock can reversibly paralyse the hormonally regulated expression of a specific gene important in development.

0916 IN VITRO STUDY ON THE PROMOTER ACTIVITY OF DICTYOSTELIUM ACTIN GENES, Masaki Iwabuchi, Shigeharu Takiya, Kazuhiro Takahashi and Yoshiaki Sazuki , Hokkaido University, Sapporo, 060, Japan and National Institute for Basic Biology, Okazaki, 444, Japan

We constructed various kinds of the chimeric genes from Dictyostelium actin and silkworm fibroin genes and examined the in vitro efficiency of transcription of these chimeric genes in the cell-free system containing HeLa cell extract by the truncate assay method. In the test of the chimeric gene (pFA5) in which the region upstream from the transcription initiation point of the actin 5 gene was joined at various positions to the corresponding region in the fibroin gene, there was no change in the efficiency of faithful transcription as compared with that of the actin gene. However, the pFA5 gene which lacked the TATA box or in which the sequence at the initiation region of the actin gene was altered caused no faithful transcription. Similar results were obtained with the reverse type of the chimeric gene (pA5F), which consists of the upstream region including the TATA box of the actin 5 gene and the downstream including the transcription initiation point of the fibroin gene. In this case, however, the transcription efficiency of the pA5F gene reached a level comparable to that of the fibroin gene. Thus, we can conclude that the extremely low efficiency of transcription of the actin gene in the cell-free system is not due to the upstream sequence of the transcription initiation site and that the nucleotide sequence surrounding the transcription initiation point plays certain important roles in raising the transcription efficiency. In the experiments in which the promoter activity of the actin 5 and 6 genes was compared in vitro using two chimeric genes, pA5F and pA6F, the 5 gene had the stronger promoter than the 6 gene.

0917 c-AMP-DEPENDENT PROTEIN KINASE DURING DEVELOPMENT OF D. discoideum. B.H. Leichtling*, K. L. Schaller*+, I. H. Majerfeld*, C. Woffendin* and H. V. Rickenberg*+, *National Jewish Hospital & Research Center and + Univ. of Colorado School of Medicine, Denver, CO 80206

During development of the cellular slime mold D. discoideum mRNA complexity increases approximately two-fold. Lodish, Firtel and Ratner showed that most of this increase occurred at the end of aggregation. The accumulation of a large subclass of this developmental mRNA, after the formation of sustained cell-cell contacts, appeared to depend on cAMP insofar as disaggregation of tipped aggregates resulted in the loss of this subclass of mRNA which was prevented or reversed by exogenous cAMP. This cAMP-dependent subclass of mRNAs accumulated only in the prespore (posterior) cells of the migrating slug.

We partially purified the cAMP-dependent protein kinase (cAMP-d PK) from D. discoideum and studied its behavior during development. Both the regulatory and catalytic subunits of the kinase were found in vegetative amoebae; the activities of both subunits increased in parallel approximately four-fold during development. The increase represents de novo synthesis of the kinase and did not require sustained cell-cell contact. The peak of kinase activity occurred during aggregation, prior to the accumulation of the prespore specific mRNAs. Prespore cells, separated by centrifugation through a Percoll gradient from presumptive prestalk cells, had approximately four times as much cAMP-d PK as did the prestalk cells. These findings are compatible with a role of cAMP and the cAMP-d PK in the accumulation of prespore specific mRNAs of D. discoideum.

0918 REGULATION OF THE TYPE II PROCOLLAGEN GENE DURING CHICK LIMB CARTILAGE DIFFERENTIATION, William B. Upholt, Dean Kravis, Linda J. Sandell, and Val C. Sheffield, University of Chicago, Chicago, IL 60637

Expression of the type II procollagen gene during chick limb cartilage differentiation has been analysed by cell-free translation, Northern blot analysis, quantitative solution hybridization, nuclear transcription studies, and DNA methylation analysis. These experiments show that the major mechanism of regulation of the type II procollagen gene during limb development is transcriptional. Steady state levels of type II procollagen mRNA increase approximately 90-fold during the differentiation of stage 24 limb mesenchyme to cartilage in culture. Low but significant amounts of type II procollagen RNA are present in stages 20-24 chicken limb prior to immunologically detectable synthesis of type II collagen. A large portion of this RNA is associated with polysomes. Although a pattern of decreased methylation is characteristic of the type II procollagen gene in tissues producing large amounts of type II collagen such as sternal or epiphyseal cartilage, this change in methylation is not necessary for expression of the gene during limb cartilage differentiation in culture. Supported by National Institutes of Health Grants HD-09402, HD-04583, GM-7543, GM-07281, and HD-07009.

1919 REGIONAL CHANGES IN PH FOLLOWING THE INDUCTION OF DICTYOSTELIUM DISCOIDEUM MIGRATING SLUGS TO FORM FRUITING BODIES. Katherine Deml Rand, Dept. of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

In D. discoideum, the choice between 2 morphogenetic pathways can be controlled by environmental conditions, including the ambient pH. For instance, after aggregates form (in the dark) on a substrate containing NH_3 at a low pH (6.0-6.4), they construct fruiting bodies directly at that site. However, aggregates formed on a substrate containing NH_3 at high pH (7.2-7.5) assemble migrating slugs which move to another site. These slugs are cylindrical masses of cells with narrow anterior tips, which can be induced to form fruiting bodies by low ambient pH or overhead light. Based upon the effects of pH and NH_3 on the slug/fruit decision as well as cAMP signalling, Sussman and Schindler (Differen. 10:1, 1978) proposed a model of morphogenesis which makes predictions about developmental changes in pH. Using microelectrodes, I have measured the pH in both migrating and induced slugs. Within 15 uninduced slugs, there was no difference in mean pH between the tip, middle, and back regions. However, in 17 slugs which were induced to fruit, the mean pH in the tip decreased by about 0.4 pH units, and in the middle by about 0.15 pH units; the pH in the back did not decrease significantly. These changes occurred within 10 minutes of induction, whether slugs were induced with overhead light alone or by transfer to a low pH buffer in addition to the light. The pH difference between the tips and backs within individual induced slugs was -0.50 ± 0.15 pH units. The pH of the tip remained low during further development to the Mexican hat stage. I am grateful to Maurice Sussman in whose laboratory these experiments were conducted.

1920 STRUCTURE AND EXPRESSION OF A DROSOPHILA TROPOMYOSIN GENE: A SINGLE GENE ENCODES DIFFERENT MUSCLE ISOFORMS, Guriqbal S. Basi, Mark Boardman and Robert V. Storti. University of Il. Health Sci. Ctr., Chicago, Il. 60612

We have identified three tropomyosin (Tm) genes clustered in an 18 kbp region of Drosophila melanogaster (Dm) DNA. Two of these genes, mTmI and mTmII encode muscle forms of Tm, whereas the third encodes a cytoplasmic form. The mTmI gene expresses a major transcript of 1.3 kb and lesser amounts of a 1.6 kb transcript during muscle development in early embryos. In the adult thoracic muscle however, the mTmI gene expresses parallel levels of a 1.6 kb and a 1.8 kb transcript. The switch in mRNA expression of the mTmI gene in these different muscle tissues is reflected by the appearance of a new mTm isoform. The mTmII gene expresses a single 1.4 kb transcript in both embryonic and thoracic muscle.

The DNA sequence of the mTmI gene and flanking regions has been determined, and the deduced amino acid sequence of the mTmI protein shows a high degree of homology with rabbit skeletal muscle alpha tropomyosin. The expression of the different embryonic and thoracic transcripts has been analyzed by S1 nuclease mapping. Both sets of transcripts share identity at the 5' end of the gene but exhibit differences in the 3'end of the gene. This results in differences in the C-terminal portions of the two muscle Tm isoforms.

Thus in Drosophila a single gene encodes different muscle Tm isoforms in different muscle tissues during development.

0921 ACTIVATION OF A SILENT HPRT ALLELE IN HELA CELLS BY 5-AZACYTIDINE, Robert Ivarie, Iain Farrance, & Julie Morris, Molecular and Population Genetics, University of Georgia, Athens, GA 30602

HeLa H23 cells are a mutant female human tumor cell line resistant to 6-thioguanine (Milman et al. 1976. Proc. Nat. Acad. Sci. 73,4589). They harbor defective hypoxanthine phosphoribosyl transferase (HPRT) as the result of a mutation that also shifts the isoelectric point of the enzyme. Spontaneous HAT^+ revertants arise at a frequency of about 10^{-8}. Such revertants contain both mutant and wild type HPRT polypeptides when assayed by immunoprecipitation and two-dimensional gel electrophoresis; thus, they appear to result from activation of a silent $HPRT^+$ allele. To determine whether such revertants arose via hypomethylation of genomic DNA, H23 cells were treated with various concentrations of the DNA hypomethylating agent, 5-azacytidine (azaC), and revertants scored by HAT selection. At an optimal dose of 5 μM, the reversion frequency was increased up to 60-fold over the spontaneous rate. Two of the azaC-induced revertants have been shown to produce both mutant and wild type HPRT polypeptides; they also contain active HPRT. Thus, the azaC revertants appear to have arisen via activation of a silent $HPRT^+$ allele. Like the spontaneous HAT^+ revertants, azaC revertants were unstable and reverted to 6-thioguanine resistance at about 10^{-4} frequency. Rereversion results from inactivation of the wild type $HPRT^+$ allele because expression of the wild type, but not the mutant, enzyme was lost. These observations support the hypothesis that DNA methylation plays a central role in mammalian X chromosome inactivation.

0922 EXPRESSION OF LATE HISTONE VARIANTS IN LYTECHINUS pictus, Jim Knowles and Geoff Childs, Albert Einstein College of Medicine, Bronx, NY 10461

The sea urchin genome contains several different multigene families which encode temporally regulated and tissue specific histone proteins. The best characterized histone gene family contains clustered sequences encoding all five histones repeated in about 500 nearly identical tandem arrays. These tandemly repeated histone genes (early genes) have been shown to encode the histone mRNAs found in the early blastula stage of development. Another histone gene family (late genes) has recently been isolated (Childs et. al. 1982, Cell 31:383; Maxson et. al., Nature 301:120). These genes are repeated 8-10 times in the sea urchin genome, and unlike their early gene counterparts, are not tandemly repeated.

We have studied the temporal expression of the late histone variants H3 and H4 in developing embryos of Lytechinus pictus. Using S1 analysis, mRNA for both histones was found in maternal RNA in approximately 2000 copies per egg. The genes are activated at blastula and reach a maximum concentration of 100-500 times that present in the egg, at 24 hours post-fertilization. Both genes are expressed in a similar temporal fashion, that differs from that of the early genes. Additional studies with elongation of nascent transcripts in isolated nuclei show transcription from the early genes to decrease 21-fold between 7 and 15 hours post-fertilization, while late gene transcription increases 6.5-fold. Late gene transcripts continue to be generated at 24 hours, far after early gene transcription has ceased.

0923 CELL TYPE-SPECIFIC EXPRESSION OF PGK ALLELES IN MAMMALIAN TESTIS DEMONSTRATED BY IN SITU CYTOHYBRIDIZATION, J.R. McCarrey, M.W. Welsing, D.H. Keith, A.D. Riggs, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

The cell type-specificity of expression of two alleles of phosphoglycerate kinase (PGK-1 and PGK-2) in mammalian testis was studied by in situ cytohybridization using a PGK-1 cDNA clone and a 25-bp synthetic oligonucleotide as probes. Mouse testis was analyzed after either quick freezing and cryostat sectioning or plastic embedding and microtome sectioning. Sections were probed with either the PGK-1 cDNA clone which is known to crosshybridize with PGK-2 sequences or with the synthetic 25-mer which is homologous to a region in the 5'-untranslated region of the PGK-1 mRNA and hybridizes specifically to this allele under conditions of high stringency. PGK-2 expression was indicated in those cells that were labelled by the former but not the latter probe. These results indicate that there is a dramatic increase in the number of PGK-2 transcripts per cell at the elongated spermatid stage or later in spermiogenesis. This represents the first analysis of PGK expression in mammalian testis based directly on detection of specific RNA sequences. This is important in distinguishing between modulation of gene expression at the transcriptional versus post-transcriptional levels. Thus it can now be concluded in this case that the previously reported increase in PGK-2 enzyme activity in postmeiotic spermatogenic cells is the result of de novo PGK-2 transcription in these cells. The occurrence of postmeiotic, haploid expression of genes in mammals has been the subject of a great deal of debate based on both developmental and evolutionary considerations. These results corroborate earlier analyses of PGK-2 expression carried out at the post-transcriptional level, thus establishing a very strong case in favor of the occurrence of haploid transcription.

0924 DNA SEQUENCES CONTROLLING TRANSCRIPTION OF SV40 PROMOTERS, AND THEIR DIRECT INTERACTION WITH A CELLULAR PROTEIN, Ulla M. Hansen[1], Phillip A. Sharp[2], and Alison A. Bertuch[1], 1)Lab of Eukaryotic Transcription, Dana-Farber Cancer Institute, Boston, MA 02115, 2)Center for Cancer Research, MIT, Cambridge, MA 02139

A series of deletion mutants of SV40 were tested for early and late promoter activity in vitro in a transcription extract prepared from HeLa cells (Hansen and Sharp (1983) EMBO J. 2, in press). These mutants had previously been characterized for expression in vivo. Transcription in vitro from both the SV40 early and late promoters was strongly dependent on an upstream region of DNA that contains six direct GC repeats. Sequences spanning two or more of these repeats stimulated transcription in a bidirectional fashion, at initiation sites between 50 and 200 base pairs away from the repeats. These sequences may function by mediating the activity of a SV40-specific transcription factor.

With this in mind, we have developed a nitrocellulose filter-binding assay for use in the purification and characterization of a cellular protein which specifically binds the region of the SV40 promoters containing the GC repeats. The assay is rapid and readily quantitated, and therefore ideal for use in purification. A cellular protein with extremely high affinity to the SV40 DNA is detected in whole cell extracts. The region of DNA containing the GC repeats is required and apparently sufficient for the binding. We are presently examining other cellular and viral promoters for interactions with this cellular protein, since it is likely to be involved in promoting transcription of a class of cellular genes. Class-specific transcription factors would provide an attractive means of regulating stages in development.

0925 AN ENZYME ACTIVITY WITH ANALOGIES TO TOPOISOMERASE I IS ASSOCIATED WITH THE REGULATORY REGIONS OF rRNA GENES IN TETRAHYMENA, Bjarne J. Bonven, Elmar Gocke and Ole Westergaard, Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Århus C, Denmark.

The chromatin structure of developmentally regulated genes undergoes profound alterations in connection with gene activation. A key feature of "activated", transcriptionally competent chromatin is the presence of DNAse I hypersensitive sites at the 5' and 3' regulatory regions of the genes.
We have recently demonstrated DNAse I hypersensitive sites on actively transcribing rRNA genes from Tetrahymena. Three hypersensitive sites, each spanning approx. 100 bp, are centered at -100, -600 and -1000 bp with respect to transcription initiation. An additional site is found near the terminator of the gene. The DNA at each of the hypersensitive sites is associated with an endogenous activity, which for the reasons outlined below has been tentatively categorized as a topoisomerase I: (i) SDS-treatment of r-chromatin causes site-specific cleavage of the rDNA; (ii) the cuts are single-stranded (confined to the non-coding strand); (iii) the protein binds covalently at the 3' end generated in the cleavage reaction. By these criteria, the protein-DNA complexes are analogous to the reaction intermediates trapped by SDS-treatment of topoisomerase I bound to its DNA substrate. Sequencing studies indicate that the putative topoisomerase recognizes an oligonucleotide appearing in tandem repeats around the hypersensitive sites. Our present goals are direct identification of the enzyme and clarification of its role in regulation of transcription.

0926 CYCLOHEXIMIDE INHIBITS THE STEROID STIMULATED ACCUMULATION OF YOLK POLYPEPTIDE TRANSCRIPTS IN DROSOPHILA, John H. Postlethwait, University of Oregon, Eugene, OR 97403

Yolk polypeptides are synthesized by the fat body cells of female insects, secreted into the blood, and sequestered into the oocytes to form yolk. In some species, injections of the steroid hormone ecdysterone stimulate the accumulation of yolk polypeptide (YP) transcripts in female fat body cells, and this stimulation can be mimicked in males by injecting the hormone. The question is whether the hormone acts directly on the YP genes or indirectly, mediated by the translation of some regulatory protein whose synthesis is directly controlled by ecdysterone. To test this possibility, cycloheximide was used to block the incorporation of labelled amino acids into protein either in animals treated with hormone or in controls, and the quantity of YP transcript was assessed using Northern blots probed with labelled DNA sequences homologous to the YP genes. It was found that at levels of cycloheximide sufficient to block protein synthesis, the levels of YP transcript were also extremely low, with or without the addition of hormone. It is concluded that protein synthesis is necessary to permit most of the hormonally stimulated increase in YP transcript.

0927 SHORT NUCLEOTIDE SEGMENTS IN NON-CODING REGIONS OF COORDINATELY EXPRESSED MUSCLE GENES ARE LOCATED IN A SINGLE LOW-MOLECULAR-WEIGHT REPETITIVE GENE TRANSCRIPT. M.A.Q. Siddiqui, Chandrika Saidapet, Pramod Khandekar, Ana-Maria Zarraga, Charmaine Mendola and Diarmuid Nicholson, Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110
It has been proposed that eukaryotic gene expression can be controlled by the network of repetitive DNA. Repetitive DNA is known to be transcribed and is represented in both nuclear and cytoplasmic RNAs. Recently, we have isolated a low molecular weight RNA, 7S RNA, from the early chick embryonic cardiac muscle tissue. The 7S RNA sequence was subsequently cloned into Escherichia coli, strain RR1, and characterized. The hybridization of 7S specific cloned DNA (pSS48) to chick genomic DNA suggests that 7S RNA is the product of repetitive DNA and has a repetition frequency of 300-fold in the haploid chick genome. Under low stringency, pSS48 DNA also hybridizes with high specificity, to the chick cardiac muscle genes for myosin heavy (MHC) and light chain (MLC), and possibly to other coordinately expressed genes for muscle proteins. The sequence analysis of recombinant plasmids specific for MLC and MHC indicated that short nucleotide stretches common to 7S RNA reside in the non-coding regions of the respective genes. The 7S RNA sequence appears to be highly tissue specific, since DNA and RNA from several sources did not hybridize to pSS48 DNA. Furthermore, the 7S RNA-like sequence(s) appear in the chick blastodermal cells preferentially earlier than the onset of transcription of genes for major muscle proteins. These results, taken together, suggest an important role for 7S RNA in regulation of expression of muscle genes during early chick development.

0928 SYNTHESIS AND PROCESSING OF LIPOVITELLIN IN TROPICAL ANOLINE LIZARDS. Magda H. Morales, Carmen Baerga and Anabel Hernández. University of Puerto Rico, Rio Piedras, P.R. 00931.

In non mammalian vertebrates, vitellogenin, synthesized in the liver, is the precursor of the major egg yolk proteins. A lipovitellin-like protein has been purified and characterized from the egg of the tropical lizard Anolis pulchellus and the hormonal regulation of its synthesis is being studied. We have shown that 17 B estradiol stimulates the synthesis of a vitellogenin-like protein in both female and male explants ((A.M. Vallés. Master Thesis 1981, UPR) This induction is prevented by 1 mg/ml α-amanitin (R. Osuna. Master Thesis 1983). Even though vitellogenesis in tropical lizards appears to follow the general scheme described for amphibians, SDS-PAGE molecular weight analysis of the immunoprecipitated proteins indicates that the newly synthesized protein has a similar mol. weight as the egg component (110-120,000 d) instead of the expected 200-240,000 d. This observation suggests either that a higher molecular weight precursor does not occur in tropical Anolis or that its site of cleavage is the liver instead of the follicle. In order to distinguish between these two possibilities we have analyzed the protein synthesized and secreted by the liver at different intervals after the injection of 2 μ Ci of ^{3}H-leucine. The results indicate that up to 60 min the label is in the liver and is detected mainly in the 110-120,000 mw region and in several other bands of higher mol. weights. After one hour a dramatic decrease occurs in the intracellular labeled proteins, concurrently with a significant increase of labeled proteins in the serum. This suggests that a vitellogenin precursor is cleaved at the site of synthesis.

0929 REPLICATION AND REMETHYLATION AT SPECIFIC SITES IN CHROMATIN, Mark A. Marchionni, Richard L. Cate and Walter Gilbert, Harvard University, Cambridge, MA 02138

We have used rat hepatoma tissue culture(HTC) cells to examine the timing of insulin and fibrinogen gene replication and subsequent methylation at particular sites in chromatin. We synchronized HTC by replating mitotic cells and density-labelled newly-replicated sequences with 5-BrdUrd in serial cultures. We then analyzed their DNAs by restriction enzyme digestion, isopycnic CsCl centrifugation and Southern blots. Results of probing such blots showed that all three fibrinogen genes replicated during the middle of S, as did insulin II. Insulin I, however, replicated at the very end of S. Furthermore, we have examined remethylation of the single HpaII site (resides on a 1300 bp SacI-PvuII fragment) upstream of insulin I. We hybridized SacI-PvuII digestion products with excess recombinant M13 phage DNA and analyzed the reaction by HpaII digestion. Daughter strands that remained unmethylated at the time of harvest generate a HpaII cleavage site. However, sites that were already remethylated are not cut. Hence, this assay can be used to measure the time lag between replication and remethylation. Results of our initial experiments indicate that remethylation occurred within 1 hour of replication at this site

0930 METHYLATION LEVELS AT CG DINUCLEOTIDES IN THE RAT INSULIN II GENE DETERMINED BY SEQUENCING, Richard L. Cate, Harry Nick, George M. Church, and Walter Gilbert, Harvard University, Cambridge, MA 02138

We have employed the technique of genomic sequencing developed by Church and Gilbert to measure the level of methylation at a cluster of CG dinucleotides in the rat insulin II gene. This procedure allows one to determine the level of methylation at all CG dinucleotides, not just those that reside in restriction enzyme sites. A cluster of five CG dinucleotides spanning 40 bp in the third exon of the insulin II gene was analyzed in the DNA of five tissues. All five CG dinucleotides were completely methylated in spleen and thymus. The level of methylation at these sites in liver, kidney, and an insulin-producing tumor was variable, ranging from 0 to 80%. In these tissues, genomic sequencing revealed a much lower level of methylation at a Hha I site, than was previously indicated by conventional enzyme analysis. We are currently examining the reasons for this discrepancy, and are also analyzing a cluster of CG dinculeotides near the promoter of rat insulin I.

0931 ACTIVE TRANSCRIPTION OF REPEAT SEQUENCES DURING DMSO-INDUCED DIFFERENTIATION OF HL-60 CELLS. Chuan-Chu Chou, Richard C. Davis, Janet P. Slovin, Glenn Yasuda, Sunil Chada and Winston Salser, UCLA, Los Angeles, CA.90024

cDNA clones for genes regulated during terminal differentiation of DMSO-treated HL-60 cells were selected using differential screening techniques and then probed using nick-translated total human DNA to detect the presence of repeat sequences. It is remarkable that not only do more than 50% of the cDNA clones contain repeat sequences but also, the repeat sequence-containing mRNAs are regulated during terminal differentiation of HL-60 cells. Examples of mRNAs containing one copy, two copies or a partial copy of Alu repeats have been found. The Alu sequences in these cDNAs are sufficiently diverged that some of them cross-hybridized poorly in dot blot tests. One gives undetectable hybridization with the BLUR 2 probe and little hybridization with a human total DNA probe. Conversely, among the other repeats that have no sequence homology to the Alu repeat is one which gives as strong a signal with a bulk human DNA probe as any Alu repeat we have tested (signal corrected for repeat sequence length). We hope that further studies will clarify why the appearance of mRNAs with repeat sequences is correlated with terminal differentiation in these cells.

0932 DEVELOPMENTAL REGULATION OF RIBOSOMAL RNA TRANSCRIPTION: MOLECULAR MECHANISM AND TEMPLATE SEQUENCES INVOLVED, M.R. Paule, P. Kownin, C. Iida, D. Knoll and S. Brown-Shimer, Colorado State University, Fort Collins, CO 80523.
Ribosomal RNA transcription is regulated by a stable modification of RNA polymerase I (RNAP I). rRNA transcription is down regulated in response to starvation in Acanthamoeba. This regulation can be reproduced in an *in vitro* transcription system capable of faithful initiation at the homologous rRNA start site: S100 extracts from vegetative cells actively transcribe rRNA while extracts from starved cells cannot unless they are supplemented with RNAP I purified from vegetative cells. The levels of both the transcription initiation factor(s) [TIF-I] and the levels of RNAP I assayed on nonspecific templates (i.e. calf thymus DNA) are constant, but the RNAP I from the starved cell cannot specifically initiate transcription. This property is retained in RNAP I purified to near homogeneity from the starved cell even though its specific activity on calf thymus DNA matches that of the vegetative polymerase. RNAP I from starved cells has the same subunit composition as that from vegetative, but is 5X more heat labile than vegetative enzyme. BAL 31 deletion mapping has shown that the sequence from -46 to about +16 is necessary and sufficient to promote rRNA transcription - no far upstream sequence requirement can be detected *in vitro*. This sequence region has been shown to contain the binding site for TIF-I and to be sufficient to demonstrate regulation *in vitro*. Therefore, the steps in transcription involving this DNA sequence and RNAP I are impaired by the modification of the enzyme from starved cells. Supported by NIH GM26059 and GM22580.

0933 GENE EXPRESSION IN DEVELOPMENTAL MUTANTS OF *DICTYOSTELIUM DISCOIDEUM*, C.L. Saxe III, and R.A. Firtel, University of California, San Diego, La Jolla, CA 92093

Previous work in this laboratory has included the description of a series of developmentally regulated genes that display differential expression in either prespore or prestalk cells. The patterns of prespore and prestalk-specific gene expression have been examined in the rapidly developing mutant of *Dictyostelium discoideum*, Fr17, and a derivative strain, JC-5, which is temperature-sensitive for cell cohesion late in development. In Fr17 and JC-5 the timing of several aspects of prespore gene expression is accelerated relative to that of the wild type strain, NC-4. In the case of the prestalk-specific genes, the period of maximal expression is also accelerated relative to NC-4. However, in contrast to prespore expression, the timing of initial expression is unaffected in the mutants. The possible role(s) of cell cohesion in the expression of these developmentally regulated genes was studied by performing temperature shift experiments on JC-5. Under the conditions of standard filter development, the loss of specific cell contact does not appear to affect any aspect of the expression of the genes examined in this study. Experiments involving development in liquid shake culture are being performed to address the question of whether nonspecific cell contacts are necessary or sufficient to maintain the pattern of gene expression seen on filters.

0934 REGULATION OF EARLY GENE EXPRESSION IN DICTYOSTELIUM DISCOIDEUM, Sandra K. O. Mann and Richard A. Firtel, University of California, San Diego, La Jolla, CA 92093

We are examining a set of genes expressed early in the developmental cycle of Dictyostelium. These genes are not expressed in vegetative cells and transcripts are first detectable at 2.5 hours during normal development on filter pads. The complementary mRNA levels peak between 5 and 7.5 hours and then decrease. The expression of these genes is stimulated by pulsing cells with low levels of cAMP, a condition that mimics the in vivo pulsing during aggregation (4 to 8 hours into development). Expression is inhibited by high, continuous levels of cAMP, a condition found later in the developmental cycle at a time when expression of these genes decreases in vivo.

We are currently examining the structure of these genes, particularly sequences at the 5' end, in an effort to identify regions of homology that may be involved in their regulation. We hope to use DNA- mediated transformation to better determine the function of these regions.

0935 REGULATION OF HEAT SHOCK GENES IN DICTYOSTELIUM, Elliot Rosen, Annegrethe Sivertsen, Wolfgang Nellen and Richard A. Firtel. University of California, San Diego, La Jolla, California 92093.

Using in vivo labelled RNA from heat shocked cells we have isolated three heat shock inducible genes from Dictyostelium. The first hybridizes to two mRNAs of ~2.5kb. The larger message is induced greater than 50 fold by heat shock while the smaller one disappears. DNA sequence analysis indicates that this fragment encodes the hsp70 gene of Dictyostelium and shows that the amino acid sequence of the Dictyostelium and Drosophila hsp70 genes are ~70% homologous. The second heat shock inducible gene also hybridizes to an ~2.5kb mRNA which is different from the hsp70 gene. In addition we have isolated a 4.9kb transposon, Tdd-1, which encodes a 0.9kb heat shock inducible transcript. Using these cloned genes we have examined heat shock gene expression. Heat shock transcripts are induced by 20 minutes after the temperature shift, reach maximal expression by 3 hours, and then begin to disappear. In contrast, actin and four other non-heat shock messages which have been examined, are rapidly lost following the temperature shift. Examination of polysomes indicates that the hsp70 transcript is associated with large polysomes by 20 minutes after heat shock. We have begun studying the effects of heat shock during Dictyostelium development. Heat shock causes the rapid disintegration of cell aggregates during the first twelve hours of development. In contrast, the integrity of later developmental structures is maintained after heat shock and the cells complete development upon return to 22°C.

0936 ADULT α CARDIAC ACTIN IS THE MAJOR SARCOMERIC ISOFORM EXPRESSED IN EMBRYONIC CHICKEN BREAST MUSCLE, Juanita D. Eldridge, and Bruce M. Paterson, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205

During myogenesis, synthesis of the beta cytoplasmic actin isoform is greatly reduced whereas expression of the alpha sarcomeric actins commences with the onset of differentiation. We have isolated and characterized three different actin genes in the chicken: the beta cytoplasmic gene, the alpha cardiac gene, and the alpha skeletal gene. The genes have been unambiguously identified on the basis of nucleotide sequence and the amino acids encoded by the amino terminal exon. Utilizing a specific restriction enzyme fragment from the 5' portion of each gene as a primer, we have developed a primer extension assay that is diagnostic for the steady state transcript level from each of the actin genes. This assay has been used to determine the level of expression of each actin isoform in embryonic chick breast muscle during myogenesis in tissue culture, and muscle formation in vivo. The results reported here demonstrate the adult alpha cardiac actin is the predominant sarcomeric isoform expressed in 15 day embryonic chick breast muscle, in vitro and in vivo. Low levels of the adult skeletal actin mRNA are detectable in fused cultures, embryonic breast muscle, and in one day post hatch cardiac muscle. At five weeks post hatch there is no detectable alpha cardiac mRNA in breast muscle but low levels of beta actin mRNA are still present. At this stage, adult alpha skeletal actin mRNA is the only detectable sarcomeric actin transcript. Based upon the primer extension assay, no sarcomeric actins are expressed in embryonic chick brain tissue although beta actin is abundantly expressed.

0937 POST-TRANSCRIPTIONAL CONTROL OF THE SYNTHESIS OF α-TUBULIN AND A 94,000-DALTON PROTEIN DURING DIFFERENTIATION OF EMBRYONAL CARCINOMA CELLS, Chin C. Howe, David K. Lugg and G. Christian Overton, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

Changes in the expression of the genes encoding α-tubulin and a 94,000-dalton protein specified by a PYS-2 cDNA clone, p4-30, were examined in a differentiated teratocarcinoma-derived parietal endoderm cell line, PYS-2, and an undifferentiated embryonal carcinoma cell line, F9. Relative to other proteins or mRNA species, the rate of the α-tubulin and p4-30 protein synthesis as well as the levels of their corresponding cytoplasmic mRNAs are lower in PYS-2 than in F9 cells. Similarly, induction of differentiation of F9 cells by retinoic acid and dibutyryl cyclic AMP results in reduced relative levels of the cytoplasmic mRNAs for these proteins. The relative levels of α-tubulin and p4-30 RNAs in the nucleus of PYS-2 cells are also lower than those in F9 cells. However, the relative numbers of α-tubulin and p4-30 gene copies as well as the apparent relative rate of RNA transcription are similar in both cell types. These data suggest that the relative levels of the α-tubulin and p4-30 RNAs in the nucleus and cytoplasm depend on the relative stability of the two RNAs and not on the relative rate of transcription. This interpretation is supported by the faster disappearance of the two RNA species relative to other RNAs from actinomycin D-treated PYS-2 cells.

0938 CHARACTERIZATION OF PROMOTER STRENGTH & INDUCIBILITY BY HYBRID GENE CONSTRUCTION, J.D. Mosca, K-T. Jeang, G.S. Hayward and P.M. Pitha, The Johns Hopkins Un. School of Med., Baltimore, MD 21205

In order to determine the structural features which influence gene expression, comparisons have been made between Immediate-Early(IE) and Delayed-Early(DE) genes from Herpesvirus. These structural features include poly A site, removal of splice sites, influence of enhancing elements, formation of hybrid proteins and upstream putative control elements. In order to normalize the system, the structural gene for human β interferon(IFN) was inserted after the IE/DE herpesvirus promoters. Whereas the thymidine kinase(Tk)-IFN construction (HSV-DE) synthesized 90U/ml β IFN after microinjection into oocytes, the IE175-IFN (HSV-IE) and the IE94-IFN (CMV-IE) hybrid constructions synthesized 2000 and 18,000U/ml IFN, respectively. Constitutive synthesis of β IFN in Ltk+ cells transfected with these hybrid genes does not correlate with the expression observed in oocytes, suggesting that either genomic integration or cellular factors effect expression. However, all three hybrid genes are inducible after superinfection with wild-type HSV-1, (Tk-IFN, 1000U/ml; HSV IE175-IFN, 3500U/ml; CMV IE94-IFN, 1350U/ml). IE175-IFN is inducible by pre-IE factor whereas the Tk-IFN requires expression of IE genes. The mechanism of induction of CMV IE94-IFN is still to be determined. Removal of SV40 enhancing elements from CMV IE94-IFN demonstrated little effect on expression in oocytes, whereas removal of the splice site in the 5' leader sequence, construction of a plasmid without formation of a hybrid protein and changing the poly A site from SV40 poly A to the CMV endogenous poly A in CMV IE94-IFN results in a 10-fold enhanced expression of β IFN in oocytes when compared to the unmodified CMV IE94-IFN.

Gene Structure II

0939 BOVINE ESTROGEN RECEPTOR BINDS CHROMATIN AT PRE-EXISTING NUCLEASE HYPERSENSITIVE SITES, Karen Pratt, James V. Wierowski, Russell Hilf and Robert A. Bambara, Dept. of Biochemistry and Cancer Center, University of Rochester Medical Center, Rochester, N.Y. 14642 and Laboratory of Molecular Carcinogenesis, National Cancer Institute, NIH, Bethesda, MD 20205

Partially purified estrogen receptor prepared from heifer uterine cytosol, and labelled in vitro with tritiated estradiol, was used to locate receptor binding sites in target and non-target nuclei from various bovine tissues. Nuclei were digested to various extents with bovine pancreatic deoxyribonuclease I, micrococcal nuclease, or endogenous nuclease and then assessed for their ability to bind charged estrogen receptor. After very brief digestion with DNase I, such that only hypersensitive sites were cleaved, calf uterus nuclei were no longer able to bind estrogen receptor. Brief digests with micrococcal nuclease or endogenous nuclease, such that most DNA was still of polynucleosomal length, eliminated the binding ability of all nuclei types assayed. These results suggest that estrogen receptor binds to pre-existing nuclease hypersensitive sites. Since nuclease hypersensitive sites occur in regulatory regions of actively transcribed genes, including estrogen inducible genes, binding of estrogen receptor at these sites, in vivo, may be part of the mechanism by which transcription is induced.

0940 NUCLEOPROTEIN:DNA HYBRIDIZATION AS A METHOD FOR ISOLATING SPECIFIC GENES AS CHROMATIN, John P. Langmore and Jerry L. Workman, University of Michigan, Ann Arbor, MI 48109.
Single eukaryotic genes can be isolated as discrete high molecular weight chromatin fragments, based upon the fact that the termini of such target fragments are able to hybridize to complementary DNA. First, a piece of the desired genetic element is a) cloned, b) cut with a restriction endonuclease, c) trimmed with a 3' specific exonuclease in order to expose a few hundred specific bases and d) mercurated. Then the chromatin is prepared by a) efficiently restricting the nuclei with the same endonuclease used to prepare the probe, b) prefractionating the solubilized restriction fragments according to size on a glycerol gradient and c) digesting the prefractionated chromatin with a 5' specific exonuclease. This chromatin is then incubated with an excess amount of mercurated probe for several hours at 37°C in 0.1M NaCl in order to hybridize the termini of the targets with the complementary termini of the probe. The probe and probe:target hybrids are subsequently immobilized on a sulfhydryl Sepharose column and specifically eluted with 2-mercaptoethanol. The techniques have been developed using SV40 minichromosomes as a model eukaryotic target. 1000X purification of minichromosomes from a mixture with embryonic chicken chromatin has been achieved. The nucleoprotein:DNA hybridization step alone provided 150X enrichment of minichromosomes.

The early histone genes of S. purpuratus are now being isolated. Prefractionation on glycerol gradients have achieved about 22X enrichment of the 6.5 kb histone repeat. Hybridization of these fragments with mercurated probe should be capable of yielding 100 ng quantities of histone gene chromatin for study of the regulation of these genes during embryogenesis. Single-copy regulatory proteins on such fragments should be detectable.

0941 CHROMOSOME ORGANISATION OF THE MOUSE MAJOR URINARY PROTEIN GENE COMPLEX A.J. Clark and J.O. Bishop, University of Edinburgh, Edinburgh EH9 3JN

The multigene family which codes for the mouse major urinary proteins (MUPs) consists of about 35 genes. Most of these are members of two different groups, Group 1 and Group 2, which can be distinguished by nucleic acid hybridisation. The mouse genome contains approximately equal numbers (15) of Group 1 and Group 2 genes and most, if not all, of these are located on chromosome 4. Here we describe the chromosomal organisation of the MUP gene complex. We show by 'chromosome walking' that Group 1 and Group 2 genes are linked to each other in a head to head fashion, with 13 Kb of DNA between the two 5' ends of the genes. We also show that MUP genes are linked to each other in a tail to tail fashion, and that there is approximately 24 Kb separating the 3' ends of two genes. Overall the MUP gene cluster(s) accounts for at least 650 Kb of mouse chromosomal DNA.

0942 δ- AND α-CRYSTALLIN GENES OF THE CHICKEN: STRUCTURE OF TWO DEVELOPMENTALLY REGULATED GENE LOCI, James W. Hawkins, John M. Nickerson, Teresa Borrás, Charles R. King and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20205

The crystallins comprise four families (α, β, γ and δ) of proteins in the eye lens. The δ-crystallins are confined to birds and reptiles while the α-crystallins are ubiquitous. In the chicken, δ-crystallin gene expression begins at lens induction and ceases 3-5 months after hatching; by contrast, α-crystallin gene expression begins later in development and continues during lens maturation. Moreover, the spatial distributions of δ- and α-crystallin differ within the lens and change during development. We have characterized the δ-crystallin gene locus and one of the α-crystallin genes in the chicken. There is one δ- and one αA-crystallin locus per haploid genome. The δ-crystallin locus has been analyzed by blot hybridization, R-looping and partial DNA sequencing over a continous stretch of 50 Kb. There are two δ-crystallin genes, 4.2 Kb apart, which are 6.2 Kb (δ1, upstream) and 9.0 Kb (δ2, downstream) in length. Both genes have a similar polarity of transcription and a similar structure with at least 18 exons and 17 introns. Four δ-crystallin restriction polymorphisms have been isolated from three different sources of chicken DNA. Analysis of δ-crystallin cDNAs established that δ1 produces mRNA.No direct evidence has been obtained yet for the production of δ2 mRNA. The αA-crystallin gene has been isolated on a 20 Kb stretch of DNA and analyzed as δ1 and δ2. Particular attention is being given to the 5' flanking regions of these two gene loci in order to gain insight into the basis of their sequential and developmental regulation.

0943 INSERTION OF AN INTERSPERSED SHORT REPETITIVE SEQUENCE IN A GENE OF THE SEA URCHIN S. DROBACHIENSIS, Steven A. Johnson, Eric H. Davidson, Roy J. Britten, California Institute of Technology, Pasadena, CA 91125

Comparison has been made of the DNA between the sea urchins Strongylocentrotus purpuratus (Sp.) and Strongylocentrotus drobachiensis (Sd.) for the region of a gene (SP88) which is transcribed to yield a rare-class message in maternal RNA. A short repetitive sequence is present in Sd. but absent from Sp. near the 3' terminus of the transcribed region. The immediate region was sequenced in both species. An 8 nucleotide S. purpuratus sequence is perfectly duplicated at both ends of the repeat in S. drobachiensis, suggesting that the repeat is mobile and was inserted in the Sd. genome. Other members of this family of repeated sequences occur in many interspersed locations in the genomes of both species.

0944 SERUM AMYLOID A GENE STRUCTURE AND EXPRESSION; GENE CONVERSION, Clifford Lowell and John Morrow, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

Serum amyloid A (SAA) is a plasma lipoprotein produced by the liver in response to inflammatory stress (endotoxemia or tissue injury). In mice, plasma SAA levels increase at least 50 fold within 24 hrs after induction while liver SAA mRNA increases 500 fold. To study SAA tissue specific expression and induction, we have obtained genomic clones for the three genes, and a pseudogene, that make up the murine SAA gene family. Two of the genes are 96% homologous over their entire length (2.5kb), including intervening sequences (IVS), with a similar degree of homology extending 300bp 5′ and 420bp 3′ to the genes. This extensive homology is probably the result of gene conversion between these two SAA genes. The 3rd SAA gene has the same intron/exon structure as the converted pair, but is only 73% homologous in the coding regions, and has nonhomologous 5′/3′ untranslated regions and IVS. Using probes constructed from 3′ untranslated regions, we have found that all three genes are expressed to approximately the same level and with similar kinetics in response to induction. Mature SAA mRNA appears in the liver 3 hrs after induction, peaks at 12 hrs (1% of poly A+ RNA) and begins to decay 24 hrs post-induction. Comparison of the upstream sequences of the homologous genes with the distantly related gene reveals a 16bp homology, which may play a role in the coordinate expression of these genes. SAA mRNA levels are under transcriptional control, as measured by transcription in isolated liver cell nuclei. The transcriptional response is exceedingly rapid, reaching a peak 3 hrs post-induction (9 hrs before mRNA levels peak) and falling at 9 hrs. All three genes are transcribed with the same efficiency. We are using nuclear transcription to investigate the mechanism of SAA induction.

0945 YEAST HEAT SHOCK GENES ARE SUBJECT TO SEVERAL TYPES OF REGULATION, Thomas J. McGarry, Stephen E. Kurtz, Lawrence Petko, and Susan Lindquist, The University of Chicago, Chicago, Illinois 60637.

In yeast, a temperature shift from 25°C to 38°C causes the coordinate induction of a small number of proteins, the heat shock proteins, while the synthesis of most other proteins is diminished. Although similar heat shock responses have been described in many organisms, the exact function of the heat shock proteins is unclear. We have found that the heat shock genes are also developmentally regulated and are expressed at 25°C during certain stages of the yeast life cycle. Analysis of purified RNA by _in vitro_ translation and Northern blots showed induction of heat shock proteins during the stationary phase of vegetative growth and during sporulation. In contrast to the heat induction, sporulating cells synthesize the various proteins in a definite temporal order, suggesting that each heat shock protein is independently regulated. We have isolated genomic clones for the yeast heat shock proteins. Recombinant lambda phages containing inserts of yeast DNA were screened by differential plaque hybridization to ^{32}P labelled cDNAs from heat shocked and control cells. Subclones from the heat-inducible phages were used to purify heat shock mRNAs by hybridization selection, and the products of these messages were identified by _in vitro_ translation. We are using the clones to create mutations in the heat shock genes in order to clarify their role in normal growth and development.

0946 CHARACTERIZATION OF A TRANSCRIPTION FACTOR ISOLATED BY EQUILIBRIUM DNA AFFINITY COLUMN CHROMATOGRAPHY USING PROMOTER DNA, Ming-Jer Tsai, Michael A. Sanzo, Sophia Y. Tsai and Bert W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.
A DNA affinity column containing a concatemer of the 5'-flanking sequence of the ovalbumin gene was used to purify a transcription factor from partially fractionated total chick oviduct cellular extracts [Tsai _et al._, J. Biol. Chem. 256:13055 (1981)]. We have identified a fraction which bound specifically to this promoter DNA column. This fraction showed a clear preference for the cloned 5'-flanking sequence of the ovalbumin gene as compared to pBR322 sequences when examined by nitrocellulose filter binding assays. When this fraction was used in an _in vitro_ transcription assay using a truncated ovalbumin DNA template and reconstituted HeLa cell transcriptional components it stimulated the specific initiation of synthesis of ovalbumin RNA. The stimulating activity was found to be heat stable but sensitive to trypsin digestion. Therefore, a protein factor presumably is responsible for the stimulatory activity. The stimulation factor is not specific for the ovalbumin gene promoter since it also enhanced chick globin gene transcription. Finally, this stimulation factor also increased total RNA synthesis by calf thymus RNA polymerase when native chick DNA was used as a template. These results demonstrate that we were successful in using DNA-affinity column to purify a DNA-specific binding protein which maintained a functioning transcriptional activity.

0947 MULTIPLE TROPONIN T PROTEINS ENCODED BY A SINGLE GENE. DEVELOPMENT AND TISSUE-SPECIFIC REGULATION BY DIFFERENTIAL RNA SPLICING. H.T.Nguyen, R.M. Medford, A.T. Destree, N. Ruiz-Opazo, E. Summers and B. Nadal-Ginard, Dept. of Pediatrics, Harvard Medical School Dept. of Cardiology, Children's Hospital, Boston, MA 02115.
Troponin T (TnT) is one of the major regulatory proteins of the striated muscle apparatus. Immunological studies and peptide mapping analyses indicate that there are several tissue-specific isoforms of TnT. Two forms of TnT are found in skeletal muscle. We used the techniques of S1 nuclease mapping and DNA sequencing of TnT lambda genomic and TnT cDNA clones to determine precisely the nature of TnT polymorphism in skeletal muscle. From S1 nuclease mapping, two isoforms of TnT, differing in an internal peptide near the COOH-terminus, are identified in rat skeletal muscle. The expression of these two isoforms is tissue-specific and developmentally-regulated. Southern blotting analyses, however, show that there is only a single TnT gene in the rat genome. Analysis of the isolated TnT gene shows that within a 3kb region of the TnT gene, termed the isotype switch region(ISR), there are two separate exons (α-and β-), that code for structurally distinct peptides covering the same region of the TnT protein. These results represent the first demonstration of the interchangeability of exons contributing to the heterogeneity of a protein. Moreover, a unique type of "choice-discard" mechanism of differential RNA processing must be invoked to account for the developmentally-regulated formation of mature α- or β- TnT mRNA. The NH_2-terminus of TnT exhibits even greater structural heterogeneity. 4 classes of TnT cDNA clones were isolated, each encoding a different NH_2-terminal peptide sequence. The mode of regulation of the developmentally-controlled expression of these TnT isoforms is under analysis.

0948 THE STRUCTURE AND EVOLUTION OF THE DROSOPHILA HSP82 GENE, Ronald K. Blackman and Matthew Meselson, Harvard University, Cambridge, MA 02139

We have sequenced the gene encoding the 82,000 dalton heat shock protein (hsp82) from four species of Drosophila: melanogaster, simulans, pseudoobscura, and virilis. Altogether, 14,000 nucleotides were identified. In the distantly-related Drosophila species pairs, 30% of the codons contain nucleotide substitutions although the amino acid sequences have diverged by only 1.1-3.2%. Half of the amino acid changes have occurred within a 15 amino acid region which lies within an unusually polar domain of the hsp82 protein. Two closely-related species, melanogaster and simulans, have identical amino acid sequences.

The DNA sequence between the start of transcription and nucleotide -130 is generally conserved in all four species. Within this region is the TATA motif and, of great interest, a conserved sequence of 28 bp which contains multiple dyad symmetries. This DNA sequence includes the 14 bp element which regulates the heat-induced expression of the hsp70 gene (Pelham and Bienz, EMBO J., 1, 1473, 1982). In pseudoobscura, the -130 to -20 region is directly repeated with 90% fidelity at positions -830 to -720. However, the TATA motif of this repeated element has been changed at three of its seven positions and there is no evidence for an additional transcription unit in this region. The other three species do not contain repeats of this sequence. Another transcription unit, designated 63BC-T3, lies about 650 bp from the 5' end of the hsp82 gene in melanogaster and simulans. This gene is not induced by heat shock and is transcribed divergently from the hsp82 gene.

0949 SITE-SPECIFIC PHASING IN THE NONTRANSCRIBED REGIONS OF THE rDNA CHROMATIN OF DICTYOSTELIUM DISCOIDEUM. C.A. Edwards and R.A. Firtel, Department of Biology, Univ. of California, San Diego, La Jolla, CA 92093

The rDNA in Dictyostelium is organized in linear, extrachromosomal, palindromic dimers with 2 coding regions per 44 kb repeat. The dimers are repeated about 90 times per haploid genome. Using indirect end-labeling, we have mapped micrococcal nuclease and DNase I-sensitive sites in both coding and noncoding regions. In at least 40 kb of the noncoding spacer region, we observe specific phasing of nuclease-sensitive sites relative to the underlying DNA sequence. Comparison of the sites in chromatin with those in naked DNA reveals a striking pattern: the sites in naked DNA that are most readily attacked by both nucleases are precisely the same sites that are most protected in chromatin. The coding region shows a regular, 180 bp repeating pattern much like that observed for bulk chromatin.

We have also examined the effect of increasing salt concentration on nuclease-sensitive sites in chromatin. Although not all of these exhibit a regularly repeating pattern, these data are consistent with the presence of nucleosomes. These results support a model for phasing in spacer regions in which the sequence preferences of micrococcal nuclease and DNase I may be useful tools in predicting nucleosome placement. We are currently examining the chromatin organization in other regions of the Dictyostelium genome.

0950 STRUCTURE AND ORGANIZATION OF HUMAN γ CRYSTALLIN GENES. Martin Breitman and Lap-Chee TSUI, Genetics Department, Research Institute. The Hospital for Sick Children and Department of Medical Genetics, University of Toronto, Toronto, Ontario M5G 1X8 Canada.

Crystallins constitute about 90% of the soluble protein of the vertebrate eye lens and are developmentally regulated. In mammals, these evolutionarily conserved proteins are divided into 3 classes, α, β, and γ, each of which comprises several closely related polypeptides. We have used a mouse γ-crystallin cDNA (Shinohara et al, 1983) to identify and characterize corresponding members of the human γ-crystallin gene family. Using the mouse cDNA as a probe, 8 EcoRI fragments were detected in a blot analysis of human DNA. Five of these fragments were subsequently recovered in recombinant phages following screening of a human λCH4A library. Detailed phage analyses revealed that these 5 EcoRI fragments encompassed 5 very similar but distinct γ- genes. Three of these genes were contained in overlapping phages spanning approximately 25 kb, indicating clustering within the gene family. Sequence analysis of one of the genes revealed that it consisted of a short 5' exon followed by 2 major exons, each of which encodes 2 of the 4 related structural motifs of the predicted polypeptide. Furthermore, this γ-crystallin shares extensive amino acid sequence homology with a human β-crystallin (our unpublished data), although the genes encoding these 2 proteins are considerably different in structure and nucleotide sequence. These observations are consistent with a model in which β and γ crystallin genes evolved from a common ancestral sequence by divergent pathways of exon duplication. However, comparison of the human β and γ-crystallin genes has revealed conserved sequences which might be important for expression of these lens-specific genes.

0951 CLONING OF THE MONKEY ERYTHROPOIETIN GENE. F.K. Lin, C. H. Lin, P. H. Lai, J. Egrie, G. Goldwasser, F. F. Wang and M. Castro, AmGen, Thousand Oaks, California.

We have obtained a partial amino acid sequence of highly purified human erythropoietin (EPO). Regions suitable for synthesis of DNA probes were identified and mixed oligonucleotide probes corresponding to these regions were prepared. Kidneys obtained from monkeys treated with phenylhydrazine were used as a source of EPO mRNA. RIA analysis, using antibodies directed against human EPO, demonstrated a high level of EPO in the serum of phenylhydrazine treated monkeys. Northern analysis, using the probes described above, was performed on kidney poly A^+RNA. Positive results were obtained when RNA was extracted from the anemic monkeys induced to produce EPO but not from control monkeys. A monkey cDNA library was prepared and screened with a mixture of 128 sequences of a 20 nucleotide probe. Several putative EPO clones have been identified.

0952 APPLICATION OF A DIRECT GENOMIC SEQUENCING METHOD TO MOUSE IMMUNOGLOBULIN GENE METHYLATION AND DNA-PROTEIN INTERACTIONS.

George M. Church, Harry Nick, and Walter Gilbert, Biological Laboratories, Harvard University, Cambridge, MA 02138.

Direct sequencing of genomic DNA allows determination of cytidine methylation by hydrazine reactivity and sites of DNA contact with proteins using dimethyl sulfate treatment of intact cells. A denaturing gel separates by size mixtures of unlabeled DNA fragments from complete restriction and partial chemical cleavages of the entire genome. These lanes of DNA are transferred and UV-crosslinked to nylon membranes. Hybridization with a short 32-P labeled single-stranded probe produces the image of a DNA sequencing ladder extending from the 3' or 5' end of one restriction site in the genome. Numerous different sequences can be obtained from a single membrane by reprobing. Sequence data for mouse immunoglobulin heavy chain genes from several differentiated cell types will be presented.

0953 A UNIQUELY CONSERVED POLYPEPTIDE BETWEEN COWS AND BLOWFLIES SUGGESTS HORIZONTAL TRANSFER OF GENETIC INFORMATION, Alan Thorpe, London University, London E1 4NS, Tim Hunkapiller and Lee Hood, California Institute of Technology, Pasadena, CA 91125

A 32-residue peptide isolated from the pancreas has been isolated and sequenced from various mammals (human, cow, sheep and pig) as well as a possibly related sequence from chickens. The mammalian sequences differ one to two residues between any two examples and the chicken sequence is about 45% similar to the mammalian. The function of pancreatic polypeptide (PP) is unknown, but it appears to have regulatory effects on gastrosecretory functions. Using a bovine PP-specific antisera, an antigenically similar peptide was detected in the heads of blowflies. When isolated and sequenced it was found to be identical to bovine PP. Such conservation is surprising given the divergence between the mammalian and avian sequences and the extreme evolutionary distance between cows and blowflies. This conservation along with the parasitic relationship of blowflies to bovines suggests the strong possibility of a bovine to blowfly horizontal transfer of genetic information along with its regulated expression. Using synthetic oligonucleotide probes derived from the possible PP coding sequences, we are now attempting to isolate the appropriate genes from cow and blowfly. Comparisons at the DNA level should answer the question of horizontal transfer and, if positive, perhaps indicate something regarding the sequences that were involved.

0954 THE CONSTRUCTION OF NUCLEOSOMES FROM DEFINED DNA SEQUENCES OF REGULATORY INTEREST, Gad Yagil and Roni Seger, Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, Israel

There is increasing evidence that DNA sequences recognized by regulatory molecules may have a reduced tendency to form nucleosomes under appropriate physiological conditions. The question arises whether this is because these regulatory DNA sequences have an innate reduced affinity to histone octamers, or whether more specific proteins displace the histone cores from the DNA sequences. To examine this question, we have reconstituted nucleosome cores from several defined pieces of prokaryotic and eukaryotic DNA, including the origins of replication of both PBR322 and SV40 utilizing the salt gradient procedure of Tatchell and Van Holde. All the defined pieces examined do form nucleosomes, as manifested by their sedimentation rate through sucrose, and their DNAse I digestion patterns. The yields are however low compared with the nucleosome cores reconstituted from 146 bp calf thymus DNA and chicken erythrocyte core histones; this is probably connected with relatively low concentration of the defined sequences employed. The equal yields from all DNA sequences examined (between 141 and 175 bp long) indicates however the regulatory DNA regions do not have a reduced tendency to be packaged in nucleosomes.

Gene Expression II

0955 ESTRADIOL INDUCES DNA SYNTHESIS WHICH IS REQUIRED FOR MAXIMAL PRODUCTION OF VITELLOGENIN IN XENOPUS LIVER PARENCHYMAL CELLS, Barry S. Aprison and Lawrence J. Wangh, Department of Biology, Brandeis University, Waltham, MA 02254

Primary cultures of purified adult male *Xenopus* liver parenchymal cells were established and maintained for more than one week in a serum-free medium. These cultures produced vitellogenin in response to estradiol at concentrations greater than 10^{-10}M. Cultured cells also synthesized DNA in an estrogen dependent manner. As revealed by autoradiography an average 18.4$\pm$ 1.6% of the cells in control cultures and 29.1$\pm$ 0.9% of those in estradiol (10^{-8}M) treated cultures had ^{3}H-thymidine labelled nuclei. Within 48 hours of adding the estrogen(10^{-6}M), ^{3}H-thymidine incorporation into DNA measured 4 fold and there was a 20% increase in the total amount of DNA per culture. Steroid enhancement of DNA synthesis is estrogen specific, since progesterone and 5α-dihydrotestosterone failed to increase ^{3}H-thymidine incorporation. Tamoxifen, an antiestrogen, inhibited estradiol dependent DNA synthesis and also inhibited the induction of vitellogenin. Cytosine arabinoside(araC) blocked all DNA synthesis in these cultures, but did not inhibit estradiol induction of vitellogenin. However, the amount of vitellogenin produced in the presence of araC was reduced by 50%. Southern blot analysis of genomic DNA showed estrogen did not induce amplification of the vitellogenin A1 gene. Therefore, DNA synthesis is not required for initial induction but is required for maximal production of vitellogenin in cultured *Xenopus* liver parenchymal cells.

0956 ANTI-SENSE TRANSCRIPTS DIMINISH THYMIDINE KINASE GENE EXPRESSION, Jonathan G. Izant, Fred Hutchinson Cancer Research Center, Seattle, WA 98104.

The potential of anti-sense DNA strand transcription to inhibit gene activity by RNA duplex formation is being investigated as a novel genetic tool for identifying and analyzing the cellular functions associated with cloned DNA sequences. Excision of the coding portion of a cloned gene and reinsertion of the same fragment in reverse orientation will result in the normal (or any other) promoter directing the transcription of the nonsense DNA strand, thereby producing complementary sequence mRNA. When such flipped gene constructions of the Herpes Simplex virus thymidine kinase (HSV TK) gene are co-microinjected with the wild type gene at a 100:1 ratio, there is a reduction in the extent and efficiency of transient TK expression in TK^- mouse L cells: The proportion of viable cells with demonstrable TK activity 24 or 48 hours following injection drops to an average of 9% as compared to 45% for neighboring cells co-injected with the HSV TK gene and a 100 fold excess of control plasmid containing either promoter alone, coding sequence alone, or pBR322. Futhermore, autoradiography of the cells still expressing TK shows that ^{3}H-thymidine incorporation is reduced. No decrease in viability is detected in cells containing the putative RNA duplexes. TK^- ,$APRT^-$ L cells cotransformed with APRT and flipped TK gene constructions have a reduced capacity to express subsequently microinjected TK genes, suggesting that the phenomenon is due to a trans-inhibition of TK, and is probably not an artefact of rearrangements following microinjection. Whereas, the presence of HSV TK anti-message has no effect on the expression of the non-crosshybridizing chicken TK gene, preliminary evidence suggests that analogous anti-sense constructions of the chicken TK gene inhibit chicken but not HSV TK expression.

0957 CHANGES IN KERATIN GENE EXPRESSION DURING HORMONE INDUCED DIFFERENTIATION, Dennis R. Roop, NCI/NIH, Bethesda, MD 20205. Mark S. Kronenberg and James H. Clark, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

cDNA clones corresponding to the major keratins synthesized in mouse epidermis, the 50, 55_b and 60 kd keratins (synthesized in proliferating basal cells) and the 55, 59 and 67 kd keratins (synthesized in terminally differentiating cells) have been used to study the expression of keratin genes in vaginal epithelium. This epithelium is of interest because the state of differentiation and thus the degree of keratinization is hormone dependent and this allows the induction of keratin gene expression to be studied in ovariectomized animals after exposure to estradiol. Vaginal epithelium isolated from ovariectomized animals prior to exposure to estradiol is thin and the very low level of expression of both subsets of keratin genes in these cells is consistent with the low degree of keratinization observed morphologically. There is a dramatic induction of the keratin genes expressed in proliferating epidermal cells (the 50, 55_b and 60 kd keratin genes) within 24 hr after exposure to estradiol and this correlates with the onset of proliferation in the vaginal epithelial cells. The expression of the keratin genes expressed in differentiating epidermal cells (the 55, 59 and 67 kd keratin genes) only becomes pronounced 48 hr after exposure to estradiol and this correlates with the observation of stratification and keratinization in the epithelium at this time. These results indicate that expression of these subsets of keratin genes is correlated with the differentiation state of epithelial cells and that there is a coordinate induction of the 55, 59 and 67 kd keratin genes during terminal differentiation.

0958 TISSUE-SPECIFIC EXPRESSION OF TYPE I COLLAGEN GENES, Sherrill L. Adams, Eileen S. Allebach and Richard J. Focht, University of Pennsylvania, Philadelphia, PA 19104
The collagens are a large family of closely related extracellular matrix proteins whose synthesis is regulated in a tissue-specific way. For example, Type I collagen is synthesized at very high levels in bone and tendon, intermediate levels in skin, low levels in smooth muscle, and not at all in cartilage. We have examined the basis for this tissue-specific variation, using highly purified populations of primary cell cultures from tissues of chick embryos and have found that there are several parameters which determine this wide range of synthetic rates. (1) RNA levels differ considerably among the various cell types, indicating that the Type I collagen genes are probably transcribed at different rates. However, there is not a strict correlation between the amount of mRNA and the rate of protein synthesis. (2) The Type I collagen mRNAs differ qualitatively from one cell type to another, due at least in part to selection of alternative polyadenylation signals, giving rise to RNAs which differ by hundreds of nucleotides in the length of the 3' untranslated region. There is a very strong correlation between the type of transcript present and the translatability of the mRNAs, both in vivo and in vitro, implying that the 3' untranslated region may determine translational efficiency. (3) In at least one type of cell we have examined, the Type I collagen mRNAs are present and can be translated in vitro; however, no synthesis of Type I collagen is observed in the intact cells, indicating that there may also be cell-specific factors required for recognition of the mRNAs.

0959 TISSUE-SPECIFIC EXPRESSION OF TYROSINE AMINOTRANSFERASE, Lisa A. Balogh, Kai-Lin Lee and Francis T. Kenney, Univ. Tenn.-Oak Ridge Grad. Sch. Biomed. Sci. and Biol. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37830.
Previous studies have shown that tyrosine aminotransferase (TAT) activity is detected almost exclusively in the liver, but low levels of TAT are also reportedly present in kidney, brain, and heart. With the development of more sensitive methods for detection of enzyme and the isolation of a cDNA clone it has recently become possible to investigate the mechanisms whereby TAT is expressed in one tissue and not another. We have selected 3 tissues for the study of tissue-specific expression of TAT: liver (a known expresser), kidney (a proposed expresser) and testis (a proposed non-expresser). Using 2 parameters, presence of enzyme and transcripts, as our basis for study we have the following results: (1) Using the Briggs' assay for TAT we found the highest activity in liver, 20 to 40 fold less activity in kidney, and barely detectable activity in testis homogenates. (2) Utilizing western blot analysis of partially purified TAT from each tissue we were able to detect TAT only in liver. (3) Hybridot measurement of TAT sequences in total RNA showed sequences present in kidney nearly to the extent detected in liver, whereas a low level was detected in testis. (4) Northern blot analysis demonstrated that sequences detected in dot blot analyses were the same size as sequences in liver. (5) Hybridot measurement of sequences in total cytoplasmic RNA, however, indicated that the level of sequences in kidney was far below that seen in liver, while the level in testis was low as seen before. These data suggest several mechanisms for tissue-specific expression of TAT.
(Supported by NIH CA 09104 and U.S.D.O.E. under contract W-7405-eng-26 with Union Carbide Corp)

0960 CYCLIC AMP INDUCTION OF TYROSINE AMINOTRANSFERASE: TRANSCRIPTIONAL OR TRANSLATIONAL REGULATION, Leslie A. Stringfellow, Kai-Lin Lee, and Francis T. Kenney, Univ. of Tenn. Oak Ridge Grad. Sch. of Biomed. Sci. and Biol. Div., Oak Ridge Natl. Lab., Oak Ridge, TN 37830.

Cyclic AMP (cAMP) increases the functional level of rat liver tyrosine aminotransferase (TAT), as assayed by in vitro translation. This increase in mRNA activity could be due to either increased translation of an unchanged quantity of mRNA (by an increase in the rate of initiations/mRNA or an increased rate of elongation) or an increase in mRNA levels (either by increased transcription or release of mRNA from a non-translatable pool). We have performed experiments which appear to rule out translational regulation of TAT by cAMP. 1. The translation elongation rate of TAT mRNA is unchanged by cAMP treatment. We have not examined the rate of initiation. 2. cAMP increases TAT mRNA in all cytoplasmic compartments and thus does not appear to cause the movement of TAT mRNA from RNP particles into the polysome fraction. 3. cAMP causes an increase in hybridizable TAT mRNA in total cellular RNA, cytoplasmic poly(A+) RNA, and nuclear RNA preparations, which is concurrent with and completely accounts for the increase in translational activity of TAT mRNA. Thus, cAMP increases the quantity of TAT transcripts rather than improving their translational efficiency (Res. supported by NIH grant CA 09336 and by U.S. Dept. of Energy under contract W-7405-eng-26 with Union Carbide Corp.)

0961 δ-CRYSTALLIN GENE EXPRESSION IN THE EMBRYONIC CHICKEN LENS, Teresa Borras, John M. Nickerson, Jim W. Hawkins, Gokul Das and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda,MD.

δ-Crystallin comprises 70-80% of the protein synthesized in the developing chicken lens. δ-Crystallin mRNA begins to accumulate during lens induction, is the predominant lens mRNA during embryogenesis and dissappears 3-5 months after hatching. There are two similar polypeptides present in a ratio 1:3 in the lens, and there are two linked δ-crystallin genes. The basis for the different expression of the δ-crystallin genes is not known. Here we present the full-length sequence of a δ-crystallin cDNA and the sequence of the 5' regions of the δ-crystallin genes. Interestingly, the δ-crystallin cDNA has two possible translation initiation sites. Striking alterations in the ratio of synthesis of the two δ-crystallin polypeptides as a function of ion concentration, leave open the unconventional possibility that both AUG codons have the capacity to initiate translation. A 1.3 Kb fragment containing the promotor region of δ-crystallin gene 1 has been sequenced. Characterization of this region includes a CCAAT box, a TAAA box, the cap site, and two exons, 34 and 63 bp respectively, separated by a 100 bp intron. Translation is initiated from sequences encoded in the second exon of the gene. Other interesting features of the δ-crystallin promotor include enhancer-like core sequences and stretches of alternating pyrimidines and purines. Initial sequences of the promotor region of gene 2 show many similarities to that of gene 1. In vitro transcriptional studies are being performed to compare functional properties of the two δ-crystallin gene promotors.

0962 DEVELOPMENTAL CONTROL OF α-MANNOSIDASE-1 SYNTHESIS IN DICTYOSTELIUM DISCOIDEUM, George P. Livi, James A. Cardelli, Robert C. Mierendorf and Randall L. Dimond, University of Wisconsin, Madison, WI 53706.

The lysosomal enzyme α-mannosidase-1 (αMAN) is one of the earliest developmentally controlled gene products in D. discoideum. Monoclonal antibodies prepared against purified αMAN were used to analyze different enzyme forms synthesized in vivo (Mierendorf et al., J. Biol. Chem. 258: 5878-5884, 1983) and to study developmental regulation of enzyme synthesis, processing and stability. αMAN is first synthesized as a large (140K) precursor protein which is modified and proteolytically processed to mature enzyme with subunits of 58K and 60K. The developmental increase in cellular αMAN activity results from an increase in the relative rate of de novo enzyme synthesis, which is subject to pre-translational control. Aggregation-deficient (AGG⁻) mutants prematurely terminate the developmental accumulation of αMAN activity indicating that normal enzyme expression requires the proper functioning of many developmentally essential genes. This defect results from an enhanced rate of enzyme inactivation which correlates with the failure to induce a developmentally controlled change in the post-translational modification system, affecting several lysosomal enzymes. A direct screening for αMAN-deficient mutants has generated AGG⁻ strains with reduced rates of enzyme synthesis. One strain synthesizes no detectable αMAN protein and lacks functional αMAN mRNA. These mutants define a small number of very early developmentally controlled genes required for the induction of αMAN biosynthesis, some of which are involved in the post-translational modification or processing of the enzyme precursor.

0963 THE STRUCTURE AND EXPRESSION OF A QUAIL TROPONIN C GENE, Peter C. Maisonpierre and Charles P. Emerson, Jr., Dept. of Biology, Univ. of Virginia, Charlottesville, 22901

Troponin C (TnC) is the calcium-binding subunit of the troponin complex in vertebrate striated muscle. Where many contractile proteins are represented by different, muscle-type-specific isoforms, TnC in cardiac and slow skeletal muscle has the same amino acid sequence - leading Wilkinson (Eur. J. Biochem. 103, 179) to suggest that there is a single slow/cardiac TnC gene. We find that in quail there is indeed a single slow/cardiac TnC gene. This implies that, unique among other contractile protein genes, this TnC gene is subject to a means of dual regulation whereby it is expressed during the development of both heart and skeletal muscle. In order to examine the basis for this dual regulation we have used a 420 bp embryonic quail breast muscle cDNA (cC111), encoding a major portion of the slow/cardiac TnC, to isolate an homologous 13.8 kb quail genomic clone. This clone, λ24B, has been partially sequenced, revealing three exons which are co-linear with corresponding regions in cC111 and account for codons 19 through 140 of the 161 that encode the slow/cardiac TnC. So far, four introns have been found in the gene. Their relative size and placement within the codons for the well-defined calcium-binding domains show interesting similarities and differences with that described for a calmodulin gene. (Putkey et al. J. Biol. Chem. 258, 11864) We are now sequencing the promoter region of λ24B and comparing the sequence of cardiac- versus slow-TnC mRNA 5' non-translated regions in order to determine whether promoter structure, or more specifically, the choice between a separate cardiac- and a slow-specific promoter, serves as a basis for the dual activation of this TnC gene in developing heart and skeletal muscle.

0964 GENE REGULATION DURING EARLY DEVELOPMENT OF THE CELLULAR SLIME MOLD DICTYOSTELIUM DISCOIDEUM, J.A. Cardelli, G.P. Livi, R.C. Mierendorf, and R.L. Dimond. University of Wisconsin, Madison, WI 53706
Major changes occur in the pattern of protein synthesis during early development of D. discoideum as revealed by 2 dimensional gel electrophoresis of pulse labeled proteins. The relative rate of synthesis of 41 proteins increase while 16 proteins decrease in the rate of synthesis all during the first 90 minutes of development in cells previously grown in axenic culture. Eight additional proteins increase in rate of synthesis 2-3 hours in development if cells are grown first on bacteria. Similar studies done with developmental mutants have indicated that at least half of these proteins are under developmental control. Many of these regulated proteins can be identified when RNA prepared from developing cells is translated in vitro and the resulting products displayed on 2D gels. Four control mechanisms acting at pretranslational and translational levels regulate the synthesis of proteins during early development. Most proteins increasing in rate of synthesis are accompanied by parallel increases in the mRNAs. However at least 1 protein increases in rate of synthesis because its mRNA is translated more efficiently in developing cells. The majority of proteins decreasing in rate of synthesis are under translational control. Finally at least 2 proteins stop being produced because of a rapid degradation/inactivation of the mRNAs. The lysosomal enzyme α-mannosidase (α-man) is an example of a protein which increases in rate of synthesis during early development in cells previously grown on bacteria. α-man is initially synthesized in vivo as a 140K protein which is rapidly processed into 2 subunits of 58K and 60K. In vitro translation of the α-man mRNA produces a 120K protein which can be modified by microsomal membranes co-translationally to the 140K form. The dramatic increase in the rate of α-man synthesis during development is paralleled by an increase in functional α-man mRNA suggesting control at the level of transcription.

0965 DEVELOPMENTALLY LINKED TRANSCRIPTION OF THE SEX-LINKED ACTIN GENE IN DROSOPHILA MELANOGASTER, Ann Sodja, and Rasheeda S. Zafar, Department of Biological Sciences, Wayne State University, Detroit, Michigan, 47202.
Actins perform a number of diverse and important functions in the development and life cycle of an organism. As a major component of microfilaments, actin influences the distribution of informational molecules during early development. Because of our interest in eukaryotic gene regulation and how it influences a developing system, we have chosen to examine more closely the sex linked actin (5C3-4) expressed in embryogenesis and the sequences which surround it. In order to establish how much of the actin mRNA is actually used in translation and to determine the unit(s) of transcription at 5C, we have analyzed by Northern blotting/hybridizations the poly A$^+$ mRNA isolated from polyribosomes. We observe that the 3' end probe from 5C gene which contains some transcribed but untranslated actin specific sequences as well as 3' adjacent sequence hybridizes, in addition to the 2kb actin mRNA from which it is transcribed, to a smaller poly A$^+$ RNA. This putative transcript does not cross-react with actin coding probe. Hybridizations with the 3' end strand specific probe have shown that as expected, the transcript shares same orientation as the actin gene. The sequence appears to be unique on the genome. We are intrigued by its extreme vicinity to the actin gene and its apparent temporal coexpression with the 5C actin gene. We are mapping it more precisely on the DNA by S1 nuclease and are sequencing to locate putative regulatory signal sequences. We plan to test its translatability in an in vitro system. Ultimately, genetic analysis of the region will be used to determine whether the short transcript plays a role in temporal and/or spatial specificity of the particular actin gene.

0966 ORGANIZATION AND REGULATION OF THE ACTIN MULTIGENE FAMILY OF DICTYOSTELIUM DISCOIDEUM. Patricia Romans and Richard A. Firtel, UCSD, La Jolla, CA 92093

Actin gene expression in the cellular slime mold Dictyostelium discoideum is differentially regulated during vegetative growth and throughout normal development as well as under a variety of other physiological conditions. These genes constitute a family of approximately 20 members, only a few of which are closely linked. Genomic clones of 15 family members have been isolated and sequence analysis of the 5' and 3'non-translated flanking regions indicates subfamily homologies among them. Only two or three of the 15 are pseudogenes, while of the rest, only two contain substitutions altering the predicted amino acid sequence in the regions examined. The most unusual feature of the 5' flanking regions is a series of short G-C rich palindromic sequence families located in the region 100 to 200 base pairs 5' to the methionine initiation codon. Overall, this region has a base composition averaging 16% G-C. A given actin gene may have zero, one, or two of these specific sequences whereas they have not been found associated with any of the other developmentally regulated genes examined in our laboratory. The pattern of expression during development of several of the actin genes has been examined previously (1,2). Individual genes are differentially regulated both with respect to the timing and extent of expression. We are presently examining the expression of the remainder of the cloned genes during development and during prespore and prestalk cell differentiation. We will correlate specific expression patterns with the unusual conserved 5' upstream sequences.

1. M. McKeown and R.A. Firtel (1981). Cell 24, 799-807.
2. M. McKeown and R.A. Firtel (1982). CSHSQB 46, 495-505.

0967 PATTERNS OF KERATIN GENE EXPRESSION ARE TISSUE SPECIFIC FOR DIFFERENT STRATIFIED SQUAMOUS EPITHELIA DURING FETAL AND NEONATAL DEVELOPMENT Charles F. Shuler, Rajesh Aggarwal, Stephen A. Schwartz, The University of Chicago, Chicago, IL. 60637.
Keratin gene expression serves as a specific parameter of epithelial cell differentiation. The 67kd keratin has been proposed to be a specific marker for stratified squamous epithelium (SSE) terminal differentiation. However, the pattern of temporal keratin expression in simple prenatal epithelia during fetal SSE growth and differentiation is unknown. The keratins from 4 SSE types were characterized during pre and post-natal development. Backskin, footpad, palatal and tongue epithelia were isolated from 13-17 days(d) in utero, 1d neonatal, and adult rats. The keratins were compared with the corresponding histology of the respective tissues. Backskin and footpad epidermis from 13-17d in utero had simple patterns of 4-6 keratins with molecular weights (MW) between 48 and 58kd. 19d in utero, and especially postnatal backskin and footpad, displayed an abundance of 3 keratins with MW greater than 60kd including one of 67kd. In backskin and footpad the appearance of the higher MW keratins coincided with the development of the stratum corneum, implying that 67kd keratin is synthesized in terminally differentiating cells. However, palatal and tongue epithelia had 6 and 7 keratins respectively ranging in MW from 50 to 68kd. Identical keratins were present at all respective stages of embryogenesis and epithelial differentiation. Keratin phenotypes from palate and tongue did not correlate with the tissue histology until the adult when some of the keratins increased proportionally to the thickening of the stratum corneum. Therefore, in contrast to recent proposals, it is apparent that all SSE do not progress through identical programs of keratin gene expression during epithelial differentiation.

0968 REGULATION OF ACTIN GENE EXPRESSION AFTER DIFFERENTIATION AND ADENOVIRUS INFECTION. K. Khalili, C. C. Howe and R. Weinmann, The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104

We have studied the pluripotent F-9 teratocarcinoma cell line and its differentiated counterpart PYS to understand the lower levels of actin protein synthesis in the latter. We have monitored the rate of actin RNA biosynthesis with human genomic probes previously characterized (Khalili and Weinmann, Gene, 1982). The analysis reveals that although the rate of actin RNA synthesis is identical, the stability of the hnRNA precursor in nuclei is markedly reduced on differentiation. Thus, the lower level of translatable actin mRNA in the cytoplasm of PYS cells can be explained by the differential instability of the nuclear hnRNA precursor.
Another case we analyzed for the rationale for differential expression of actin genes was the adenovirus-induced shutoff of cellular gene expression. In this latter case we found the rate of actin RNA transcription and accumulation of nuclear precursors was unaffected. Since the cytoplasmic levels of actin mRNA were unaltered, we studied the defect at the translational level. At least part (one-half) of the actin mRNA is shifted to smaller polysomes and becomes untranslatable. Furthermore, cell-free translational extracts from infected cells discriminate against cellular actin mRNAs. Since these effects are only observed in adenovirus-infected cells, we suggest that these alterations are virus-induced.

0969 DEVELOPMENTAL REGULATION OF ALBUMIN AND α-FETOPROTEIN (AFP) GENE EXPRESSION IN THE RAT, Joseph Locker and Louis Muglia, The University of Chicago, Chicago, IL 60637

Albumin and AFP are major serum proteins of similar structure, transcribed from closely linked genes. They comprise a developmentally-regulated gene family with four distinct modes of expression: **A fetal liver mode.** Both genes are transcribed at maximal levels; both show intermediate DNA methylation and high chromatin sensitivity to DNaseI. **A fetal yolk sac mode.** AFP is transcribed at maximal levels while albumin is transcribed at very low levels. The AFP gene is unmethylated and shows high chromatin sensitivity; while the albumin gene is generally methylated but has a demethylated region near the 3'-end, and intermediate chromatin sensitivity. **An adult liver mode.** Albumin is transcribed at maximal levels while AFP is transcribed at very low levels. The albumin gene is unmethylated and shows high chromatin sensitivity, while the AFP gene is generally methylated but has two demethylated regions near the 3'-end and intermediate chromatin sensitivity. **An inactive mode** (e.g. adult kidney). Neither gene is transcribed, and both have very high DNA methylation and low chromatin sensitivity. The gene switching of fetal liver involves a gradual reduction of AFP but not albumin mRNA that begins about 16 days gestation and precedes the rise in serum albumin by 2 to 3 days.

0970 PARTIAL PURIFICATION OF THE ADENOVIRUS EARLY 1A (E1A) PROTEIN, A EUKARYOTIC TRANSCRIPTIONAL REGULATOR, Ann Tsukamoto and Arnold J. Berk, Molecular Biology Institute and Department of Microbiology, U.C.L.A., Los Angeles, CA 90024

Studies with adenovirus early region 1A (E1A) mutants have shown that the 289 amino acid protein encoded by this region is a positive regulator of transcription because it is required for normal induction of the other adenovirus early region genes. Transcriptional activation by this protein is not limited to these adenovirus early genes since activation has been demonstrated for the rat insulin gene and the rabbit β-globin gene. We will describe the partial purification of the 289 amino acid E1A protein from adenovirus 2 infected human cells. In addition, the assay which will be used to demonstrate the functionality of the purified protein will be presented.

0971 DNA SEQUENCES COMPLEMENTARY TO SPORULATION-SPECIFIC INDUCED TRANSCRIPTS ARE NOT NECESSARILY ESSENTIAL FOR DIFFERENTRATION, Elizabeth Gottlin Ninfa, Larry Feldberg and David B. Kaback, Dept. of Microbiology, UMDNJ-New Jersey Med. Sch., Newark, N.J. 07103

Cultures of yeast heterozygous for mating type (MATa/MATα) can be induced to undergo synchronous meiosis and spore formation. At least 95% of the transcripts that have been found in sporulating cells have also been found in vegetative and asporogenous cells. Thus only a limited number of DNA sequences are complementary to sporulation-specific expressed transcripts. To isolate these genes, a yeast: λ607 recombinant DNA library containing 2-4 kb inserts was constructed and screened by differential plaque filter hybridization. The small inserts minimize the possibility that adjacent transcripts found in both vegetative and sporulating cells will obscure the differential hybridization signal from a sporulation-specific transcript. So far, four unique DNA sequences complementary to sporulation-specific induced transcripts have been isolated. To determine their role in sporulation, these genes were disrupted in the yeast chromosome by inserting a nonspecific DNA fragment into the middle of the transcribed region. So far, homozygous insertion mutants of three genes appeared to both sporulate normally and give 4 viable spores suggesting these sporulation-specific expressed sequences are not obligatory for differentation. In related experiments, we examined the expression of cloned vegatative genes thought nonessential for sporulation. CDC10 is a gene required for mitotic growth but not sporulation. Surprisingly, sporulating cells contained 10 fold more CDC10 transcript than either vegetative or asporogenous (α/α and a) cells. Higher levels of transcripts complementary to the inducible galactose catabolic genes (GAL7,10,1), which serve no apparent function in sporulation, were also found in sporulating cells. These results suggest that genes preferentially expressed during cellular differentiation may not always be essential for the process.

0972 REGULATION OF THE YEAST STE6 GENE BY THE MATING TYPE LOCUS, Katherine Wilson and Ira Herskowitz, University of California, San Francisco, CA 94143

The mating type locus (MAT) of the yeast Saccharomyces cerevisiae codes for regulatory proteins which control the expression of unlinked genes required for mating and sporulation. One of these, encoded by the MAT@2 gene, is a negative regulator of a family of genes (the "a-specific" genes): STE6, STE2, STE14, BAR1, and the a-factor structural gene.

To determine the mechanism by which the @2 protein regulates this gene family, we have cloned the STE6 gene to use as a probe for STE6 expression. We find that STE6 RNA is not detectable in strains which have wildtype @2 function, but is only detectable in strains where @2 protein is absent (as in MATa strains) or defective (as in mat@2 mutants). @2, therefore, inhibits the production of stable STE6 RNA. In addition, analysis of a STE6-lacZ fusion has revealed a region, located approximately 100 basepairs upstream of the transcription initiation site, which is necessary for @2 control over STE6. In other words, deletions which remove this region allow STE6 expression even in the presence of @2. We are determining whether this putative @2 control region is sufficient to confer cell-type specific regulation over heterologous genes. In addition, we are determining whether proteins from crude extracts of @ cells bind to STE6 DNA in DNA protection studies.

0973 SEQUENCE ORGANIZATION AND DEVELOPMENTAL EXPRESSION OF AN INTERSPERSED, REPETITIVE ELEMENT WITH ENHANCER-LIKE CHARACTERISTICS IN DICTYOSTELIUM. Alan R. Kimmel, LCDB, NIADDK, Bethesda, MD 20205

We have examined the genomic organization and developmental expression pattern of a short, interspersed repeat element and associated single-copy sequences. 1% of the poly(A)+RNA from vegetative cells contain sequences which hybridize to this repeat. The complementary RNA is heterogeneous in size and 90% of its mass hybridizes to single-copy DNA. A series of genomic DNAs and cDNAs derived from poly(A)+RNA have been isolated which are complementary to the repeat. Comparisons of the various genomic and cDNA sequences indicate that $(AAC/GTT)_n$ but in 100 short (-35 to 150 bp) tandem blocks per genome interspersed with single-copy DNA. DNA and RNA blot hybridizations with probes from regions adjacent to this element or probes specifically deleted of $(AAC/GTT)_n$ sequences hybridize to unique restriction fragments and unique poly(A)+RNA species. The $(AAC/GTT)_n$ is asymmetrically transcribed with only $(AAC)_n$ sequences represented in RNA. One stretch has been localized 70 bp 5' to transcription initiation of a particular gene. While in other cases the M4 repeat lies toward the 5'-end of isolated cDNA clones. The $(AAC)_n$ gene family is expressed with a specific developmental pattern. Individual AAC-containing RNAs have a pattern of expression during development suggestive of coordinate expression of many AAC gene family members. The M4 repeat is enhancer-like in its sequence, position independence and pattern of expression.

0974 YEAST RIBOSOMAL PROTEIN GENES AND YEAST GENES NECESSARY FOR PRE-mRNA PROCESSING, John L. Woolford, John C. Larkin, Robert L. Last, and Mitch O. Rotenberg, Carnegie-Mellon University, Pittsburgh, PA. 15213

We are investigating the mechanism whereby the yeast ribosomal protein genes are coordinately expressed, using several cloned rp genes. None of six cloned rp genes are clustered with other rp genes in the yeast genome; some of these rp genes are single copy and some are duplicated. DNA sequence analysis of four of these genes has revealed some sequence motifs in common 5' to the genes. We are studying one particular single copy rp gene with a readily assayable phenotype, resistance or sensitivity to the antibiotic cryptopleurine. Gene doseage studies indicate that the expression of this gene is balanced with that of other rp genes by a post-transcriptional regulatory mechanism. Nucleic acid sequences involved in the expression of this gene *in vivo* are being determined by assaying the cryptopleurine phenotype of yeast transformed with mutagenized copies of the gene. We are examining the duplicated rp39 genes in order to determine whether a special mechanism exists for balancing their expression with that of single copy rp genes. The rp39 genes are similar but not identical in sequence,and both are expressed. LacZ gene fusions with the 5' sequences of the rp39a gene have been constructed whose expression in yeast is regulated like that of the intact rp39a gene. Mutations have been isolated that affect the expression of this rp39a gene fusion *in vivo*.

We have cloned three yeast genes, RNA2, RNA3, and RNA11, that are necessary for pre-mRNA splicing in yeast. We are identifying the products of these genes and isolating antibodies against them in order to determine their subcellular localization and biochemical properties.

0975 PROTEINS INVOLVED IN THE DEVELOPMENTALLY REGULATED IN VITRO TRANSCRIPTION OF 5S RNA GENES FROM A CHROMATIN TEMPLATE, Mark S. Schlissel and Donald D. Brown, Carnegie Institution of Washington, Department of Embryology, Baltimore, Md. 21210

There are 40,000 copies of the oocyte-type and 800 copies of the somatic-type 5S RNA gene per diploid genome in *Xenopus laevis*. Both types of 5S RNA genes are transcribed *in vitro* from purified genomic DNA. Chromatin purified from somatic cells, however, supports the synthesis of only somatic-type 5S RNA *in vitro*. We present evidence that the somatic-type genes in chromatin are present in stable transcription complexes lacking only RNA polymerase III. This chromatin can be perturbed by a variety of means *in vitro* so that the oocyte-type 5S RNA genes become active when they are incubated in complete transcribing extracts. In each case, the activation of the oocyte-type genes correlates with the removal of histone H1 from the chromatin template. The activated oocyte-type genes can be re-repressed by reconstitution with purified H1 at a ratio of about 1 H1 molecule per nucleosome. Other histones at greater concentrations can also re-repress the oocyte-type genes. When oocyte-type genes in activated chromatin are programmed into active transcription complexes *in vitro*, they become refractory to H1 addition. This leads us to a model of the differentiated state of 5S gene expression which consists of active genes that are packaged in stable transcription complexes and repressed genes that have no factors associated with them. The inactive genes are inaccessible to added factors because they are complexed in a chromatin structure containing histone H1.

0976 STRUCTURE OF SOME DEVELOPMENTALLY REGULATED GENES IN DICTYOSTELIUM. C. Reymond, S. Datta, R. Hernandez, and R.A. Firtel, University of California San Diego.

We are presently investigating a series of genes preferentially expressed in prestalk cells late in Dictyostelium development which appear to be coordinately regulated under a number of developmental conditions. Transcription from these genes is first detectable in mid-aggregation, at the time physiological levels of cAMP are rising. Their mRNAs can also be induced precociously in shaking culture by cAMP. When late developing aggregates are dissociated, the level of complementary RNA decreases rapidly; addition of cAMP to these cultures causes a rapid reaccumulation of the mRNAs to their original levels. These results suggest that the expression of these genes is modulated by cAMP (1).

Genomic clones for these mRNAs have been isolated and are currently being characterized. Two of the clones hybridize to 2 transcripts of different molecular weights, even though the corresponding genes are unique. Preliminary evidence suggests that this may be the result from differential splicing. In depth studies of the expression of these genes during development as well as sequence comparisons of their possible 5' regulatory regions will be presented. The goal is to use DNA mediated transformation to determine the biological function of such regions.

(1) Mehdy, M.C., Ratner, D., Firtel, R.A. (1983). Cell 32, 763.

0977 EXPRESSION OF A TRANSPOSON DURING HEAT SHOCK AND DEVELOPMENT IN DICTYOSTELIUM. Annegrethe Sivertsen, Elliot Rosen and Richard A. Firtel. University of California San Diego, La Jolla, CA 92093.

We have identified a 4.9kb transposable element, Tdd-1, containing 313 base pair inverted repeats. There are ~50 complete copies and 150 partial copies of the element in the genome. Tdd-1 hybridizes to two sets of transcripts: one is induced during development and the other is induced by heat shock. The developmentally regulated transcripts are heterogeneous in size and of the same polarity. With the exception of the inverted repeats all subfragments of Tdd-1 hybridize to this set of transcripts. Due to the presence of partial Tdd-1 elements we do not know the origin of heterogeneity in this set of transcripts. Induction of the developmental transcripts occur coordinately during aggregation. Heat shock of Dictyostelium induces synthesis of a 0.9kb transcript from Tdd-1 which has the opposite polarity of the developmentally regulated ones. Induction of the heat shock message occurs within 30 minutes and expression is maximal at 2-3 hours. In order to study the heat shock inducible transcript from a single element we have cloned parts of Tdd-1 in yeast. We have identified a heat shock inducible RNA in yeast transformants that has the same polarity as the heat shock inducible message hybridizing to Tdd-1 in Dictyostelium. This would suggest that the cloned Tdd-1 sequence includes regulatory sequences required for heat shock induction in Dictyostelium and that these are being recognized in the heterologous yeast system.

0978 REQUIREMENTS FOR PROMOTER ACTIVATION IN TWO HUMAN CELL LINES. D. Lewis, S. Fu and J. Manley, Dept. of Biol. Sci. Columbia University, New York, NY

We are examining the function of the cloned adenovirus type 2 (Ad2) late promoter in two human cell lines, HeLa and 293 (293 cells are human embryonic kidney cells transformed by, and which constitutively express, Ad5 early region IA and IB). The Ad2 promoter (-403 to +33bp relative to the late mRNA start site) was fused to SV40 T antigen encoding sequences to monitor expression. The promoter is not active in transient expression assays in HeLa cells unless an enhancer element (the SV40 72 base pair repeat) is present in cis, consistent with the results of others. However, this same promoter is expressed efficiently in 293 cells in the absence of an enhancer sequence. These results establish that this promoter can be activated by two distinct mechanisms; i.e., through the presence of either a cis-acting enhancer element in HeLa cells, or else a (presumably) trans-acting factor in 293 cells.

We have also mapped the boundary of the nucleotide sequences required for expression in the two cell types. In both 293 and HeLa cells, wild-type levels of transcription are obtained with 5' deletion mutants terminated at -66bp from the mRNA start site, although further deletions result in dramatic decreases in expression. Deletions which impinge upon the promoter from the 3' site, however, have quite different effects in the two cell types. Deletions to position -2 have no effect on promoter function in HeLa cells. In 293 cells, deletion to +7 reduces expression greater than ten fold, while deletion to +5 or -2 essentially abolishes initiation. Interestingly, these deletions destroy a sequence, first noted by Ziff and Evans, capable of forming a stem-loop structure. We speculate that this sequence may form a recognition signal for a factor required for promoter activation in 293 cells, but not in HeLa cells.

0979 ANTIBODIES TO SMALL NUCLEAR RNPs INHIBIT SV40 RNA SPLICING IN XENOPUS LAEVIS OOCYTES:
A. Fradin, R. Jove, C. Hemenway, T. Michaeli, J.L. Manley, and C. Prives, Dept. of Biol. Sci., Columbia University, New York, NY, 10027

Microinjection of SV40 DNA into Xenopus laevis oocyte nuclei results in the expression of both the viral late and early region transcripts, and the synthesis of capsid proteins VP-1 and VP-3, as well as the large and small tumor antigens. As observed during the late phase of infection in permissive monkey kidney cell, late-region transcripts are present in approximately ten-fold excess over early transcripts. However, unlike the infected monkey cell, oocytes do not replicate SV40 DNA, and their choice of RNA splicing pathways differs considerably from that observed in infected or transformed mammalian cells. This suggests that different cell types regulate expression of exogenously added genes by alternate mechanisms.

To investigate the role of snRNP particles in RNA processing, serum of several SLE (lupus) patients containing antibody to U1-RNP were coinjected into the oocyte nucleus along with SV40 DNA. The presence of antibody resulted in a virtual complete inhibition of late mRNA splicing. Although unspliced RNA was found in the cytoplasm, synthesis of VP-1 was virtually abolished. While all antisera tested affected late RNA splicing and VP-1 synthesis, some had no detectable effect on early RNA splicing and protein synthesis. However, some antisera resulted in a drastic inhibition of large T antigen, but not small t antigen, synthesis. In fact, in no case has an effect on the small t antigen splice been detected. These results suggest that not only do U1-RNPs play a crucial role in RNA splicing, but also that SV40 early and late pre mRNAs have different requirements for processing.

Developmental Genetics

0980 SEXUAL REGULATORY GENES IN DROSOPHILA MELANOGASTER, Deborah J. Andrew, UCSD, La Jolla CA 92093

Sex determination is one of the most dramatic examples of the differential control of gene expression during development. The past few years have witnessed substantial progress in elucidating the regulatory hierarchy controlling sexual differentiation in Drosophila melanogaster. Genetic functions at three levels have been identified. These include (1) the primary determinant of sex: the X chromosome to autosome ratio; (2) regulatory genes (e.g. dsx, tra, tra-2, ix) whose actions are set in response to this ratio and whose functions are to control the actions of (3) terminal sexual differentiation genes. Recent work in our lab focused on the genetic characterization of regulatory genes in this hierarchy. To begin to test models derived from genetic studies as well as to elucidate the molecular basis of sex determination, our lab has cloned dsx and is well along in the cloning of both tra and tra-2. Since nearly all known genes that function in the regulation of sex determination have been found fortuitously, the major thrust of my work is a molecular search for additional genes that function with tra, tra-2, dsx, and ix in the control of sex determination. My approach has been to isolate genomic sequences homologous to the cloned dsx gene. Several sequences have been isolated and their cytological positions determined. Genetic screens of these regions are being done as well as molecular assays for sex specific transcripts. Moreover, one "dsx cognate" sequence hybridized close to the cytological position of tra and another close to tra-2. These sequences are being used in the cloning of these two genes.

0981 A SALIVARY GLAND MARKER FOR VARIEGATED POSITION-EFFECT IN Drosophila melanogaster.
J. Stephen Kornher and Stuart A. Kauffman. University of Pennsylvania, Philadelphia, PA 19104.

Variegation in Drosophila occurs primarily in adult diploid tissues, yet the compaction of variegating genes is manifest in the polytene nuclei of the larval salivary gland. We have evidence for a salivary gland specific marker which variegates in a translocation stock called $T(1;4)w^{m258-21}$. The marker chosen was the gene Sgs-4 which encodes a glycoprotein specific for the larval salivary gland. A cloned probe specific for the Sgs-4 structural gene (pacDm1513) was utilized, thus permitting measurement of Sgs-4 transcript levels and the extent of polytenization for the Sgs-4 locus as a function of variegation permissive (17°C) and variegation restrictive (29°C) temperatures.

$T(1;4)w^{m258-21}$ larvae raised at 17°C accumulated approximately 50% less Sgs-4 transcript than larvae raised at 29°C as determined by RNA dot blot and Northern blot analysis. Control larvae showed no dramatic temperature effect. These results are consistent with measurements of Sgs-4 protein levels on polyacrylamide gels. This data correlates with an increase in the percentage of salivary gland nuclei compacted at the Sgs-4 locus in $T(1;4)w^{m258-21}$ larvae raised at 17°C when compared to 29°C.

Whole genome Southern analysis of salivary gland DNA from this strain suggests that the Sgs-4 locus is less polytene at 17°C than at 29°C. Thus reductions in Sgs-4 protein and transcript levels are correlated with increased compaction and less polytenization of the Sgs-4 locus in salivary gland nuclei of $T(1;4)w^{m258-21}$ larvae raised at 17°C.

0982 DISPROPORTIONATE REPLICATION OF THE HISTONE GENES DURING THE DEVELOPMENT OF DROSOPHILA MELANOGASTER. Linda D. Strausbaugh, Genetics and Cell Biology Section, The University of Connecticut, Storrs, Ct. 06268.

The histone genes of *Drosophila melanogaster* are a tandemly repeated multigene family of approximately 100 repeats per haploid genome. These repeating units occur in two sizes of 4.8 and 5.0 kb., and each repeat contains one gene for each of the five histone protein subtypes. The vast majority of the histone repeats are clustered at a single site on the left arm of chromosome 2, at cytogenetic position 39DE. These tandemly repeated histone genes are shown to undergo a disproportionate replication when present in a single, rather that the usual double dose. This deficiency for the histone locus occurs in individuals which are geneticly constructed to be heterozygous for the chromosome Df(2L)161, a deficiency chromosome with breakpoints at 38A6-B and 40A4-B1 on 2L, which deletes the entire histone cluster. Quantitative dot hybridizations and genomic blots with reconstruction controls reveal that there is a substantial increase in histone repeats in DNA samples from adult deficiency heterozygotes. This amplification of histone DNA has occurred during the ontogeny of the fly in a single generation. Results from nitrocellulose blots of fragments generated using a number of different restriction enzymes show that both major repeat types are included in the increased copies in adult DNA. A hemizygous genetic state for this histone region is also shown to delay development to climbing larval stages and to delay adult eclosion times, suggesting that the combination of amplified copies and delayed development may compensate for a histone deficiency. Experiments are in progress to address possible tissue specificty of the amplification event. Supported by PHS grant 1 R01 GM28680 and the Univ. of Conn. Research Foun.

0983 CELLULAR DEFECTS IN TS EMBRYOGENESIS MUTANTS OF THE NEMATODE CAENORHABDITIS ELEGANS, Randall Cassada[1], Kenneth Denich[2], Edoardo Isnenghi & Einhard Schierenberg[3], 1 Zoology, University of Freiburg, West Germany; 2 Genetics, University of California, Berkeley; 3 MCD Biology, University of Colorado, Boulder.

To analyze the genetic program for embryogenesis in this simple metazoan, we have isolated temperature sensitive lethal mutants defining 30 "emb" genes, essential for embryogenesis. Ten *emb* genes map together on chromosome III in a possible cluster. We estimate there will be 200-500 total emb genes from complementation data for 54 such genes so far known (Cassada et al., 1981; Hirsh et al., 1977; Miwa et al., 1980; Wood et al., 1980). The maternal genome plays the dominant role in embryogenesis, since for most *emb* genes maternal expression can be shown to be necessary or sufficient (or both). We can order the 30 mutants according to temperature sensitive period and arrest stage.

The cellular anatomy and lineages in living embryos in the normal 12-hour, 550-cell embryogenesis are now known (Sulston et al., 1983). Before the 100-cell stage, we see some defective cell process(es) microscopically in most *emb* mutants, e.g., in meiosis (polar-body formation), pronuclear migration & fusion, egg shell formation, morphology and behavior of mitotic spindles or cell membranes, timing or orientation of cell divisions. Timing defects include changes in the cell cycle clock (a 3 x reduction for all cells, or alteration of the slope of the anterior-posterior gradient of division rates or division as if both poles were posterior) or alteration of the order of division relative to other developmental events, e.g. migration in gastrulation. Defects may be related to histone defects in some of the mutants previously reported here (Certa et al., 1981).

0984 ANALYSIS OF NORMAL AND TRANSDUCED GENES OF CATECHOL METABOLISM IN DROSOPHILA DEVELOPMENT, J. Lawrence Marsh, U.C. Irvine, Irvine, CA 92717

Although the foundation of our understanding of gene regulation comes from studies of the biochemical genetics of enzymes in a variety of metabolic pathways in prokaryotes and lower eukaryotes, most of the gene systems studied in metazoans have been non catalytic structural genes which are terminal differentiation products. The 37B,C region on the left arm of chromosome II in *Drosophila* contains a cluster of genes which affect cuticle formation and at least three genes which affect catechol and biogenic amine metabolism directly. At least one of these genes (dopa decarboxylase, *Ddc*) must go through five cycles of expression and repression in response to developmental cues. Genetic evidence has located the *l(2)amd* locus adjacent to the *Ddc* locus and suggested that it may show a similar pattern of control. We have identified clones containing the *amd* gene by p-mediated gene transfer and transformation complementation and have analyzed the expresssion of this locus in relation to *Ddc* expression. We have also analyzed the expression of 15 new transformation induced chromosomal locations of the *Ddc* gene with respect to tissue and temporal regulation. All inserts identified show a qualitatively normal pattern of expression although several inserts show abberant levels of expression at particular stages which may result from their new chromosomal location. The implications of these position effects are discussed and transformation with modified genes is described.

0985 ISOLATION OF COSMIDS FROM THE t COMPLEX OF THE MOUSE USING HOMOLOGOUS RECOMBINATION IN E. COLI, Anna-Maria Frischauf, Annemarie Poustka, Hans R. Rackwitz, Bernhard Herrmann, Elliot Ehrich and Hans Lehrach, European Molecular Biology Laboratory, Heidelberg, FRG

Cosmids from the proximal half of chromosome 17 from wild type and t complex bearing mouse genomes have been isolated by screening of cosmid libraries by homologous recombination in E. coli. The probe plasmids were cloned DNA fragments in pUC9 derived by microcloning of individually cut metaphase chromosomes (D. Röhme, H. Fox, B. Herrmann, A.-M. Frischauf, J.E. Edström, P. Mains, L. Silver, H. Lehrach, submitted for publication).
Cosmid libraries were constructed in vectors having an R6K origin and no homology to pUC9. In addition, cosmid vectors were designed to allow chromosome walking using homologous recombination techniques. Handling and transfer of cosmid stocks relied largely on in vivo packaging of cosmids into phage lambda particles by induction of a resident lysogen.

0986 STRUCTURE AND REGULATION OF THE YELLOW GENE OF DROSOPHILA, Harald Biessmann Department of Biochemistry and Biophysics, Univ. of California, San Franciso,CA94143
The yellow gene, located at the tip of the X chromosome (y: 1-0.0) is one of many genes whose normal function is regulated to produce a precise spatial pattern. Its product is required to synthesize or deposit black melanin pigment into the cuticle of the developing fly during the second half of the pupal stage. Deposition of melanin is first visible in the dorsal bristles of head and thorax in 70 hrs old pupae and continues for the next 20 hrs in a defined sequence. To study the underlying principles of tissue specific expression and developmental control, I have isolated some 70 kb of DNA from the y gene region. The structural gene could be localized to a 4 kb DNA fragment by mapping the breakpoints of 11 different alleles of the y^1-type which give rise to a totally mutant (yellow body color) phenotype. With this DNA fragment I could identify a $polyA^+$ mRNA transcript present in early pupae but not in embryos, late pupae or adults. It appears that the y gene is transcribed during the first two days of pupal development, prior to the onset of visible melanin deposition in the cuticle. In contrast to y^1-type alleles, y^2-type alleles exhibit distinguishable patterns of cuticle coloration which is typical for every allele and resides in the yellow locus itself. By genomic Southern analysis I have mapped the breakpoints of 8 different of these pattern mutations and found that they map very close to the coding region, with one notable exeption(y^{bg}) which maps some 20 kb away. Further analysis of these breakpoints and insertion sequences will be done to establish a correlation between the various DNA lesions and the resulting tissue specific mutant phenotypes.

0987 A ZYGOTIC GENE WHICH SPECIFIES DETERMINATION OF SEGMENTS IN THE DROSOPHILA BLASTODERM Teresa R. Strecker, Judith A. Lengyel and John R. Merriam, University of California, Los Angeles, CA 90024. -- Tailless is a uniquely interesting gene because it is the only known zygotic lethal gene which affects both the anterior and the posterior of the embryo pattern, without affecting the middle segments. We believe that the tailless gene acts early and is involved in maintenance and/or elaboration of the maternal organization of the anterior-posterior axis of the embryo. -- Homozygous tailless embryos have a defective head and lack the telson and the eighth abdominal denticle band. These mutant embryos never hatch from the chorion. The overall length of the body does not appear to be shortened, despite the loss of the telson and associated eighth denticle belt, which contribute at least ten percent to the body length of a normal embryo. The body length of the mutant embryo appears to be achieved by an increase in length of the remaining abdominal denticle belts. In contrast, there is a range in severity of the head phenotype in tailless embryos, which is probably due to an arrest at various times during the process of head involution. Eleven maternal effect mutants, of which polehole is an example, with an embryonic lethal mutant phenotype appear similar to tailless (A. Mahowald, pers. comm.). -- The tailless gene maps to the chromosomal region 100A. Deficiencies of the region which uncover the $tailless^1$ allele appear, from preliminary results, to have an exaggerated phenotype. Work is continuing to characterize the phenotype more fully by SEM and to obtain additional tailless alleles. By combining polehole and tailless in a double mutant embryo we will learn what results when the contribution to pole organization from both the maternal and zygotic genome is defective.

0988 RECESSIVE DEVELOPMENTAL MUTATIONS EXPRESSED FOLLOWING HEAT SHOCK, Nancy S. Petersen and Herschel K. Mitchell, Division of Biology, Caltech, Pasadena, CA. 91125

Heat treatment of Drosophila pupae at specific stages of development causes defects which resemble mutant phenotypes. This has led to the suggestion that the heat induced developmental defects or phenocopies are due to failure of gene expression at a specific stage in development. Recently we have found that we can uncover a recessive mutant phenotype by heat treatment at the appropriate developmental stage. This shows that some phenocopies at least are due to failure of a specific gene to be expressed. We have worked with two recessive mutants, forked which affects bristles and multiple wing hair, which affects hair cells all over the body. We have been able to define sensitive periods when pupae which are heterozygous for the recessive mutant gene and a wild-type gene can be heated, and the resulting adults show the mutant phenotype. The sensitive period for uncovering the recessive phenotype is different for different parts of the animal, allowing us to use heat shock to determine the time of gene action in specific tissues. Furthermore, the ability to define the mutant gene responsible for a phenocopy opens the way for molecular analysis of the mechanism of phenocopy induction.

0989 CELLULAR MORPHOGENESIS IN THE YEAST CELL CYCLE, J.R. Pringle, K. Coleman, A. Adams, S. Lillie, B. Haarer, J. Robinson, C. Jacobs, C. Evans, and P. Shiels. Division of Biological Sciences, The University of Michigan, Ann Arbor, MI 48109

We are studying the morphogenetic events of the yeast cell cycle using three main approaches. (1) Biochemical and morphological analyses of known cytoskeletal proteins. For example, visualization of actin and tubulin in wild-type yeast and morphogenetic mutants using fluorescent probes reveals spatial correlations suggesting that both cytoskeletal elements may be involved in generating the polarization of growth and cell-wall depostition that occurs in these cells. However, elimination of the microtubules with the inhibitor nocodazole does not prevent either the emergence or the selective growth of the bud, although it does prevent the migration of the nucleus to the mother:bud neck that normally occurs prior to nuclear division. (2) Continuing mutational analyses of cell-cycle events. Presently we are focusing on the analysis of second-site suppressors of available *ts* or *cs* cell-cycle (*cdc*) mutants with morphogenetic defects. To date, extragenic suppressors have been isolated for *cdc3*, *10*, *11*, *12*, *24*, and *42* mutations. It appears that some of the suppressors of *cdc10* mutations are alleles of *CDC3*, and vice versa. (3) Identification of the products of the *CDC* genes involved in morphogenesis. We are isolating the genes of interest on recombinant plasmids, constructing fusions of the genes to the *E. coli lacZ* gene, raising antibodies against the fusion proteins, and using these antibodies to identify and localize the *CDC* gene products. To date, *CDC24-lacZ* fusions have been obtained, *CDC11* and *12* have been isolated and unequivocally identified, and plasmids complementing *cdc3* and *10* mutations have also been obtained.

0990 ALTERATIONS IN PROTEIN MODIFICATION IN A *DROSOPHILA* MUTANT SHOWING ZYGOTIC LETHALITY AND MATERNAL EFFECT LETHALITY, Clarissa M. Cheney, Kathryn G. Miller and Allen Shearn, Department of Biology, The Johns Hopkins University, Baltimore, MD 21218.

The temperature-sensitive *Drosophila* mutation, l(3) c21R^{RW630}, causes zygotic lethality and maternal effect lethality. At the restrictive temperature, 20°, homozygous mutant larvae die during the pupal stage. At the permissive temperature, 20°C, homozygous mutant larvae complete development and become fertile adults. When these mutant adult females are shifted to 27°, the eggs they produce fail to hatch, even when fertilized by non-mutant males. Furthermore, the eggs produced by mutant females at 27° show gross defects in egg structure, nuclear migration, and in formation of the syncytial blastoderm. These embryos cease development at the time of cellularization. Proteins synthesized in mutant ovaries and in mutant imaginal wing discs were examined by two-dimensional gel electrophoresis. At 27°, alterations in the synthesis of a small group of abundant 45-50k proteins was seen. Four of the affected proteins can be grouped into two pairs. The members of each pair differ in isoelectric point, but have the same molecular weight. In wild type tissue at 20° and 27° and in mutant tissue at 20°, synthesis of the acidic member of the pair is high and synthesis of the basic member is reduced or absent. In the mutant at 27°, the situation is reversed. Partial proteolytic digests show that each pair represents one protein species. The alterations in isoelectric point are probably due to a post-translation modification. Thus, the mutation l(3) c21R^{RW630} could produce a mutant modifying enzyme. The zygotic lethality and maternal effect lethality could be the result of improper modification of specific proteins.

0991 ANALYSIS OF THE IAB-2 REGION OF THE BITHORAX COMPLEX. Barbara Weiffenbach and Welcome Bender, Harvard Medical School, Boston, MA 02115

The genes of the bithorax complex of Drosophila are involved in determining the developmental pathway of the thorax and abdomen. Mutants of this complex transform structures of one segment into those of another segment. We are examining the phenotypes of several abdominal mutants in various combinations. We hope to define the individual bithorax gene products by transforming segments of the bithorax complex genes into mutant embryos. We are concentrating on one region, Iab-2, which specifies the normal development of the second abdominal segment. We chose Iab-2 because its function appears to be contained within 30kb -- a small region by bithorax complex standards. We have built a cosmid vector for P element transformation which can carry inserts of foreign DNA between 25 to 35 kb. Because this plasmid carries the att site of phage lambda it can be stably maintained by integration into the chromosome of an E. coli strain carrying a special lambda prophage (constructed by M. Gottesman and B. Howard). In addition, this cosmid carries the Drosophila alcohol dehydrogenase gene (Adh^+) as a marker to identify Drosophila transformants. Cosmids containing the Iab-2 region will be injected into Adh^- ; iab-2 homozygous embryos and the progeny screened for Adh^+ and $Iab\text{-}2^+$ transformants.

0992 MOLECULAR ANALYSIS OF THE ABDOMINAL REGION OF THE BITHORAX COMPLEX IN Drosophila melanogaster, Francois Karch and Welcome, W. Bender Harvard Medical School, 250 Longwood av. Boston 02115 Mass.

The products of the bithorax complex (BX-C) in D. melanogaster direct the fate of all the segments posterior to the second thoracic segment (T2). The genes which direct the development of the third thoracic and first abdominal segments (Ubx and bxd), have been extensively studied at the genetic and molecular level. However, the genes which control the second to the eighth abdominal segments (the iab genes), are less well characterized and are now the focus of our molecular studies. We have extended the chromosomal walk to collect the DNA of the iab genes and have localized the breaks of many chromosomal rearrangements causing recessive loss of function. As expected from the genetic data, the DNA lesions for iab-2, iab-5 and iab-6 mutations are in the same order as the segments they affect. The abdominal region of the BX-C is huge; we have isolated more than 200 kb of DNA and we just reached a break which defines the iab-8 gene, 180 kb away from the iab-2 region. We have started to analyze the transcriptional organization of these genes and have isolated cDNA clones complementary to genomic segments derived from the iab-2 region. We found at least two kinds of cDNA, and one of them appears to be spliced over 25 kb of DNA. Our major interest is to define what is the smallest functional unit of particular iab genes.

0993 POSITIVE AND NEGATIVE CONTROL OF THE BITHORAX AND ANTENNAPEDIA GENE COMPLEXES IN DROSOPHILA. P.W. Ingham. Imperial Cancer Research Fund. London. England.

Segmental identity in Drosophila is controlled by selective expression of selector genes, principally those comprising the bithorax complex (BX-C)and Antennapedia complex (ANT-C). The appropriate pattern of expression of these two complex loci is established during embryogenesis and maintained throughout the proliferation of imaginal cells. Several genes appear to be involved in the regulation of expression of the BX-C and ANT-C. Mutations of the supercombs (scb) locus result in the homeotic transformation of many body segments by causing the inappropriate expression of BX-C and ANT-C genes. Thus scb, like extra sex combs (esc) and Polycomb (Pc) appears to be involved in the negative control of the BX-C. I have found that (i) maternally derived scb activity is sufficient for normal larval development (ii) zygotic scb expression expression is essential for normal adult development. Thus the requirement for scb differs from both esc, required only maternally early in development, and Pc, required zygotically throughout development. Analysis of animals mutant for both scb and bx or pbx, suggest the latter mutation identifies a regulatory function within the BX-C. The trithorax (trx) gene is required for the positive regulation of the BX-C and ANT-C genes. Whilst it functions primarily during imaginal proliferation, its absence during embryogenesis results in a reduction in BX-C expression. The phenotype of esc; trx double mutants suggests that the two genes probably act independently. It is concluded that the BX-C and ANT-C loci are regulated by a network of genes expressed both maternally and zygotically.

Molecular Biology of Development

0994 MOLECULAR ANALYSIS OF THE BXD LOCUS WITHIN THE BITHORAX COMPLEX OF DROSOPHILA,
Debra A. Peattie, Department of Biochemistry, Stanford University, Stanford CA 94305

There is a specific class of mutations in Drosophila that affects the developmental fates of cells within the different body segments of the fly, partially or entirely transforming one type of body segment into another. These homeotic mutations lead to such oddities as flies with four wings instead of two or with legs in the place of antennae, and such mutations define genes that direct cells along different and specific developmental pathways. The bithorax gene cluster regulates developmental strategies within the thoracic and abdominal segments of the fly, and my work concentrates on the bithoraxoid (bxd) mutants which possess a first abdominal segment identical to the preceeding third thoracic segment. Extreme bxd mutants possess eight legs rather than six, and, like the other bithorax mutations, this phenotype can be explained by the absence or inactivation of a gene product necessary for specific differentiation. Genetic and molecular analyses indicate that the bxd locus is approximately 20 kb in size, and DNA sequence determination of this locus has begun. bxd-specific poly-(A)+ RNAs of 0.9 (Goldschmidt-Claremont, unpublished data), 1.2, and 1.3 kb have been found, and hybridization analysis of three bxd-specific cDNA clones indicate there are at least four exons within the bxd region. The DNA sequences of these cDNAs reveal no significant open reading frames, so another cDNA library is being constructed, and experiments are being done to determine whether or not the functional bxd gene product is actually a protein. To date, all data relevant to bxd function and to the known effects of other bithorax alleles on this locus are compatible with the possibility that the functional gene product is an RNA or RNA-protien complex

Plant Molecular Biology

0995 INHERITANCE OF FUNCTIONAL FOREIGN GENES IN PLANTS,
Robert B. Horsch, Robert T. Fraley and Stephen G. Rogers, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, MO 63167

Agrobacterium tumefaciens, the causative agent of crown gall disease is capable of transferring a DNA segment (T-DNA) located between specific border sequences from its tumor-inducing plasmid (Ti plasmid) into the nuclear DNA of infected plant cells. Expression of T-DNA encoded tumor genes in the transformed cell provides a selectable marker for recognition of those cells in culture: the ability to grow on medium without added phytohormones. Unfortunately this trait interferes with regeneration of normal, fertile, transformed plants.

Recently, we and others have obviated the need for the tumor genes for identification of transformants by construction of chimeric genes that function as dominant selectable markers in plant cells. Our chimeric gene contains the coding sequence of the bacterial gene for neomycin phosphotransferase II joined to the 5' and 3' regulatory regions of the nopaline synthase gene which is known to be expressed constitutively in higher plant cells. We have shown that petunia and tobacco cells transformed with this chimeric NOS/NPTII/NOS gene are readily selected and are highly resistant to kanamycin.

Morphologically normal plants have been regenerated from Nicotiana plumbaginifolia cells and Petunia hybrida cells transformed with an A. tumefaciens strain containing a Ti plasmid carrying a chimeric kanamycin resistance gene. The regenerated plants contain a functional chimeric gene as demonstrated by Southern hybridization analysis and the ability of leaf segments to callus on media containing kanamycin. Progeny derived from several transformed plants have been shown to inherit the foreign gene in a Mendelian manner.

0996 DEVELOPMENT OF INTRANUCLEAR MICROINJECTION INTO PLANT PROTOPLASTS,
Terry J. Reich, Larry A. Holbrook*, Brian L. A. Miki, and V. N. Iyer. Carleton University and *Agriculture Canada, Ottawa, Ontario, Canada

The technique of intranuclear microinjection of plant protoplasts for the purposes of genetic transformation is currently being developed. Protoplasts of Medicago sativa, are attached to grid patterns photoengraved on cover slips after nuclei have been stained with fluorescent dyes. A specially-designed syringe system can start and stop the flow of DNA after micropipettes are inserted into the nucleus. The entry of DNA is visualized by the swell of the nucleus. As compared to controls, 90% of the injected protoplasts go into first cell division. Single cell analytical techniques for following the short term fate of injected Ti-plasmid of A. tumefaciens are under development. An environmental chamber that controls humidity, temperature and sterility enclosed the microinjection apparatus and ensures long-term survival of protoplasts after injection.

0997 EMBRYONIC AND POST-EMBRYONIC REGULATION OF RuBPCASE. JUNE MEDFORD AND IAN SUSSEX. Dept. of Biol. Yale Univ. New Haven CT. 06511

We are studying embryonic and early post-embryonic regulatory mechanisms in the bean, Phaseolus vulgaris using the geneticexpression of RuBPCase (ribulose 1,5-bisphosphate carboxylase oxygenase). Immunological quantitation shows the protein changes during early embryogeny, reaching a peak at 20 days and subsequently decreases in the latter part ofembryogeny. The protein then reappears in the same tissue following seed germination at a level 5X that of embryogeny. Quantitation of mRNA (for both the chloroplast encoded large subunit and the nuclear encoded small subunit of RuBPCase) is currently in progress and is expected to reveal similar expression. This particular pattern of expression found in the cotyledon tissue reflects transcription in two different developmental stages that are separated by a period in which there is no DNA synthesis or cell division. We will present data which suggests that RuBPCase, in contrast to most other systems where it has been studied, does not require a light stimulus for expression. In addition preliminary data indicate RuBPCase expression in bean cotyledons is retarded by absisic acid, a known regulator of late embryonic developmental programs. Isolation of all members of the nuclear encoded small subunit gene family is in progress. Hybridization of mRNA under stringent conditions will allow discrimination of expression from distinct family members. Such discrimination of expression of individual family members should allow us to examine signals responsible for embryonic and post-embryonic expression.

0998 CHLOROPLAST MEMBRANE PROTEINS AND CYTOPLASMIC MALE STERILITY, Li Jigeng, Li Jia-yang. Institute of Genetics, Academia Sinica, Beijing, China.

M201A and M201B of sugar beets and 3197A and 3197B of sorghum were used as experimental materials in this study. "A" means sterile line, and "B" maintainer.

Preparation of chloroplast and membrane proteins was performed as previously. Membrane preparation was lysed by NP-40 and SDS and separated by electrofocus. Second dimensional electrophoresis was run in SDS-PAGE. Slabs were stained with silver method.

No significant differences were observed in the separation patterns obtained by one dimensional electrophoresis of either electrofocus or SDS-PAGE between A and B of sugar beets. However, under the two dimensional electrophoresis consisting of these two methods, obvious distincts were revealed between these two lines. In comparison with B, one spot of about 43kd protein in A was moved to alkali direction. Two spots in the range of 38-42kd at pH5-6 occurred slight but accurate changes in their positions. Similar variation were also observed in separation patterns between A and B of sorghum. In the range of pH5-6, five spots in A were more bigger and heavier in color than that in B. In the region near pH6 there was one spot in A which was absent in B, and in the same pH region two spots of about 110kd only present in A also.

These results showed that chloroplast membrane proteins may be related to the pollen fertility in higher plants.

0999 THE ORGANIZATION AND EXPRESSION OF TUBULIN GENES IN HIGHER PLANTS, M.J. Guiltinan, R. Cyr, M. Bustos and D.E. Fosket, University of California, Irvine, CA 92717

A soybean genomic library in lambda was screened with Chlamydomonas α- and β-tubulin c-DNAs. Forty-five clones containing sequences hybridizing to the β-tubulin probe and four hybridizing to the α-tubulin probe were identified and isolated. Restriction fragments of the lambda inserts which hybridized to the probes were subcloned in PUC 13 for further analysis. Restriction maps of the cloned fragments were constructed for all four α-clones and eight of the β-clones. A comparison of these maps indicate that four of the β-clones represent different genes while three of the α-clones appear to be different. Southern hybridization to restricted soybean genomic DNA with either the Chlamydomonas c-DNAs or the homologous cloned probes suggested that the soybean genome contains four each of α- and β-tubulin genes. In contrast, Southern hybridization to restricted tomato DNA gave additional bands of hybridization to the labeled probes. We verified that the genomic clones contained tubulin gene sequences by using them to hybridization-select soybean poly A + RNA which was translated in a wheat germ cell-free system. The translation products were shown to react with antibodies specific for higher plant tubulin.

1000 COORDINATE INCREASE IN MAJOR TRANSCRIPTS FROM THE HIGH pI αAMYLASE MULTIGENE FAMILY IN BARLEY ALEURONE CELLS STIMULATED WITH GIBBERELLIC ACID (GA). John C. Rogers, Washington Univ., St. Louis, MO 63110. Barley aleurone cells provide an extraordinary system for studying developmental and hormonal regulation of gene expression. Aleurone cells form a layer surrounding the starchy endosperm in barley seeds. During germination of the seed, the embryo secretes the plant hormone GA which specifically induces the previously quiescent aleurone cells to secrete large amounts of αamylase as well as other hydrolases. This response is developmentally regulated because the embryo does not synthesize amylase in response to GA, and it is hormonally regulated because the aleurone cells make no amylase in the absence of GA. To study this system we have cloned and sequenced a full-length cDNA for amylase from aleurone cells (JBC 123, 4567, 1983). Protein sequence data from others show that this cDNA corresponds to the high-pI αamylase isozyme. Southern blot data localize the gene for this clone to a single HindIII fragment and indicate that there may be as many as 6 more homologous genes. Primer extension experiments used as a primer the terminal 5' coding sequence from our cDNA clone; this primer would not cross hybridize with low pI αamylase transcripts. Two major transcripts were identified that were coordinately induced by GA. These shared a conserved 23 base sequence immediately 5' to the ATG start codon, but a C-G transversion and 3b deletion were present therein. An unusual 8bp GC palindrome immediately preceded the ATG in both. Distal to the conserved sequence there was no apparent homology. It is likely that there are transcripts from 2 separate genes. These data indicate that there is a high pI αamylase multigene family with at least 2 active members, both of which are regulated in some manner by the plant hormone, GA.

1001 ANALYSIS OF BRASSICA NAPUS STORAGE PROTEIN GENES, Steven Scofield and Martha Crouch, Indiana University, Bloomington, IN 47405

Brassica napus has two types of embryo-specific storage proteins, napin and cruciferin. These strictly regulated genes are expressed at high levels during seed development. It is possible to experimentally modulate the level of napin and cruciferin expression in cultured Brassica embryos. Embryos cultured on 10^{-6} M ABA or high osmoticum synthesize normal mid-maturation levels of storage proteins. We are studying napin and cruciferin genes with the ultimate goal of exploiting this experimental system to define DNA sequences involved in their developmental regulation. Genomic Southern blots reveal that both napin and cruciferin are coded by families of genes. The napin family has at least 12 hybridizing bands in Eco Rl digests. This enzyme does not cut within any of the cloned napin genes. These bands span a range of 2.1 to 18 Kbp. Several of the bands have intensities corresponding to 2 copies. Similar experiments done with a cruciferin probe show 3 hybridizing bands between 9 and 10 Kbp. Two of these bands have multi-copy intensities. We have constructed a genomic library for Brassica napus, an allotetraploid with 4N = 3.2 pg. Fragments from Sau 3A partial digests were cloned in the lambda vector EMBL 4. The library has been probed with napin and cruciferin cDNA clones. Comparison of restriction patterns of napin genes with a napin cDNA clone indicates no introns are present within the translated portion of the gene. Cruciferin phage have been detected but have not been isolated, apparently due to an instability problem.

1002 ORGANIZATION AND EXPRESSION OF THE COMPLEX HOR 1 AND HOR 2 LOCI IN DEVELOPING BARLEY GRAIN, Brian G. Forde, Martin Kreis, Sadiq Rahman, Richard P. Fry, Martin S. Williamson, Hilary M. Lewis, Jacqueline Pywell, Peter R. Shewry and Benjamin J. Miflin, Biochemistry Department, Rothamsted Experimental Station, Harpenden, U.K.

Over 90% of the storage proteins ('hordeins') in the barley endosperm are encoded by two genetic loci (Hor 1 and Hor 2) about 8 centimorgans apart on the short arm of chromosome 5. Each locus consists of at least 10-15 genes and specifies a heterogeneous group of polypeptides. We have studied the expression of these gene families during seed development. The coordinated, tissue-specific expression of the two loci is modulated by temporal and nutritional factors and by a trans acting 'regulatory' gene. Each of these secondary controls have been found to act differentially on the two loci and even differentially on two sub-families of genes within the Hor 2 locus. Sequence analysis of cDNA clones has revealed the occurrence of short repeated sequences at both loci and similarities between these repeats at the two loci suggest the possibility of a common evolutionary origin for the two gene families.

1003 DIFFERENTIAL EXPRESSION OF THE CHLOROPLAST GENOME IN A C_4 PHOTOSYNTHETIC PLANT.
Betty J. Wood and James R. Y. Rawson, Botany Department, University of Georgia, Athens, GA 30602 USA.
Cloned chloroplast DNA fragments were used as hybridization probes to identify the in vivo transcriptional products of the chloroplast genome that are present during the development of the chloroplast in the C_4 photosynthetic plant pearl millet (Pennisetum americanum). RNA was isolated from etiolated plants that were exposed to light for varying periods of time and from mesophyll and bundle sheath cells of plants that contained fully differentiated chloroplasts. The different RNAs were size fractionated by electrophoresis in denaturing gels and Northern blots were prepared. ^{32}P-labeled plasmids containing specific chloroplast DNA fragments were hybridized to the immobilized RNA. The stable RNAs in the chloroplasts were identified on the basis of their size and their origin on the chloroplast genome. The stable transcripts in the chloroplast were regulated in several ways during the development of the chloroplast. One, the majority of the transcripts were constitutively expressed in both the dark and the light as well in both types of cells. Two, several transcripts increased in concentration when dark grown plants were placed in the light. Three, at least 3 transcripts decreased in concentration when dark grown plants were placed in the light. Four, several transcripts appeared to be cell specific. Of particular interest is the fact that the gene for the large subunit of ribulose 1,5-bisphosphate carboxylase is represented as 2 stable transcripts in bundle sheath cells and a single stable transcript in mesophyll cells. Supported in part by grants from the USDA Competitive Grants Program (80-CRCR-1-0489) and the NSF (PCM-8200949).

1004 REGULATION OF RIBOSOMAL PROTEIN mRNA LEAVES IN AUXIN-TREATED SOYBEAN HYPOCOTYLS.
J. Stephen Gantt and Joe L. Key, University of Georgia, Athens, GA 30602.
Application of auxin to soybean hypocotyls results in a massive accumulation of protein and RNA. We have shown that 24 hours after treatment of soybean seedlings with the synthetic auxin (2,4-dichlorophenoxy) acetic acid (2,4-D) the levels of translatable cytoplasmic ribosomal protein (RP) mRNAs are increased 8-fold above those in non-treated tissue. To more thoroughly investigate the effects of auxin on RP gene regulation we have isolated RP cDNA clones. cDNA was made to poly(A)RNA isolated from auxin-treated hypocotyls. Of the approximately 150 clones screened by hybrid selection-translation, we have identified 2 that contain cDNA capable of hybridizing mRNA coding for small subunit proteins and 7 that hybridize mRNA coding for large subunit proteins. Preliminary hybridization studies show that poly(A)RNA isolated from 2,4-D treated hypocotyls contains approximately 10-fold more RP mRNA than does poly(A)RNA isolated from non-treated tissue. These cDNA clones are currently being used to further investigate the regulation of the soybean RP genes.

1005 EXPRESSION OF CHLOROPLAST GENES DURING EMBRYOGENESIS, Katyna E. Borroto, James R.Y. Rawson, Leon Dure III, University of Georgia, Athens, GA 30602

The expression of chloroplast genes during cotton embryogenesis was followed by measuring the dot blot hybridization of total mRNA to different chloroplast cDNA clones. RNA was extracted from cotton embryos at different stages of development ranging from "young embryo" (5 mg) to the mature dry seed and seeds germinated for several days. This represents a 25 fold increase in embryo size and was comprised of 19 dots. Among the clones used in the hybridization reaction were those for RUBP carboxylase LSU, chloroplast rDNA and the "photo gene 32". All of these are products of the chloroplast genome.
The results of these experiments show that these genes are expressed constitutively during embryogenesis and that this expression increases up to ten fold during germination. In some cases it appears that these genes are expressed at a higher level in very young embryos than in more mature embryos. These results are surprising since these embryos are not exposed to light during embryogenesis and are not green.
The possibility that the increased expression of these genes observed during germination is a result of increased chloroplast genome per cell rather than enhanced expression of the gene was tested by hybridization of the clones to total DNA from different developmental stages.

1006 CELLULAR DIFFERENTIATION AND PHOTOSYNTHETIC GENE EXPRESSION IN DEVELOPING MAIZE LEAVES, Belinda Martineau, University of California, Berkeley, CA 94720

In maize leaves, cell divisions occur primarily in the leaf's basal meristem, older cells being displaced by younger cells below them. This process results in a positional gradient of cell ages along the length of a young leaf. This simple basipetal pattern of graminaceous leaf development is somewhat complicated by the differentiation of bundle sheath cells; a photosynthetic cell type of maize leaves in addition to the leaf mesophyll. This "complication" of cellular dimorphism is interesting because the two cells functionally cooperate in the multi-step scheme of CO_2 fixation called C_4 photosynthesis. Maize uses two CO_2-fixing enzymes (PEPCase and RuBPCase) which are located exclusively in mesophyll and bundle sheath cells, respectively, of the plant's leaves. I have found that PEPCase and RuBPCase mRNAs are also restricted to these respective cells types in mature leaf tissue. I have taken advantage of the leaf positional gradient to compare changes in gene expression involved in C_4 photosynthesis with leaf morphological development monitored using light microscopy. Accumulation of RuBPCase (large and small subunit), PEPCase and chlorophyll a/b binding protein (a component of the "light reaction" of photosynthesis), and that of the mRNAs associated with these proteins, increases dramatically as bundle sheath cells become morphologically differentiated. Measurable quantities of all four mRNAs are, however, found in the relatively undifferentiated basal portion of the leaf. I am currently using *in situ* hybridization techniques to determine whether RuBPCase mRNAs are present in the entire population of basal cells or exclusively in cells predetermined to become bundle sheath cells.

1007 CAROTENOID BIOSYNTHESIS INFLUENCES EXPRESSION OF PHOTOSYNTHETIC GENES IN DEVELOPING MAIZE LEAVES, Timothy M. Nelson, Stephen P. Mayfield, and William C. Taylor, University of California, Berkeley, CA 94720

Maize mutants which accumulate carotenoid precursors are arrested early in plastid development.[1] A subset of these carotenoid mutants are viviparous (possess altered seed dormancy). Analogous chlorophyll biosynthetic mutants arrest at later stages of plastid development.[2] We find that certain maize carotenoid mutants which accumulate colorless precursors (e.g., phytoene) also fail to accumulate normal levels of nucleus-encoded mRNAs for chlorophyll-binding proteins, ribulose bisphosphate carboxylase small subunit, and other light-regulated photosynthetic proteins. The same mutants have normal levels of phytochrome, the pigment-protein which is thought to mediate most light effects on transcription in leaves. The effects of carotenoid deficiency on mRNA accumulation can also be generated by blocking carotenogenesis with inhibitors such as norflurazon. We have purified pigments and biosynthetic enzymes from wild type and mutant leaves. The carotenogenic activities are loosely associated with chloroplast membranes and can be solubilized by sonication. The carotenoid products apparently affect both the accumulation of photosynthetic mRNAs and proteins and the synthesis of earlier enzymes in the carotenogenic pathway. Lack of these products in mutants may be the source of blocks in plastid development.

1. DS Robertson (1975) J. Heredity 66, 67-74.
2. P Mascia and DS Robertson (1978) Planta 143, 207-211.

1008 INDUCIBLE EXPRESSION OF THE VIRULENCE GENES OF THE A6 *AGROBACTERIUM TUMEFACIENS* TI-PLASMID, Scott Stachel, Gynheung An, and Eugene Nester, University of Washington, Seattle, WA 98195

A. tumefaciens is able to transfer a specific segment of plasmid DNA from itself to plant cells. The transferred DNA becomes integrated into the plant nuclear genome and expressed, and this expression results in the phenotypic transformation of plant cells. The genes responsible for the transfer and integration steps are not contained on the DNA which is integrated, but are located on a separate thirty kilobase region of bacterial plasmid DNA designated the virulence (*vir*) region. We have analyzed the expression and regulation of twelve separate *vir* loci. *LacZ* fusions were randomly generated throughout the *vir* region by employing a *Tn3:LacZ* system, and the expression of these fusions was determined in *Agrobacterium* under a variety of growth conditions. Both actively growing dicot and monocot plant cells produce a small molecular weight factor that induces *vir* gene expression. A regulatory *vir* locus has been tentatively identified. We are currently characterizing the molecular changes that occur within induced bacteria as part of the virulence process.

1009 EXPRESSION OF TISSUE SPECIFIC EXTRACELLULAR PROTEASE ACTIVITIES IN CORN SCUTELLA AND DERIVED CULTURES, Ning-Sun Yang, James Burmester, Collin Park, Cetus Madison Corp., Middleton, WI 53562

A casein agar-indicator gel assay was optimized for quantitative measurement and screening of plant protease activities present in organ explants, tissue cultures and tissue extracts. Using this assay method, we have studied the developmental and differential expression and biochemical properties of a specific protease activity present in corn. This enzyme is tissue (scutellum)-specific, secreted extracellularly, and its expression is regulated in the early sporophytic development of corn. Readily distinguishable differences in the level and time course of expression were detected among different genotypes tested. These or similar protease activities were also detected in unorganized callus tissue cultures derived from corn scutella, but little to no activity was expressed in the organized or regenerating (ie. shooty or rooty) calli of corn tissue cultures. Positive correlation between seed germination/seedling vigor and expression of the scutellar protease activity was observed. Biochemically, the enzyme exhibits the following characteristics: molecular weight = 94000. A broad pH optimum between pH 4 to 6, completely resistant to soybean trypsin inhibitors and readily degrade high molecular weight maize scutellar proteins. Attempt will be made to isolate gene(s) coding for this protease and to characterize the regulatory sequences involved in the expression of the enzyme.

1010 DEVELOPMENTAL AND HORMONAL REGULATION OF GENE EXPRESSION IN PLANTS, Joe L. Key, John Walker and Philip Kroner, University of Georgia, Athens, GA 30602

We are investigating the regulation of gene expression in seedling tissue of soybean using cloned cDNAs to genes whose expression is regulated both during normal development (i.e., progressing from zones of cell division, to cell elongation and finally to mature fully differentiated cells) and to the hormone auxin when applied to one of those tissues. One set of two cloned sequences is expressed maximally in elongating cells, depletes rapidly upon excision, and increases rapidly upon auxin addition to the growth medium. These sequences seem to be a closely linked component of the auxin-regulated cell elongation process. Another set of the sequences are non-abundant in dividing cells and reach high abundance (up to 1% each of the total poly(A) RNA) in mature cells. Auxin addition to these cells causes these sequences to deplete to .01% or less of the total poly(A) RNA. Another poly(A) RNA sequence is present in dividing cells in one mature size and increases in size during the transition to mature cells; auxin treatment rapidly shifts the mature form back primarily to the smaller size. Physiological and molecular studies, including sequencing of genomic clones, to this set of developmentally auxin-regulated gene products are continuing. This work is supported by NIH grant GM30317.

1011 A *R. MELILOTI* LOCUS THAT REGULATES SYMBIOTIC NITROGEN FIXATION GENES, Wynne Szeto, Lynn Zimmenman, Venkatesan Sundaresan & Frederick Ausubel, Harvard Medical School, Boston, MA 02114.

Symbiotic nitrogen (N_2) fixation between *Rhizobium meliloti* and alfalfa is a complex process controlled by both bacteria and plant genes. In order to understand the molecular mechanisms underlying the reciprocal signalling which culminates in symbiosis, the role of *Rhizobium* in the developmental process was first studied. By site-directed mutagenesis (using transposon Tn5) of cloned *R. meliloti* DNA, several gene regions essential for symbiosis have been identified. To investigate the actual functions of these loci during symbiosis, a series of strains carring Tn5 insertions in different symbiotic regions were used to induce indffective (Fix^-) nodules in alfalfa. Specific proteins as well as RNA were purified from these Fix^- nodules and analysed. Using this approach, a DNA region approximately 5 kilobases upstream of the nitrogenase (*nif*) operon was found to be essential for *nif* production: strains carrying mutations in this retion differentiate into bacteroids which do not accumulate detectable amounts of *nif* RNA nor *nif* polypeptides. Further RNA hybridization studies showed that this locus affects not only the expression of *nif* RNA, but also that of another symbiotic operon adjacent to the *nif* locus. Other experiments indicate that this locus codes for an activator of *Rhizobium* synbiotic genes. In addidion, this activator has some properties which are analogous to the regulatory protein (*nif* A protein) which controls the entire N_2 fixation pathway in *Klebsiella pneumoniae*, an organism which fixes N_2 in the free-living state. This suggests that at least some aspects of the N_2fixation apparatus among divergent organisms are conserved.

1012 **STUDIES OF PLANT DEVELOPMENTAL GENES USING TI PLASMID VECTORS,** Robert B. Simpson, Elias A. Shahin, Thomas D. McKnight, Linda Margossian and Marcella Lillis, Molecular Biology Group, ARCO Plant Cell Research Institute, 6560 Trinity Court, Dublin, CA 94568.

We are developing techniques to genetically transform tomato cells with DNA inserted into non-tumorigenic ("disarmed") Ti plasmids, so that the cells can regenerate "normal", fertile plants containing the new DNA. The basic plan is to co-cultivate tomato protoplasts with Agrobacterium tumefaciens containing modified Ti plasmids. A series of modified vectors have been designed for this purpose and are at various stages of development. We believe that we have efficiently transformed tomato cells with a disarmed vector and regenerated plantlets from those cells. This conclusion, based on octopine synthesis by the plantlets, must be confirmed by analysis of the DNA of the plantlets.

The modified vectors are being used to introduce developmentally regulated genes into plants and study their ability to function in organized and unorganized plant tissue. One example is the small subunit (SS) of ribulose-1,5-bisphosphate carboxylase, a nuclear gene whose expression is regulated both developmentally and environmentally (photoinduction). We are identifying transcriptional control sequences using chimeric genes composed of the SS promoter DNA and terminator DNA fused to the coding region for neomycin phosphotransferase from Tn5. The mechanisms of SS protein synthesis, transport and processing are being studied by introducing the entire SS gene from pea into other plants where the endogeneous protein is distinguishable from the pea SS. We have also introduced the DNA for Robertson's Mutator, a transposable element from corn, into tobacco cells and are currently testing whether the mutator can transpose in this new environment.

1013 **INDUCIBLE TRANSCRIPTION AND PUFFING OF HEAT SHOCK GENES TRANSFORMED INTO DROSOPHILA.** Robert Cohen and Matthew Meselson, Harvard University, Cambridge, MA 02138

A cloned hsp70 heat shock gene with various amounts of 5' DNA was introduced into the genome of D. melanogaster by P element-mediated transformation. The hsp70 gene contained about 100 bp of 3' flanking DNA and was marked by substituting a 1.05 kb segment of bacteriophage λ DNA for the hsp70 region from +89 to +2027 (where +1 is the start of transcription). Genes with 5' DNA extending to -195, -146 and -70 are transcribed with essentially wild-type efficiency under faithful heat shock control to give a 1.6kb polyA+ hsp70-λ hybrid RNA. S1 mapping indicates that these transcripts initiate at the normal hsp70 start site. When only 51 nucleotides of 5' DNA are present, transcripts of the correct size and start site are induced by heat shock, but the amount observed is only about 1 percent of the wild type level. No transcripts are seen in heat shocked flies transformed with hsp70 genes having only 44 or 25 nucleotide pairs of 5' DNA. The hsp70 gene extending to -195 produces a salivary gland chromosome puff at the site of integration in response to heat shock.

Several fly lines transformed with a marked hsp26 heat shock gene have also been established. The introduced gene is transcribed in response to heat shock. Its developmental control in the absence of heat shock is being investigated.

1014 Developmental Expression of Immunoglobulin Light Chain mRNAs in Balb/c (normal) and SJL (λ1 deficient) Mice, Sharon Ogden and Ursula Storb, University of Washington, Seattle, WA 98195

Mice of the SJL and related strains produce about 100X less λ1 immunoglobulin than normal mice. We have found that the defect is genetically linked to a single basepair substitution in the Cλ1 gene which results in an amino acid change in the constant region of λ1 (Arp et al., Nature 298:184-187, 1982). Other λ1 gene exons and flanking sequences known to be involved in gene rearrangement are normal in SJL mice (op. cit.). In order to begin to determine whether the observed change in Cλ1 or an as yet unknown regulatory locus in the vicinity of the gene were the cause for the λ1 deficiency in SJL mice, we have analyzed the development of λ1 mRNA levels in SJL mice. Furthermore, to obtain an overall picture of light chain gene expression parallel studies were conducted on λ1 and κ mRNA levels in normal Balb/c mice and in transgenic mice carrying a functional, rearranged κ gene. The following results will be presented and discussed: 1) Normal fetuses have a 10X higher λ1/κ mRNA ratio than adults. 2) While SJL adults have exceedingly low λ1 mRNA levels corresponding to their low λ1 protein levels, SJL fetuses and newborns have normal levels of λ1 mRNA. 3) Transgenic mice express a microinjected κ gene at a high rate in lymphoid cells, but no other cells (Brinster et al., Nature, in press; Storb and Brinster, unpublished)). Their levels of λ1 mRNA are normal. The presence of a rearranged κ gene or of its products does thus not appear to exert a feedback inhibition on λ1 gene expression. Supported by NIH grants CA/AI 25754 and DE 02600. S.O. is supported by 67-4655.

1015 HERBICIDE RESISTANCE IN PLANTS: AN EXAMPLE OF GENE AMPLIFICATION, Howard M. Goodman, Edmund Tischer, and Guenter Donn, Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114

A variant line of alfalfa tissue culture cells has been isolated which are resistant to the experimental herbicide L-phosphinothricin, a mixed competitive inhibitor of glutamine synthetase (GS). The variant cell line contains approximately ten fold more GS activity than wild type cells. GS from the variant line has been purified to homogeneity and a partial amino acid sequence determined from a cyanogen bromide cleavage fragment. A cDNA clone coding for GS has also been isolated and its nucleotide sequence determined. Southern hybridization analysis indicates that herbicide resistance is due to the amplification of one of the structural genes for alfalfa GS.

1016 PHENOL SYNTHESIS IN INTACT PLANTS VERSUS CULTURED CELLS, John S. Fletcher and Michael J. Muhitch, University of Oklahoma, Norman, OK 73019

A comparison has been made of the secondary product accumulation in intact tissues versus that of newly-established and long-established tissue cultures of Paul's Scarlet rose. The phenols of rose stems and stem-derived cell suspensions have been analysed using reversed-phase HPLC. The major phenols found in stem tissue were gallic acid, epicatechin, the procyanidin dimer epicatechin-catechin, epigallocatechin gallate and an unidentified gallotannin (I). In contrast, cell suspension cultures which were started from stem and which have been maintained for over 20 years contained only gallic acid and epicatechin-catechin. A newly established suspension culture cell line of Paul's Scarlet rose contained gallic acid, epigallocatechin gallate, epicatechin gallate, ferulic acid and a procyanidin polymer as major phenolic constituents. The total phenol contents of the various rose extracts ranged from 0.5 μmol/gr fr wt in the 20 year old cell line to 130-170 μmol/gr fr wt in the stem tissue, with the newly established cell line containing an intermediate amount (35 μmol/gr fr wt). The inability of long-established cultures of plant cells to accumulate secondary products may reflect either a lack of gene expression or a loss of genetic capabilities during long periods of culture.

Embryogenesis and Oogenesis

1017 ISOLATION AND CHARACTERIZATION OF MOUSE GENOMIC DNA CLONES OF AN EARLY DIFFERENTIATION MARKER : ENDO A. M. Vasseur, C. Marle, P. Brûlet and F. Jacob, Unité de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Dr Roux, 75015 Paris.

The expression of a cytoskeletal protein, endo A, which is recognized by the monoclonal antibody TROMA-1 is differentially regulated during mouse early embryogenesis and embryonal carcinoma cell differentiation. A cDNA clone, isolated from a trophoblastoma cDNA library (Brûlet and Jacob, PNAS, 79, 2328, 1982), detects a specific 18S mRNA in trophoblastoma but not in embryonal carcinoma cells which directs the in vitro synthesis of proteins recognized by TROMA-1 in a pattern undistinguishable from Endo A. This cDNA hybridizes with two specific DNA bands of 2.5 and 2.3 kb on a southern blot of genomic mouse DNA digested with EcoRI. We have screened a mouse DNA library and isolated two kinds of genomic DNA clones : λ34 type contains the 2.3 kb EcoRI fragment and type λ24 the 2.5 kb fragment. mRNA mapping indicates that in the clone λ34 the gene is restricted to the only 2.3 kb fragment whether in λ24 it is encoded by a 9kb long fragment. Subcloning of the genomic clones has revealed that these two genes were closely linked with two different types of short repetitive interspersed sequences. We have isolated and characterized the two subclones which hybridize to the 18S Endo A mRNA and analyzed and compared the non coding regions at the 5' and 3' ends of the two genes. The possible role of the repetitive sequences associated with these two genes in the regulation of their expression will be discussed.

1018 TRANSLATIONAL CONTROL BY XENOPUS OOCYTE SPECIFIC PROTEINS, Joel D. Richter and Lori J. Lorenz, Department of Biochem. Univ. of Tennessee, Knoxville, TN 37996-0840

Xenopus oocytes contain proteins which are oocyte-specific, which decrease in amount as oogenesis progresses, which co-sediment with nontranslating ribonucleoprotein (RNP) particles (i.e., 40-60s), and which bind poly(A) RNA (JBC 258: 4864, 1983). These RNA binding proteins were used in a series of mRNA-protein reconstitution experiments. When the proteins were reconstituted with oocyte poly(A) RNA or rabbit globin mRNA, the resulting mRNP particles had densities of 1.34 g/cc. Native poly(A) RNP particles had an almost identical density of 1.36 g/cc. When reconstituted globin mRNP was injected into Xenopus oocytes, no globin synthesis was apparent even though injected naked globin mRNA was translated efficiently. Deproteinization of reconstituted globin mRNP resulted in the synthesis of globin following oocyte injection. Monoclonal antibodies were generated against the RNA binding proteins. One antibody was directed against an antigen which was oocyte specific and oogenetically regulated, which sedimented at 40-60s, and which bound specifically poly(A) RNA. The antibody is being used for 3 purposes: 1) to immunopurify the protein to use in reconstitution and RNA footprinting experiments, 2) to immunopurify nontranslating RNP particles in order to examine the RNA and protein components of such particles and 3) to immunoadsorb polysomes in an attempt to isolate and clone the cDNA for the binding protein. The cDNA will be used to examine the transcriptional control of the binding protein mRNA. (Supported by NIH Grant GM 32559.)

1019 ACTIN GENES ARE DEVELOPMENTALLY REGULATED IN THE AMPHIBIAN EMBRYO. Tim Mohun, Sean Brennan, Nina Dathan, Sharon Fairman and John Gurdon. CRC Molecular Embryology Group, Department of Zoology, University of Cambridge, Downing Street, CB2 3EJ, England.

Actin cDNAs have been isolated from embryonic cDNA libraries and fully characterised by DNA sequencing. Gene specific probes for both muscle (α) and cytoplasmic actin transcripts have been derived from the corresponding cDNAs. Using both RNA "Northern" blots and S1 protection assays we have quantitated the steady state levels of the various actin transcripts through early development. Cytoplasmic actin transcripts are inherited by the embryo from the maternal RNA pool whilst α-actin mRNA is first detected only in gastrula embryos.

α-actin mRNA synthesis is restricted initially to the mesoderm of the gastrula and subsequently to cells of the somite and myotome tissue. In contrast, cytoplasmic actin transcripts can be detected in all stages and regions of the developing embryo. Cardiac actin mRNA is a prominent component of mesoderm, somite, myotome and embryonic heart mRNA yet in the adult frog, cardiac mRNA cannot be detected in skeletal muscle and is confined to heart tissue. The programme of actin gene expression during Xenopus embryogenesis therefore involves both activation and repression of particular α-actin gene types. Commitment to α-actin gene expression by a restricted group of embryonic cells is first detected amongst blastomeres prior to gastrulation. α-actin mRNA synthesis is initiated by such cells in the absence of the normal cell-cell interactions arising during formation of the embryonic axis.

Gene specific probes have been used to isolate members of the actin multigene family from a λ-phage genomic library enabling actin gene organisation in the Xenopus genome to be characterised.

1020 NUCLEOCYTOPLASMIC AND TISSUE SPECIFIC DISTRIBUTION OF RNAS IN XENOPUS DEVELOPMENT, Milan Jamrich, Joseph G. Gall, Tom Sargent and Igor Dawid, NIH,NICHD,Bethesda,MD20205

Our effort in last few years has concentrated on the analysis of transcription during Xenopus oogenesis. The transcription was mainly monitored by in situ hybridization of cloned sequences to the lampbrush chromosomes of Xenopus - a technique introduced by us earlier (1). More recently we have developed a new technique for the analysis of the nucleocytoplasmic and tissue specific distribution of RNA utilizing in situ hybridization to methacrylate embedded tissue sections (2). This technique offers the advantage of superior morphology to any previously published technique and is suitable for study of RNA distribution during the entire development of the frog. Using these techniques we studied the transcription and distribution of RNAs complementary to a variety of repetetive sequences, histone genes and vitellogenin gene as well as other sequences of known and unknown function. The main focus of our current research is the analysis of the spacial distribution of gastrula specific sequences during early embryogenesis of Xenopus (3). Furthermore, experiments are underway to determine whether any of these sequences are expressed during regeneration.

1. Jamrich M.,R.Warrior, Steele R. and J.G.Gall, PNAS 1983,80,pp. 3364-3367.
2. Jamrich M., Mahon K. and J.G.Gall, in preparation.
3. Sargent T.D. and Dawid I.B., Science, in press.

1021 THE XENOPUS LAEVIS α TUBULIN GENE FAMILY, Desmond Smith and Timothy Mohun, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, England.

In order to address questions concerning the regulation of tubulin genes, a study has been undertaken of the Xenopus laevis α tubulin gene system. A blastula stage cDNA library was probed with a chicken α tubulin cDNA probe. A positively hybridising cDNA clone was obtained and sequenced using the dideoxy technique. The complete protein sequence was derived and confirms the high degree of evolutionary conservation of α tubulin. Southern blot experiments indicate that there are approximately 10 - 20 α tubulin gene copies in the X. laevis genome.

In all tissues examined, Northern blot experiments reveal three size classes of α tubulin mRNA. Compared to other tissues, brain was found to be enriched approximately ten fold for α tubulin mRNA. The 3' untranslated region of the cDNA hybridised specifically to a 1.7 kb species of the three size classes of α tubulin transcript. Northern blot analysis of various development stages demonstrates temporal control of α tubulin mRNA levels.

Positively hybridising plaques have been isolated from a genomic library screened with a coding region probe derived from the α tubulin cDNA. Some of these also hybridised specifically to a probe prepared from the 3' untranslated region of the frog cDNA. The nucleotide sequence of the α tubulin gene from one of the genomic clones that hybridised to both probes has been determined. The intron/exon structure of the gene has been deduced, and the sequence has been examined for potential regulatory regions. These genes will be introduced into oocytes and developing frogs to define regulatory regions on the basis of afunctional assay

1022 BIOGENESIS OF MEMBRANES DURING XENOPUS EARLY DEVELOPMENT, R. Tencer and M. Goldfinger, Free University of Brussels, Belgium.

The proteins of the new cell surfaces formed during cleavage have been identified by iodination of cells dissociated from Xenopus embryos at different developmental stages. The pattern of these iodinated proteins does not change significantly from early cleavage to late gastrula stage. However differences are observed when iodinated compounds of cells from the animal part,separated from embryos at blastula or gastrula stage, are compared to compounds originating from cells of the vegetative part taken from embryos at the same developmental stage.

In order to tackle the factors which control the spatial distribution of these membrane proteins, we have investigated the influence of animal - vegetative polarity on the distribution of these compounds by inverting the eggs (A.W. Neff et al, Dev. Biol. 97, 104-112, 1983).

A relation seems to exist between animal - vegetative polarity and the segregation of some membrane proteins in different parts of the embryo.

1023 PATERNAL AND MATERNAL PRONUCLEI ARE BOTH ESSENTIAL FOR NORMAL MOUSE DEVELOPMENT, James McGrath and Davor Solter, Wistar Institute, Philadelphia, PA 19104

Using recently developed techniques for nuclear transfer (McGrath and Solter, Science, 220, 300, 1983) we have analyzed possible reasons for developmental failure of mammalian parthenotes. Fertilized eggs were constructed in which the paternal pronucleus was replaced with the maternal pronucleus from another embryo (gynegenones) or the maternal with the paternal (androgenones). As a control, one pronucleus (either maternal or paternal) was replaced with the pronucleus of the same paternal sex from another embryo. Control embryos developed to adulthood, upon transfer to pseudopregnant females, but both gynegenetic and androgenetic embryos failed to do so. Our results indicate that neither the absence of an extranuclear contribution from the sperm nor excessive homozygocity are the causes of the developmental failure of parthenotes. The genomic contributions of the paternal and maternal pronuclei are not identical and both are necessary for normal development to term.

Molecular Biology of Development

1024 THE ORGANIZATION AND EXPRESSION DURING OOGENESIS OF REPETITIVE DNA IN THE NEWT,
Kathleen A. Mahon and Joseph G. Gall, Yale University, New Haven, CT 06511

We have studied two highly reiterated satellite DNA sequences from the newt Notophthalmus viridescens which have been shown to be transcribed on lampbrush chromosomes by in situ hybridization. Satellite 1, a 222 bp tandemly repeated sequence, is located in the pericentric heterochromatin of all chromosomes and at two non-centromeric sites on chromosomes 2 and 6. Both strands of satellite 1 are transcribed at the non-centromeric loci, apparently due to readthrough transcription from adjacent histone gene promotors (Cell 24, 639-647;649-659,1981) This pattern of transcription, in which there is a failure of termination at the 3' end of active genes, is probably a general feature of the lampbrush chromosome stage and can explain the extremely long transcription units typically seen on lampbrush loops. Biochemical analysis coupled with in situ hybridization of satellite 1 probes to the cellular RNA in 1μ sections of ovary have demonstrated that transcripts containing satellite 1 are almost exclusively nuclear. The hybridization is not evenly distributed over the nucleus but is localized on densely staining globular structures. Transcripts are readily detectable before the lampbrush chromosome stage in small pre-vitellogenic oocytes (late pachytene-early diplotene). Satellite 2 is an abundant, conserved 330 bp sequence which differs from satellite 1 both in its genomic organization and its pattern of expression in the oocyte. In contrast to the predominantly heterochromatic location of satellite 1, satellite 2 occurs in tandem clusters dispersed throughout the genome. Northern blot analysis has shown that satellite 2 shares homology with discrete RNA transcripts of ~300 and 600 nucleotides in the oocyte.

1025 ANALYSIS OF CHICKEN OOCYTE LAMPBRUSH CHROMOSOME TRANSCRIPTION, Nancy J. Hutchison and Harold Weintraub, Hutchinson Cancer Research Center, Seattle, WA 98104.
We are trying to answer two types of questions about lampbrush chromosome (LBC) structure and transcription: 1) What kinds of sequences are transcribed on LBC loops? and 2) What is the detailed structure of a lampbrush chromosome loop in terms of DNA sequence, associated proteins, transcripts, signals, chromatin conformation, etc.? The large genome size and presence of repetitious sequences makes these questions difficult to approach experimentally in Triturus species. By contrast, the chicken genome is small (1.2 pg) and contains little repetitive DNA. Furthermore, numerous cloned chicken gene probes are already available for use in answering our questions. We examined chicken oocytes and found the LBC feasible for in situ hybridization and immunocytochemical analyses. A preliminary experiment using nick-translated total DNA for in situ hybridization to transcripts showed labelling of about 20-25 sites, primarily on the micro chromosomes. Hybridization with pA1, a chicken β-actin cDNA plasmid, intensely labelled primarily one macro chromosome site. The insert coding fragment alone also labels this site. However, the fragment still contains GC tails used in cloning, so we are now testing subfragments to be sure the labelled site is due to actual coding sequences. The actin sequence transcription will provide an internal positive control for the other probes we are now testing (histones, TK, GPD, globin, etc.). In addition, it provides a starting point for "walking" around a LBC loop to begin detailed analysis of loop structure.

1026 ANALYSIS OF CELL LINEAGE IN MOUSE EMBRYOS BY MICROINJECTION OF HORSERADISH PEROXIDASE,
R.A. Pedersen[1], K.A. Lawson[2], Y.P. Cruz, J.J. Meneses, and K. Wu, Laboratory of Radiobiology and Environmental Health and [1]Department of Anatomy, University of California, San Francisco, CA 94143; [2]Hubrecht Laboratory, Utrecht, The Netherlands.

During preimplantation development the mouse embryo partitions its cells between inner cell mass and trophectoderm, and during early postimplantation development, partitions them between the various organ rudiments. We have microinjected horseradish peroxidase (HRP) into progenitor cells and analyzed the spatial distribution of their descendants at later stages. The results provide information about the time and mechanisms of cell allocation processes. Embryos were injected by iontophoresis (2-5 nA for 15-60 sec) with 2-4% HRP (Sigma Type VI). After culture for 2-48 hrs, embryos were stained for HRP activity and scored. The results indicate that cells are allocated during the fourth and fifth cleavages to inner cell mass or trophectoderm lineages. Cells overlying the inner cell mass (polar trophectoderm) move away from their central polar position during blastocyst expansion (approximately the sixth to eighth cell cycles). When anterior visceral endoderm cells (7.5 d.g.) were injected, their descendants appeared in extraembryonic yolk sac, suggesting that extensive endoderm cell movement occurs. Injected anterior ectoderm cells gave rise mainly to neuroectoderm. Studies are underway to determine when clonal organ domains are established within mouse embryos. This information will be important when studying the onset and regulation of organ-specific gene expression in mammals. Supported by the U.S. DOE, NIEHS Postdoctoral Traineeship No. 5-T3-ES07106, and the Netherlands Academy of Science.

1027 METHYLATION OF THE HUMAN ACTIVE AND INACTIVE X CHROMOSOMES, Susan J. Lindsay, Marilyn Monk, Lily Huschtscha and Kay E. Davies. MRC MAmmalian Development Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, England.

Reactivation of expression (Mohandas et al 1981, Science 211 393) and transforming ability (Venolia et al 1982, Proc. Natl. Acad. Sci. USA 79 2352) of genes on the inactive X chromosome by the drug 5-azacytidine strongly support the involvement of methylation of the base cytosine in the inactivation of one X chromosome in female mammals.We have used the restriction endonucleases MspI and HpaII to investigate the methylation patterns on the active and inactive human X chromosomes in the region of three X-chromosome specific clones. When the source of the X chromosomes was cultered human cells reproducible patterns of methylation were found which were similar for the active and inactive X chromosomes in the region of all three clones.In contrast, in peripheral blood lymphocytes the inactive X chromosome shows clear differences in methylation from the active X chromosome: surprisingly the inactive X chromosome is undermethylated in the regions probed.

1028 INTERMEDIATE FILAMENTS IN EARLY EMBRYONIC DEVELOPMENT OF DROSOPHILA MELANOGASTER, Marika F. Walter and Bruce M. Alberts, University of California, San Francisco, CA

We have demonstated the presence of a cytoskeleton of intermediate size filaments (10nm) of the vimentin type in tissue culture cells of D. melanogaster. Using a monoclonal antibody against a 46K dalton protein that also crossreacts with vimentin from vertebrate cells, we were able to show that this cytoskeleton exhibits features that are characteristic of the vertebrate vimentin cytoskeleton.
The function of the intermediate filament cytoskeleton is not known, but it has been suggested that it may be involved in maintaining structural organization within cells. In order to investigate its role in development we have examined the presence and distribution of the vimentin type cytoskeleton in early embryogenesis using protein blotting and indirect immunofluorescence. Four of the intermediate filament proteins recognized by our antibody could be detected in embryo and remained present throughout all stages of early embryogenesis as shown by testing protein extracts from 0.5 hr - 6 hr old embryos. The spatial organization of this cytoskeleton was investigated by indirect immunofluorescence of whole fixed embryos, revealing a distinct stage specific pattern in the embryonic cytoplasma. In the early developmental stages before cellular blastoderm (cycle 1-8) the intermediate filaments form an irregular network at the surface of the embryo. After nuclear migration the filamentous network becomes more defined, occupying the cytoplasmic space around the nuclei. After cellular blastoderm the pattern remains unchanged until gastrulation. These observations suggest that the intermediate filament cytoskeleton may be involved in the structural organization of the cytoplasm in developing embryos.

1029 DIFFERENTIAL TISSUE-EXPRESSION DURING MOUSE EMBRYOGENESIS OF TRANSPOSON-LIKE REPETITIVE DNA SEQUENCE. Philippe Brûlet, Service de Génétique cellulaire du Collège de France et de l'Institut Pasteur, 25 rue du Dr. Roux, 75724 Paris Cedex 15.

A family of long, moderately repetitive and dispersed DNA sequence in the mouse genome was recently described (Brûlet et al., PNAS, 80, 5641, 1983). They present structural analogies with integrated retroviral sequences. A 6 kb RNA transcript is detected in undifferentiated embryonal carcinoma cell lines but not in any differentiated cell lines tested. By in situ hybridization, the RNA is also differentially detected in the tissue of early mouse embryo. The inner cell mass and the embryonic ectoderm at day 6 and 7 expressed the RNA but preimplantation embryos, trophectoderm and endoderm do not at a detectable level.

Data to correlate the tissue specific expression with the DNA sequence will be presented as available.

1030 DEVELOPMENTALLY REGULATED GENES OF THE EARLY RABBIT EMBRYO, Carlisle P. Landel, Patricia Menzel, Thomas E. Gilroy and Cole Manes, Center for Neurologic Study, 11211 Sorrento Valley Rd., Suite H, San Diego, CA 92121

We have constructed a cDNA library from six-day, pre-implantation rabbit embryos. At this stage, the rabbit embryo contains 10^5 cells, 95% of which are trophoblast. 1.2 x 10^4 bacterial colonies containing cDNAs cloned into the PstI site of pBR322 were screened with homologous ^{32}P-labeled cDNA, yielding 1010 colonies representing genes whose transcripts make up at least 0.5% of the mRNA of this stage. These colonies were then screened with ^{32}P-labeled cDNA made from either the half of the six-day embryo consisting mostly of trophoblast, the half containing the inner cell mass, the twelve-day embryo (at which stage the limb buds have already formed), or the twelve-day placenta. This screen yielded 50 clones that are six-day embryo specific, and ten clones whose expression increases dramatically by twelve days. Preliminary characterization of the six-day specific clones has revealed at least fifteen different sequences among/the first twenty-six examined. Three of these clones represent unique sequences within the genome. The DNA of the six-day embryo is very highly methylated, and preliminary Southern blots of this DNA cut with HpaII and MspI and probed with these three clones suggest that the DNA in the region of these sequences remains methylated at this stage of development.

1031 TWO-DIMENSIONAL POLYACRYLAMIDE GEL ANALYSIS OF CHANGES IN PROTEIN SYNTHESIS AND PROTEIN PHOSPHORYLATION DURING OOCYTE MATURATION IN XENOPUS, James L. Maller and Diana S. Smith, Department of Pharmacology, Univ. of Colorado School of Medicine, Denver, Colorado 80262

Ripe Xenopus oocytes physiologically arrested in first meiotic prophase progress to second meiotic metaphase upon exposure to progesterone in vivo, a process termed oocyte maturation. Cycloheximide treatment of oocytes blocks oocyte maturation, and studies in several laboratories indicate increased incorporation of labeled amino acids into protein during maturation. Other laboratories have reported conflicting results on whether the increased protein synthesis reflects qualitative or quantitative changes. Pulse-labeling analysis of protein synthesis on low Bis-acrylamide two-dimensional 10-17.5% gradient O'Farrell gels demonstrates several types of changes with progesterone: de novo appearance of ^{35}S-methionine-labeled protein, quantitative increase in previously synthesized protein, and an acidic shift in previously synthesized protein. Two-dimensional gradient gel analysis of ^{32}P-labeled proteins reveals the de novo phosphorylation of at least eight resolved proteins during maturation. Some of these newly phosphorylated proteins are heat-stable, which may facilitate their purification and characterization. Phosphoamino acid analysis of the phosphorylated proteins by two-dimensional high voltage paper electrophoresis at pH 1.9 and pH 3.5 indicated 90.5% phosphoserine, 7.2% phosphothreonine, and 1.4% phosphotyrosine. These values were not significantly different than controls indicating the phosphorylation burst does not selectively increase the level of phosphotyrosine during maturation. These results indicate there are qualitative changes in both protein synthesis and protein phosphorylation during maturation of Xenopus oocytes. (Supported by NIH grant GM26743).

1032 SELECTIVE TRANSLATIONAL REGULATION OF RIBOSOMAL PROTEIN GENES DURING OOGENESIS AND EMBRYOGENESIS OF DROSOPHILA. M. Jacobs-Lorena, M.A. Kay, and Q.-Y. Jiang, Dept of Developmental Genetics and Anatomy, Case Western Reserve University, Cleveland, OH.

In Drosophila, the proportion of ribosomes associated with polysomes remains constant from late oogenesis through embryogenesis. Previous work from this lab identified a developmentally regulated mRNA (T1 mRNA)which is polysome-associated during oogenesis, selectively excluded from polysomes during early embryogenesis and again polysome-associated during late embryogenesis (Fruscoloni et al., PNAS 80:3359, 1983). To determine the corresponding protein product, the mRNA was purified by hybrid selection to its cDNA clone and translated in a rabbit reticulocyte cell-free system. The labeled translation products were mixed with stainable quantities of proteins isolated from subcellular fractions and the mixture fractionated on isoelectric focusing 2D-gels. The labeled translation product was found to co-migrate with an acidic ribosomal protein (rp). This identification is being confirmed by peptide mapping. We next attempted to determine if other rp-mRNAS also are under translational regulation. Postpolysomal and polysomal polyadenylated RNAs from egg chambers or embryos at different developmental stages were translated in vitro in the presence of ^{35}S-methionine. The radioactive products were mixed with marker rps and the mixture displayed on basic non-equilibrium 2D-gels. The results indicated that a large number of rps appear to be regulated in the manner described above. These observations are now being confirmed by in vivo labeling experiments. It therefore appears that the translation of many rp-mRNAs is temporally related to the synthesis of rRNA. This relationship is being further investigated by using bobbed mutants deficient in rRNA genes.

1033 DEVELOPMENTAL REGULATION OF DNA REPLICATION IN XENOPUS LAEVIS EMBRYOS, Robert M. Benbow, Pamela J. Hines, and Michelle F. Gaudette, Johns Hopkins University, Baltimore, Maryland 21218.

A novel mechanism has been implicated in the replication of chromosomal DNA during early embryogenesis in the frog Xenopus laevis. Replication is triggered by rapid and extensive strand separation throughout an entire domain of chromosomal DNA. Self-primed DNA synthesis catalyzed by the Xenopus laevis DNA polymerase-primase then commences at a large number of sites on the strand separated DNA. Prior to strand completion D-loop structures are synthesized which enable coordination between gene expression and DNA replication. The trigger to initiate each subsequent round of DNA replication is a conformational change in duplex DNA structure which occurs concomitant with strand completion.

Replication enhancer sequences have been implicated in the regulation of DNA replication during early embryogenesis. Replication enhancer sequences are cloned fragments of Xenopus laevis DNA which dramatically increase the efficiency of replication of the cloning vector in embryos. Under certain conditions replication enhancer sequences can also specify a site at which replication eyes are formed on the cloned DNA.

1034 PROTEIN TRANSFER IN THE DERMAL-EPIDERMAL TISSUE INTERACTION, Charlotte A. Peterson and Robert M. Grainger, University of Virginia, Charlottesville, VA 22901
During the development of embryonic chick skin, the dermis interacts instructively with the epidermis to control whether the epidermis forms feathers or scales. The dermis controls not only epidermal morphogenesis, but also gene activation since unique beta-keratins are synthesized in each tissue type. In order to study molecules transferred between the tissues which might act as developmental signals, we have been using a culture system which maintains the developmental specificity of the dermis even when it is separated from the epidermis by a Nuclepore filter. The structures formed in the epidermis, cultured transfilter from dermis, have many of the morphological and histological features expected of epidermal derivatives. Two-dimensional polyacrylamide gel electrophoresis demonstrates that the epidermis synthesizes many of the tissue specific beta-keratins. Transmission and scanning electron microscopy of transfilter cultures show that neither direct cell contact between the tissues, nor a basement membrane are required for the interaction to occur. We are applying density labeling techniques to this culture system to detect transfer of proteins between dermis and epidermis. We have tentatively identified collagen as one protein transferred from both feather and scale dermis, while other less abundant proteins appear to be transferred tissue specifically.

1035 IN VITRO TRANSLATION OF OOCYTE RNA WHICH PERSISTS IN THE EMBRYO OF DROSOPHILA MELANOGASTER, William H. Phillips, Jeffrey A. Winkles, and Robert M. Grainger, University of Virginia, Charlottesville, Virginia 22901
Characterization of Drosophila maternal effect mutants suggests that there are specific products synthesized in the oocyte which are necessary for cell determination and spatial organization in the embryo. In order to follow the fate of oocyte RNA during embryogenesis, a pulse/chase density labeling scheme was devised which permits us to collect embryos that contain density labeled RNA (^{13}C-^{15}N-^{2}H-substituted) synthesized during early oogenesis and light buoyant density RNA transcribed during embryogenesis. Heavy and light RNAs can be separated on high resolution NaI/KI equilibrium gradients which afford improvement over previously used salts in that the recovered RNAs are readily translated in vitro. In our first experiments, an embryo collection scheme was designed to follow the fate of dense RNA synthesized during early oogenesis. We have successfully retrieved heavy oocyte RNA which persists in these embryos. Since there is a significant amount of oocyte ribosomal RNA ($t_{1/2}$= 21 hours) remaining in these embryos, the assumption was made that there would also be adequate dense message to translate. However, with the sensitivity of our present in vitro system, we are unable to detect any translatable dense oocyte message. Consequently, most of the early oocyte mRNA must have a shorter half-life than oocyte rRNA. To obtain stable oocyte messages in sufficient quantity for analysis, we are now following the fate of oocyte RNAs synthesized during later oogenesis. These experiments will allow us to examine the persistance of oocyte translation products in later embryonic and larval stages and determine whether a stable subset may be localized and important in developmental processes.

1036 PROGRAMMED EXPRESSION OF CELL SURFACE DETERMINANTS DURING ENDODERM FORMATION, Raymond J. Ivatt,
Department Tumor Biology, M.D. Anderson Hospital, Texas Medical Center, Houston, TX 77030.

Embryonal carcinomas and early embryonic cells express an unusual class of carbohydrates on their cell surfaces. These carbohydrates are lost in a programmed way during early embryogenesis and have a very restricted distribution in the adult. Their disappearance during development coincides with the major period of histogenesis. In the adult, they are associated with cells of the reticuloendothelial system. Therefore in both the embryo and the adult, this unusual class of carbohydrate is associated with cellular interactions which are transient in nature. There are several lines of evidence which implicate these carbohydrates in cellular recognition in the early embryo. We have examined the regulation of these carbohydrates during the formation of endoderm using the embryonal carcinoma system as a model. We have developed a sequential lectin affinity chromatography procedure which fractionates these complex embryonic carbohydrates into discrete subclasses which share common structural features. This approach has allowed the first real opportunity to analyze these complex glycans. We have characterized the major structural changes which accompany endoderm formation, and have used two approaches to address the mechanisms which regulate these changes. The first approach has performed comparative biochemical studies on established cell lines which have the biochemical phenotype of either stem or endodermal cells. The second approach has isolated stem cells which are deficient in selected developmentally regulated carbohydrate determinants and performed comparative biochemical studies on these variants and the parental cells. We are currently exploring the role that cellular interactions may play in regulating the expression of these carbohydrates.

1037 THE REPLICON MODEL AND XENOPUS EGGS. Marcel Méchali, Stephen Kearsey and Ronald Laskey, CRC Molecular Embryology Group, Dept. Zoology, Cambridge University, England.
We have investigated further the sequence specificity for initiation of replication of DNA injected into Xenopus eggs (1,2).
First a wide range of both prokaryotic and eukaryotic DNA molecules replicate. Injected molecules initiate replication at similar specific periods to the host chromatin cell cycles. A direct relationship was observed between the size of the molecule and replication efficiency suggesting that the amount of replication is the limiting parameter. Second, we included plasmids which have been claimed to act as Xenopus replication origins in this system (3,4) but have repeatedly found that their replication efficiency is indistinguishable from their prokaryotic vectors alone. Third, we have cloned Xenopus ARS elements. They contain an 11 nucleotide consensus sequence similar to the ARS consensus sequence found in yeast. The presence of Xenopus ARS elements does not confer a selective advantage for replication inside the egg. The necessity for specific sequences for initiation of replication during early development will be discussed as well as the relation between a specific nuclear structure and initiation.

1. Harland and Laskey, 1980, Cell 21, 761-771.
2. Méchali et al., 1983, Cell 35, 63-69.
3. Hines and Benbow, 1982, Cell 30, 459-468.
4. Chambers et al., 1982, Proc. Nat. Acad. Sci. 79, 5572-5576.

1038 LATE EMBRYONIC H3 AND H4 HISTONE GENES OF THE SEA URCHIN, S. PURPURATUS, J.F. Kaumeyer* and E. S. Weinberg, University of Pennsylvania, Philadelphia, PA

A late embryonic histone H3 and H4 gene pair from Strongylocentrotus purpuratus has been cloned into λJ1. Sequencing confirms the identity of these genes. As in the case of the late H3 and H4 gene pair isolated from L. pictus (Childs et al., 1982), the genes are transcribed on opposite strands and are separated by about 800 bp. In comparison with the early embryonic H3 and H4 genes of S. purpuratus, the coding regions show a nucleotide divergence of 16%, very much as in the case of L. pictus. The sequence complementarity of the late S. purpuratus H3 and H4 genes with L. pictus late genes or early genes of either species ends abruptly at the 5' and 3' ends of the coding sequence except for the characteristic 3' histone terminator region and 5' TATA box. At least 4 late H3 transcripts are identified and show differential times of appearance during development. A subcloned fragment internal to the H3 coding region hybridizes to 5-10 copies per genome (depending on the individual sea urchin), but a 500 bp fragment containing 90 bp of the 5' end of the H3 mRNA and the upstream sequence hybridizes specifically to one copy per genome and is specific for one of the 4-5 late H3 transcripts. Other late H3 genes have been selected and their transcriptional patterns and structural organization will be reported.

1039 THE ACTIN MULTIGENE FAMILY OF STRONGLYOCENTROTUS PURPURATUS. Shott, R. J., Lee, J. J., Rose, S. J. and Davidson, E. H.

The actin family of the sea urchin, Strongylocentrotus purpuratus, consists of eight non-allelic genes, determined by genomic blotting methods using molecular probes that recognize the 3' non-translated regions of these genes. Gene-specific probes were used in RNA gel blot and DNA excess filter hybridization experiments to estimate the relative concentrations of different actin transcripts during embryogenesis and in different adult tissues. At least six genes are transcribed. All six produce relatively stable transcripts and display distinct patterns of expression. On the basis of their expression in the egg, early embryo and adult coelomocytes it is concluded that genes termed CyI, CyIIa, CyIIb, CyIIIa and CyIIIb encode cytoskeletal actin proteins. Actin gene M gives rise to mRNA that is expressed in muscle tissues. Actin genes CyI, CyIIa, CyIIb and M are expressed in both adult and embryonic tissues, giving rise to transcripts of 2.1-2.2 kb in length. Expression of the CyIII genes is limited to embryonic tissues. Gene CyIIIa encodes the major embryonic actin transcript (1.8 kb). We show that three of the actin genes, which are linked over a 30 kb distance in the genome, are not coordinately expressed.

1040 HISTONE GENES OF ARTEMIA: ORGANIZATION, EXPRESSION AND INTERSPERSION OF APPARENT 5SrRNA GENES, Joseph C. Bagshaw*, Matthew T. Andrews and Brian A. Perry, Biochemistry Dept., Wayne State University Medical School, Detroit, MI 48201

Encysted gastrulae of the brine shrimp, Artemia, develop into prenauplius larvae in the absence of measurable DNA replication or cell division. Histone synthesis is absent during development of encysted embryos and is coordinated with a wave of DNA replication in newly hatched larvae. Encysted embryos do not contain histone mRNA sequences, as judged by hybridization with cloned Artemia histone genes. Experiments are in progress to determine the time during development when transcription of histone genes begins. The Artemia histone genes are arranged in a tandemly repeated set with a repeat length of 8500 base pairs, a copy number of about 95, and a gene order of H1-H2A-H4-H3-H2B. All cloned histone gene sets isolated to date hybridize strongly with 5SrRNA as well as with histone mRNAs. In one of three nearly identical isolates the 5S-related sequence lies between the H1 and H2A genes. The fine structure restriction map of this region for 17 enzymes agrees with that predicted by the known sequence of Artemia 5SrRNA. From Southern blot analysis of genomic DNA fragments and DNA driven hybridization, we find no credible evidence that Artemia contains any 5SrRNA genes other than the sequences interspersed with histone genes. (Sponsored by NSF Grant PCM81-11485.)

*Present address: Dept. of Biology and Biotechnology, Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609

1041 PROCESSING OF RIBOSOMAL PROTEIN mRNAs IN X.LAEVIS OOCYTES, I. Bozzoni, F. Amaldi, F. Annesi, E. Beccari, P. Fragapane, P. Pierandrei-Amaldi, C.N.R.-Rome

The genes coding for the X.laevis ribosomal proteins L1 and L14 behave in a different way when injected into the nucleus of the X.laevis oocyte. The L14 gene is transcribed and correctly processed to give a mature mRNA which is translated into the correspondent protein which is accumulated in high amount. The L1 gene is transcribed with very high efficiency but the processing of the mRNA is uncomplete, yielding the accumulation of a specific precursor. This mRNA precursor can be correctly processed when injected into fresh oocytes. We are investigating the molecular mechanisms of this block in order to see whether it can be accounted for by either the ribosomal protein synthesis regulation or the oocyte apparatus of splicing. Mutants have been constructed in order to discriminate between these two possibilities.

Neurobiology

1042 CLONING AND REGULATION OF NEUROPEPTIDE GENES, Jack E. Dixon, Robert Deschenes, M. Tavianini and C. D. Minth, Purdue University, Department of Biochemistry, West Lafayette, IN 47907.

We have isolated and characterized cDNA clones and the corresponding genes encoding three widely distributed and abundant neuropeptides. The complete amino acid sequence of prepro-somatostatin, cholecystokinin, and neuropeptide Y have been deduced from their respective cDNA sequences. In each case the precursors to the various neuropeptides are much larger than the mature peptide. The amino acid sequences of the precursor also suggest that different mechanisms of post-translation processing are operative in producing the mature peptide. In order to develop a better understanding of the neuropeptide gene regulation, and to understand how these peptides might function in nerve cell differentiation the genes for the three neuropeptides have been isolated, characterized and are now being sequenced. Experiments on the mechanisms of gene regulation will also be presented. (Supported by grants AM 18849 and AM 18024.)

1043 ROLE OF ENVIRONMENT IN THE EXPRESSION OF CATECHOLAMINE BIOSYNTHETIC ENZYMES. G. Teitelman, I. Iacovitti, T.H. Joh and D.J. Reis, Lab. Neurobiology, Cornell Univ. Med. Coll., New York, NY

To determine whether the expression of an overt neurotransmitter phenotype by neural crest cells is predetermined or epigenetic, the ontogeny of appearance of a catecholaminergic (CA) phenotype in the developing sympathetic nervous system was examined. Using immunocytochemical techniques, we found that in mammalian embryos, the CA biosynthetic enzymes appear in neural crest cells after cells populate the sympathetic ganglia. To analyze the role of environment on the expression of CA traits, the cholinergic neurons of the chick embryo ciliary ganglia were placed in culture and examined for the presence of CA enzymes. It was found that in vitro, the neurons contain three of the four enzuymes of the CA pathway. It was also discovered that, in vivo, few CG neurons contain CA. These findings suggest that most neurons of the ganglion are intrinsically able to express a CA phenotype and that culture conditions enhance that expression allowing their detection is most CG neurons. How stable is the CA phenotype? A population of cells transiently expressing CA enzymes has been found to populate the gut and pancreas of mouse and rat embryos. In mouse pancreas, some transient CA cells contain both CA and glucagon while others contain CA and insulin. The fact that CA is no longer detected in pancreatic cells after midgestation suggests that the glucagon and insulin cells arise from the transformation of the transient CA cells. Thus, the initial expression of a CA phenotype is labile and can be modified in response to tissue influences. In summary, our studies indicate that the appearance and fate of a CA phenotype is regulated by environmental signals.

1044 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE CODING FOR XENOPSIN, THE NEUROTENSIN-LIKE OCTAPEPTIDE FROM XENOPUS SKIN, Marco Crippa and Irmi Sures, Molecular Embryology, University of Geneva, 1211 Geneva 4, Switzerland

Two oligodeoxyribonucleotide mixtures were synthesized containing sequences complementary to different parts of the hypothetical mRNA sequence of xenopsin, a biologically active octapeptide found in skin extracts from Xenopus laevis. The two primer pools were independently used to initiate reverse transcription on skin poly(A)$^+$RNA and the resulting cDNAs were then utilized to screen -in parallel- a cDNA library prepared from skin poly(A)$^+$RNA. One of the clones which hybridized with both probes was subjected to sequence analysis. It contains a nearly full-length DNA copy of a mRNA of ∿490 nucleotides which encodes a xenopsin precursor protein. The deduced precursor is 80 amino acids long, exhibits a putative signal sequence at the NH_2-terminus and contains the biologically active peptide at the very carboxyterminal end. The region corresponding to the N-terminal portion of the xenopsin precursor shows a striking nucelotide and amino acid sequence homology with the precursor of PYL[a], another recently described peptide from Xenopus skin. The corresponding genomic clones are now being isolated and characterized.

1045 MOLECULAR CLONING AND CHARACTERIZATION OF THE MOUSE MYELIN BASIC PROTEIN GENE, Naoki Takahashi, Arthur Roach and Lee Hood, California Institute of Technology, Pasadena, CA 91125

Myelin basic protein (MBP) is a major component of myelin. While human and other species have a single form of MBP (18.5 kd), two major forms of MBP have been found in mice, large (18.5 kd) and small (14 kd). Small MBP is identical in sequence to large MBP, except for an internal deletion of approximately 40 amino acid residues. Studies on mouse MBP genes using a rat cDNA clone as probe demonstrate that the myelin-deficient mutant mouse shiverer contains a greatly reduced amount of MBP mRNA in its brain and has a deletion of genomic MBP sequences (A. Roach et al., Cell 34, 799-806, 1983). For studies on the organization and expression of MBP genes in shiverer and wild-type mice, we have constructed a wild-type (SWV) mouse genomic library and have screened it using a rat cDNA cloned probe. Analysis of several hybridization-positive cosmid clones is in progress with a view towards establishing the number and coding structure of mouse MBP genes, and characterizing in detail the deletion of these sequences in the shiverer mouse.

1046 FROM GRASSHOPPER TO DROSOPHILA: A CELLULAR AND MOLECULAR APPROACH TO NEURONAL PATHFINDING, John B. Thomas, Michael J. Bastiani, and Corey S. Goodman, Dept. of Biological Sciences, Stanford University, Stanford, CA 94305

We are interested in how developing neurons find and eventually synapse with their appropriate targets. Studies of grasshopper embryogenesis have shown that the growth cones of developing neurons actively choose specific bundles of axons upon which to fasciculate and grow. These pathway choices are mediated by specific cell-cell interactions between a developing neuron's growth cone and axons running within the appropriate bundle. This specificity has convinced us that a number of cell surface molecules are differentially expressed by different bundles of embryonic axons. We would like to know what these molecules are and how they become differentially expressed in the nervous system. Thus, we have begun studying the embryonic development of the nervous system in Drosophila in order to capitalize on its advanced genetics and molecular biology. Using the same techniques exploited in the grasshopper, we have found that between 10 and 13 hrs. of Drosophila embryogenesis the pattern of neurons and the specific pathway choices they make are identical to those of grasshopper. We can purify 100,000s of nervous systems from this time period and are constructing a cDNA library from nervous system mRNA. We are currently trying to isolate sequences which are differentially expressed by subsets of neurons with the expectation that some of these sequences may represent genes coding for or controlling the expression of cell surface molecules used during neuronal pathfinding.

1047 REGULATION OF GROWTH HORMONE TRANSCRIPTION BY GROWTH HORMONE RELEASING FACTOR, Marcia Barinaga, Louise Bilezikjian, Gayle Yamamoto, Wylie Vale, Michael G. Rosenfeld and Ronald Evans, Salk Institute, San Diego, CA

We are studying the mechanism of regulation of GH expression by the hypothalamic peptide, growth hormone releasing factor (GRF). Growth hormone (GH) is the major product of the somatotrophs of the anterior pituitary and is released from the somatotrophs in response to GRF. Until recently, no peptide has been known to influence GH transcription. We have used a nuclear labelling assay which provides a direct measurement of transcription rate, to show that GRF rapidly stimulates the transcription rate of the GH gene, both in whole animals and in primary cultures of pituitary cells. GRF raises cAMP levels in pituitary cells, and stimulates a Ca^{++} dependent release of GH. The transcriptional induction is Ca^{++} independent, and is mimicked by treatment with cAMP analogs, or forskolin, which stimulates adenyl cyclase. Somatostatin blocks GRF stimulated release of GH, but does not block the associated rise in cAMP, nor does it block the transcriptional response to GRF. Release of GH can be achieved, without influencing cAMP levels, either by incubation of the cells in 50 mM K^+ or treatment of the cells with phorbol esters. Neither of these treatments influences GH transcription. Our data suggest that GRF regulates GH transcription by a cAMP mediated mechanism, which can be uncoupled from the GH release response to GRF.

1048 SCHWANN CELL-AXON INTERACTIONS STUDIED IN VITRO. G. Tennekoon, D. Hilt, L. Needham, and R. Schnaar. Depts. of Neurology & Pharmacology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205.

Schwann cells (SC) are known to interact specifically with axons. We report on the development of an assay for examining this interaction in vitro.

Schwann cells from newborn rat sciatic nerves were isolated. Approximately 4 x 10^6 cells were obtained from 10-12 Sprague Dawley rat pups of 1 to 3 days of age. By indirect immunofluorescence using anti-Thy 1.1, 10% of the cells were fibroblasts. One day after seeding, the rapidly dividing fibroblasts were killed with cytosine arabinoside (10^{-5} M). The remaining fibroblasts were removed using a complement mediated killing step with a monoclonal anti-Thy 1.1 (IgM antibody) and rabbit complement. The SC thus obtained were of greater than 99% purity. The Schwann cells grew as monolayers with a doubling time of 48 hours.

Schwann Cell recognition and adhesion to the axon is required for synthesis of basal lamina and eventual synthesis of myelin. We investigated this process by utilizing two different neuronal cell cultures; rat spinal cord explant cultures and dissociated chick spinal cord anterior horn cell cultures. Schwann cells, when co-cultured with these two types of tissues, appeared to bind to the neurites. In order to quantitate the adhesion phenomenon, the Schwann cells were labeled by culture with [^{32}P]orthophosphate and the method of McClay et al. (Proc. Nat. Acad. Sci 78:4975, 1981) was used. In summary, we have obtained cultured neurites and pure populations of SC and have demonstrated their interaction in vitro. We can now begin to quantify this interaction and examine its characteristics.

1049 AXON PATHWAY AND TARGET CHOICES DETERMINED BY LINEAGE, Sally A. Moody, University of Virginia, Charlottesville, VA 22908

Primary neurons of the South African clawed frog (*Xenopus laevis*) establish the first embryonic nervous connections. The axons of primary sensory (Rohon-Beard) and motor (primary motor) neurons of the spinal cord are the first to invade the trunk tissues.

Clones derived from an individual blastomere can be indentified in the frog by injecting horseradish peroxidase into that blastomere, allowing the embryo to develop and histochemically processing its tissue. The descendants of the injected blastomere appear as HRP-labeled cells throughout the various embryonic tissues. This technique has demonstrated that dorsal spinal cord (including Rohon-Beard neurons) was derived from the same blastomeres that were ancestral to the peripheral regions through which the pioneering Rohon-Beard axons course, including dorsal myotome and epidermis. Likewise, ventral spinal cord (including primary motoneurons) was derived from the same blastomeres that were ancestral to the peripheral regions through which the primary motor axons course, namely ventral myotome. The individual HRP-labeled axons of Rohon-Beard and primary motoneurons were traced and the number of clonally related (i.e., also HRP-labeled) peripheral cells within a 20um zone surrounding the axon were counted. Statistically, more clonally related peripheral cells were in close proximity to the axon pathway than non-clonally related cells, suggesting that lineage related cells may guide peripheral axons. In addition, motor axon arborizations were preferentially among clonally related myotubes and Rohon-Beard axon terminations were preferantially upon clonally related epidermal cells, suggesting that early target choice may be related to clonal membership.

Hematopoietic Gene Expression

1050 DEVELOPMENTAL EXPRESSION OF H-2 CLASS I GENES, Janet A. Sawicki, The Wistar Institute, Philadelphia, PA 19104

DNA cloning studies indicate that there are at least 36 Class I genes of the major histocompatibility complex of the mouse (H-2) (Steinmetz et al., 1981, Cell 25, 683). Only a few of these genes are known to be expressed in adult mice. To determine whether some of these genes are differentially expressed during development, we are investigating transcriptional and translational products of Class I genes in mouse embryos. Our studies indicate that β2-microglobulin, a small polypeptide associated with H-2K/D Class I antigens in adult mice, is both synthesized in and expressed on the cell surfaces of preimplantation embryos. H-2K/D antigens, however, appear not to be synthesized at these early stages. We have determined that a 40,000 dalton Class I (H-2)-related peptide which is synthesized and secreted only by liver cells in adult mice (Kress et al., 1983, Cell 34, 189) is also synthesized by preimplantation embryos. This peptide may induce immunological tolerance of the mother to the fetus. We are preparing a cDNA library derived from expanded blastocysts (day 4) and will screen this library using a Class I H-2 specific DNA probe. Similarly, we are screening a cDNA library derived from 14-day-old embryos prepared by John Monahan (Roche Institute). Positive clones from these libraries will be used to identify and characterize the products of Class I gene sequences expressed by embryos. These studies will help us understand how a highly complex genetic region is regulated throughout development.

1051 IgG AND IgA IMMUNOGLOBULIN CHAINS ARE EXPRESSED WITHOUT DELETION OF IgM SEQUENCES IN MOUSE MEMORY B CELLS. Aaron P. Perlmutter and Walter Gilbert, Department of Biochemistry and Molecular Biology, Harvard Universty, Cambridge, MA 02138

We have analyzed the genes for the heavy chains of IgM, IgG_1, and IgA immunoglobulins in normal Balb/c B lymphocytes. Splenocytes were fractionated by fluorescence activated cell sorting (FACS) on the basis of their surface-heavy-chain isotype. Cells that expressed surface IgG_1 or IgA neither were deleted for the IgM constant region gene nor showed any rearrangement 5' to the gamma 1 or alpha constant region genes. Furthermore, cells which simultaneously expressed IgM and IgG_1 or IgM and IgA were isolated and shown not to express any other isotype. We propose that prior to differentiation into large secreting blastocytes, small memory cells can express IgG_1 or IgA via a large primary RNA transcript spanning the 100-150 kb heavy chain gene locus without undergoing a further DNA rearrangement. Studies on these RNAs are in progress.

1052 PROCESSING OF THE 3' END OF PRIMARY TRANSCRIPTS OF MOUSE IMMUNOGLOBULIN GENES, *Michael Komaromy, John Rogers and Randolph Wall, UCLA, Los Angeles, Ca. 90024*

The mouse immunoglobulin μ gene is a complex transcription unit which can give rise to two mRNA species, membrane and secreted, which differ only at their 3' ends. Our studies have revealed the presence of a species, termed the "amputated transcript", or AT, which is the apparent result of a cleavage event which also generates the secreted mRNA(μs) form. Thus, in cells which produce only μ_s mRNA, transcription continues past both the μ_s and μ_m termini. The primary transcript is then cleaved to form the appropriate mRNA. The AT is the species formed by cleavage at the μ_s and μ_m termini, and has the intron between the two membrane exons excised. The AT is polyadenylated and is preferentially retained in the nucleus. Further studies to determine the precise 5' end of the AT will be presented.

1053 THE ANALYSIS OF THY-1 GENE. M. Teng, R.S. Basch, New York University, New York; J. Silver, Michigan State University, East Lansing; and J.N. Buxbaum, New York University & New York VA Medical Center, New York, 10010.

Thy-1 is found on murine neural, dermal and lymphoid tissues. Thymic T-lymphocytes fully express the antigen on their surfaces. In vitro, prothymocytes can be induced to express the antigen by several thymic peptides. The amino acid sequences of solubilized Thy-1 molecules from both rat and mouse (Thy-1.1, Thy-1.2) have been reported.

The mouse Thy-1 gene was studied by Southern blotting with a DNA probe isolated from a rat cDNA clone. Preliminary observations indicate that there is one Thy-1 gene. No restriction digestion differences were observed among brain, thymus and liver. The gene was also present in identical forms in two prototype mouse tumor cell lines which were Thy-1 (+) and Thy-1 (-). Several genomic clones containing the Thy-1 gene have been isolated from a Balb/c library and a tentative restriction map has been constructed.

The expression of Thy-1 mRNA in various cell types was studied by Northern blotting. There is a single 2kb poly A containing mRNA found in normal rat and mouse tissues and mouse tumor cell lines.

These results suggest that the Thy-1 gene is present as a single copy in DNA from all tissues studied. Gene rearrangement is not required for its expression in differentiated T-cells. The expression of the antigen in both lymphoid and non-lymphoid tissues is associated with identical single RNA transcripts which are larger than the size expected to code for a mature polypeptide of 14,000 daltons. This observation suggests a possible role for post-transcriptional regulation in the expression of Thy-1 antigen.

1054 LOCAL CHANGES IN CHROMATIN STRUCTURE IN THE GOAT B-GLOBIN GENE LOCUS DURING DEVELOPMENT, Paul A. Liberator and Jerry B. Lingrel, Univ. of Cincinnati, Cincinnati, Ohio.

We have been characterizing the chromatin conformation in and around the differentially expressed fetal (γ), juvenile (B^C) and adult (B^A) goat globin genes and have found local differences in erythropoietic tissues at various developmental stages. These differences exist even though the three genes and their flanking regions share remarkably high nucleotide sequence homology - sequences 1 kb 5' to the start of transcription are 90% homologous. The approach has been to examine the accessibility of various restriction endonuclease recognition sites and the general result is that their availability for digestion in intact nuclei correlates with the activity of these genes. The Pvu II site located 15 nucleotides upstream from the start of transcription in each of the three genes displays this pattern. That is, in bone marrow isolated from an anemic goat synthesizing juvenile hemoglobin, the Pvu II site at the 5' end of the B^C gene is far more sensitive to digestion than is the same site upstream from B^A. The homologous γ-globin Pvu II site is not at all accessible in this tissue. Conversely, in fetal erythropoietic tissue only the Pvu II site 5' to the γ gene and not those upstream from B^C or B^A is sensitive to digestion. In nonerythropoietic tissue none of the three sites is cut in intact nuclei. It appears that those DNA sequences which are involved in establishing the differences in chromatin structure detected here, and perhaps ultimately controlling the differential expression of these genes, are either very short or are well removed from the coding regions. Supported by PHS F32 HL06416 and NIH AM20119.

1055 REGULATION OF HLA-DR GENE EXPRESSION IN TWO HUMAN MONOCYTOID CELL LINES, B. Matija Peterlin, Andrew F. Calman, and John D. Stobo, University of California, San Francisco, CA 94143.

The expression of HLA-DR on the surface of human monocytes is not constitutive but varies with differentiation and is inducible by gamma interferon (IFN-gamma). We have studied the molecular events leading to HLA-DR gene expression in two human monocytoid cell lines, HL-60 and U-937. In HL-60, HLA-DR genes are expressed at low levels, as determined by immunofluorescence using the fluorescence-activated cell sorter and anti-HLA-DR monoclonal antibodies, and by Northern blot analysis. In HL-60, IFN-gamma leads to a severalfold increase in HLA-DR specific transcripts and protein products. Resting U-937, U-937 treated with IFN-gamma, and U-937 treated with 5-azacytidine, do not express detectable levels of HLA-DR mRNA or protein. In U-937 the HLA-DR alpha genes appear hypermethylated as compared with those in RPMI-4265, an EBV-transformed B cell line that synthesizes large amounts of HLA-DR antigen, and less methylated than those of Molt-4, a human T-cell line that is HLA-DR-negative, as determined by Msp I/Hpa II digestion and Southern blotting. After 5-azacytidine treatment, HLA-DR alpha genes of U-937 become demethylated; however no HLA-DR gene expression can be detected. The addition of IFN-gamma to 5-azacytidine-treated U-937 cells leads to a rapid appearance of large quantities of HLA-DR specific transcripts and cell surface protein products as verified by Northern blots, FACS, and immunoprecipitation. In addition, HLA-DR apha genes are more sensitive to DNAse I digestion in resting HL-60 than in resting U-937 cells, and this DNAse-sensitivity pattern changes with IFN-gamma activation of HLA-DR gene expression.

1056 REGULATORY SEQUENCES REQUIRED FOR κ LIGHT CHAIN GENE EXPRESSION, Yehudit Bergman, Douglas Rice, Rudolf Grosschedl and David Baltimore, Massachusetts Institute of Technology, Cambridge, MA 02139

During differentiation of B lymphocytes, the variable and the constant parts of immunoglobulin (Ig) are rearranged to form a complete functional Ig gene. Upon further differentiation of a B cell into a plasma cell, a large increase in the rate of transcription is observed. To study the mechanism of regulation of the κ chain gene, a rearranged κ wild-type gene and mutant genes containing deletions spanning the entire J_κ-C_κ intron were transfected into B and T cell lines. Deletion mutants were generated by cleavage at a unique restriction site within the intron, followed by digestion with Bal31 exonuclease. The wild-type κ gene and the deletion mutants were incorporated into a vector containing the polyoma origin of replication and a modified mouse H4 histone gene as an internal control for transcription. The effects of the different deletion mutants on the transcription of κ chain gene in transiently transfected mouse myeloma cells were measured using the S1 nuclease mapping technique. We find that intron DNA sequences located 0.4-0.86 kb upstream of C_κ are crucial for accurate and efficient transcription. This region coincides with a DNAse I hypersensitive site. Similar to viral transcriptional enhancer elements, these κ intronic sequences will also function when moved 5' to the V_κ promoter, in either orientation. We are studying the level of κ chain gene expression using the deletion mutants by transfecting the genes into B cell lines representing different stages of B cell development.

1057 IMMUNOGLOBULIN GENE REARRANGEMENT ON A DEFINED SUBSTRATE,
Susanna Lewis and David Baltimore, M.I.T. Cambridge, MA 02139.

The gene segments encoding the variable regions of immunoglobulins are assembled during the differentiation of a B cell through a series of site-specific recombinations. These involve widely separated regions of the heavy or light chain loci and appear to occur in a tightly regulated fashion. An understanding of the details of this rearrangement process as well as the mechanisms that control it would be facilitated by the ability to detect Ig gene rearrangement on defined sequences that have been exogenously introduced into a rearranging cell. To this end, we designed a recombination substrate bearing portions of the kappa Ig locus, which can be introduced into cultured cells in the form of a defective retrovirus. The substrate is constructed so that if it is rearranged, expression of a previously inactive selectable marker is achieved, and the recipient cell becomes drug resistant. By this means, we have isolated independant drug-resistant cell lines all of which appear to have recombined the introduced sequences. We have confirmed that authentic V_k region recombination occured by molecular cloning and DNA sequence analysis of the integrated substrate from the genome of one representative drug-resistant isolate. The design of the substrate enabled recovery not only of the V_kJ_k coding joint, but of the reciprocal recombination product as well. The reciprocal joint had a structure analogous to rearranged fragments which have been isolated from myelomas. This indicates that probably such fragments originate as direct by-products of V_kJ_k joining.

1058 ASSEMBLY OF IMMUNOGLOBULIN HEAVY CHAIN GENES IN CULTURED CELL LINES,
Stephen Desiderio Michael Paskind, Ned Landau, Fred Alt and David Baltimore, Whitehead Institute and Center for Cancer Research, M.I.T., Cambridge, MA 02139

During B cell differentiation, immunoglobulin heavy chain genes are assembled by reorganization of three discrete germline DNA segments, V_H, D and J_H. As one approach to understanding the mechanism and regulation of this process, we have studied cell lines derived by transformation of mouse early fetal liver with Abelson murine leukemia virus. These lines represent close analogues of B lymphoid cells at early stages of differentiation. A number of these lines undergo spontaneous rearrangement of the heavy chain locus in culture. We have analyzed the precursors and products of several independent rearrangements in one such line, 40E4-2, by molecular cloning and sequence determination. Most of these rearrangements represent the joining of a V_H gene to a D-J_H assembly to yield a complete immunoglobulin heavy chain gene. Joining of D to J_H and of V_H to D in the 40E4-2 line was accompanied by loss of nucleotides from the coding sequences and by introduction of novel nucleotides at the recombination joint. Examination of similar rearrangements in an independently derived Abelson-transformed line, 22D6-5, revealed that these were also the result of V_H joining to a D-J_H assembly. In the 22D6-5 line, however, introduction of novel nucleotides at the recombination joints was not observed. The structures of the recombination joints in each of these lines are similar to junctions present in genes which have undergone functional rearrangement _in vivo_. The existence of cell lines which carry out immunoglobulin gene rearrangement but which differ in their ability to introduce novel sequences at recombilation junctions should aid in the elucidation of the means by which such sequences are introduced.

1059 TRANSCRIPTIONAL ENHANCER ELEMENTS IN THE MOUSE GENOME,
Mark Mercola and Kathryn Calame, UCLA, Los Angeles, CA 90024

A strong transcriptional enhancing element located at the 5' end of the Jh-Cμ intervening sequence of the mouse immunoglobulin heavy chain gene has previously been described. We used a quantitative _in vivo_ assay to measure the ability of a DNA sequence to enhance transcription of the bacterial chloramphenicol acetyltransferase (CAT) gene from the SV40 early promoter. Our results using plasmacytoma cells show that only the single enhancer resides within a region 2kb 5' and 9kb 3' of a rearranged immunoglobulin Vh promoter and that no enhancer exists within 2kb of the Vh gene in the germline configuration. No enhancer activity was found within 6kb on either side of the translocated c-myc oncogene. The Jh-Cμ element exhibits greatest activity relative to the prototype SV40 viral enhancer in plasmacytoma cells and functions with slightly decreased activity in mouse fibroblast cells. No activity was detected, however, in a human fibroblast line. Cellular enhancers capable of functioning in mouse L cell fibroblasts appear rather frequently in the mouse genome but no activity was seen for any segments of the mouse genome tested in human fibroblasts. Unlike viral enhancers which function in cells of different species, the murine cellular enhancers we tested are species specific. These results suggest that cellular enhancers interact with a species specific factor.

1060 REARRANGEMENT OF IMMUNOGLOBULIN HEAVY CHAIN GENES INTRODUCED DURING GROWTH IN CULTURE,
T. Keith Blackwell and Frederick W. Alt, Columbia University, New York, NY 10032

A fetal liver-derived Abelson murine leukemia virus transformed cell line, 38B9, which assembles its immunoglobulin heavy chain V, D, and J segments during growth in culture, has been shown to make site-specific rearrangements of introduced cloned heavy chain recombination substrates. A recombinant plasmid has been constructed which contains a murine genomic D segment (DQ52), part of the J_H cluster which contains J_H3 and J_H4, and the HSV thymidine kinase (tk) gene inserted between the D and the J_H segments. It has been introduced into a tk^- 38B9 cell line by co-transfection with pSV neo and subsequent selection in geneticin. Appropriate DNA blotting analyses have shown that site-specific recombination within the integrated construct (D to J_H joining) occurs at a significant level in the absence of tk selection. After the line has been passed through BUdR medium (which selects against the introduced tk gene), the majority of surviving cells have deleted the tk gene through D to J_H3 or J_H4 recombination. Several of these rearrangements have been molecularly cloned, and DNA sequence analysis has confirmed that no more than 200 bp of sequence 3' to the D or 5' to the J_H elements is required for recognition by the recombinase system, and that the chromosomal location of these elements is not important.

1061 PREFERENTIAL USE OF V_H GENE SEGMENTS IN IMMATURE LYMPHOCYTES, George D. Yancopoulos, John F. Kearney and Frederick W. Alt, Columbia University, New York, NY 10032

Our studies of Abelson murine leukemia virus (A-MuLV) transformed pre-B lymphoid cell lines reveal that such lines derived from fetal liver can construct complete heavy chain variable region genes (V_H-D-J_H) in culture via an ordered program in which a V_H is fused to an already constructed D-J_H complex. We have noted that a particular V_H (VH81X) is used very frequently in the actively rearranging A-MuLV transformed fetal liver lines, and 9 of 15 A-MuLV transformed bone marrow lines with stable rearrangements have VH81X rearranged on at least one allele. Analysis of independently derived fetal liver hybridomas also indicates frequent use of VH81X. The relative level of VH81X mRNA in normal fetal liver when compared to unimmunized adult spleen is 10-100-fold higher than for a panel of four unrelated V_H genes from different families. VH81X has never been found in the expressed heavy chain of any mature, Ig-secreting cell. The reason for and possible functional significance of the preferentail use of VH81X in pre-B cells remains to be determined. We have mapped VH81X as the most 3' (D-proximal) V_H gene segment. Sequence analysis indicates certain unique features that may interfere with its ability to interact with light chain. Preferential rearrangement of the most 3' V_H gene segments may occur, with increased selection of more 5' V_H genes at a later stage. We are also studying the possibility that this (or other) V_H gene segments may serve as further intermediates in the V_H gene assembly process, possibly by being replaced by more 5' V_H gene segments.

1062 MOLECULAR ANALYSIS OF EARLY HUMAN ONTOGENY: EXPRESSION OF GLOBIN AND c-onc GENES IN HEMATOPOIETIC CELLS FROM 5-10-WK-OLD EMBRYOS AND FETUSES.
C. Peschle [1], F. Mavilio [1], L. Bottero [1], A. Carè [1], A. Giampaolo, G. Migliaccio [1], L. Cianetti [1], M. Marinucci [1], G. Russo [2], G. Mastroberardino [3]. 1) Department of Hematology, Istituto Superiore Sanità; 2) Division of Gynecology, Ospedale Avellino; 3) Istituto Patologia Medica (VI), Rome, Italy.
We have developed a collection of hematopoietic and non hematopoietic cells and organs from virtually intact human embryos and fetuses obtained by legal curettage abortions at 5-10 wk from conception. This allowed to investigate molecular and cellular aspects of early human ontogeny. The embryonic → fetal Hb switches ($\zeta \to \alpha$, and $\epsilon \to \gamma$) have been studied in yolk sac and liver erythroblasts: these switches progress asynchronously in the 5-8 wk period (the former precedes the latter as well as initiation of β-globin synthesis, which is detected from 7-8 wk onward). At cellular level, the asynchrony strongly suggests a monoclonal stem cell model to underlie the embryonic Hb switching. At molecular level, undermethylation of the close flanking regions of β-like globin genes is strongly correlated with their activation, thus suggesting that hypomethylation may play a key role in the Hb switches. The expression of c-onc genes (myc, myb, abl, erb A and B, ras, sis, fes) has been studied in erythroblasts and non-erythroblastic cells from embryonic livers, as well as in other control embryonic, fetal and adult cells. The general pattern observed indicates that: i) c-onc expression is often enhanced in embryonic and fetal vs adult hemopoietic cells, and ii) a coherent pattern of c-onc expression is usually observed at the level of hematopoietic tissues in the 5-10 wk post-conception period.

Transformation

1063 STEROID REGULATED EXPRESSION OF A CHIMAERIC TRYPTOPHAN OXYGENASE GENE AFTER TRANSFER INTO EUKARYOTIC CELLS. Patrick D. Matthias, Ulrich Danesch, Hans U. Bernard, Rainer Renkawitz and Günther Schütz. Inst. for Cell and Tumorbiology, German Cancer Res. Center, D - 6900 Heidelberg, FRG.

In rat liver expression of the tryptophan oxygenase (TO) gene is induced 10 fold by glucocorticoids. To study the mechanism of transcriptional control a fusion gene was constructed consisting of 2kb of 5' TO flanking sequences and the bacterial chloramphenicol acetyltransferase (CAT) gene. After transfer into mouse Ltk$^-$ or rat XC cells the transient expression of the TO-CAT recombinants is induced 6-10 fold by glucocorticoids, as analyzed by CAT enzymatic activity and S1 mapping of the RNA. Analysis of 5' deletion mutants of the TO-CAT recombinants showed that 170 nucleotides upstream of the TO cap site are sufficient for glucocorticoid induction.

To study the regulation of such a recombinant when present in a minichromosome, a BPV shuttle vector was constructed. This vector, pCGBPV9, contains a G418/Kanamycin resistance unit and is maintained unrearranged as a multicopy plasmid in transfected mouse C127 cells. The TO-CAT fusion gene was inserted at several locations in this vector and the recombinants were used to transform mouse cells to G418 resistance. In the cell lines thus obtained the plasmid DNA is present extrachromosomally as an oligomeric structure. High levels of correctly initiated RNA are found, and glucocorticoid treatment leads to a 2-4 fold induction. The binding of the glucocorticoid receptor protein and its influence on the chromatin structure will be analyzed in this minichromosome.

1064 EXPRESSION OF EXOGENOUS GENES IN TRANSFORMED XENOPUS LAEVIS EMBRYOS L. Etkin, B. Pearman, S. Balcells; Dept. of Genetics M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030

We have utilized transformation in Xenopus laevis to examine the developmental and tissue specific expression of several genes. DNA sequences coding for chloramphenicol acetyl transferase (CAT), Drosophila alcohol dehydrogenase (ADH), and sea urchin histones are all expressed at the mid-blastula transition stage of initial transformants. We feel that most of the expression at this early stage is from the extra-chromosomal plasmids. The presence of strong promotors such as the SV 40 early gene promotor or relatively weak promotors such as the adenovirus E3 promotor does not affect the time of expression of these genes, nor does injection of the DNAs as linear or circular molecules. These factors, however, do affect the expression of the genes quantitatively. The injected DNAs are integrated into the Xenopus genome and exhibit a random tissue specific pattern of expression in individual tissues of larvae and young froglets. We are currently analyzing the expression of these genes in clones of embryos produced by nuclear transplantation.

1065 AN APPROACH TO INTRODUCING FOREIGN HISTOCOMPATIBILITY GENES INTO THE MOUSE GERMLINE USING RETROVIRAL VECTORS, J. Goverman, M. Kriegler* and L. Hood, California Institute of Technology, Pasadena, CA 91125 and *UC Berkeley, Berkeley, California 94720

Class I and class II gene products of the major histocompatibility complex (MHC) are cell-surface glycoproteins that permit T cells to distinguish self from non-self cells. Cytotoxic T cells recognize antigen on virally infected cells in association with self class I molecules while self class II molecules serve as recognition structures on cells that present antigen to regulatory T cells. In both cases, T cell response to antigen on the cell surface is restricted by the requirement that the T cell simultaneously recognizes class I or II determinants. The thymus is the principle site of T-cell maturation and has been implicated in playing a critical role in determining which MHC determinants T cells receptors recognize. One hypothesis suggests that the self MHC gene products to which T cells are exposed during development determines the repertoire of T-cell receptors expressed in peripheral lymphoid tissue.

We are investigating events determining the specificity of the self recognition repertoire of T-cell receptors by introducing novel class I and II genes into the germline of mice. We have introduced a human class I gene into a retroviral expression vector and obtained high levels of expression of this gene on the surface of cells infected with helper virus. We are introducing class II genes into the vector and will use virus carrying these genes to inject mouse embryos. We will investigate the ability of these genes to function as recognition structures in transgenic mice and whether expression in particular tissues influences their ability to be recognized.

1066 DEVELOPMENTAL STAGE SPECIFIC REGULATION OF TRANSFECTED IMMUNOGLOBULIN GENES

Robert J. Deans, Ronald Law, and Randolph Wall, Dept. of Microbiology and Immunology, UCLA Los Angeles, CA 90024

The developmental stage specific expression of immunoglobulin (Ig) light and heavy chain genes is regulated in part at the level of transcriptional initiation or alternate RNA processing. The re-introduction, into murine lymphocytes, of Ig genes with specific domain or control element alterations is a strong approach to identifying the molecular basis for this control.

The enhancer elements of both mu heavy chain and kappa light chain genes function differently in pre-B cells when assayed for rescue of polyoma T-Ag expression in an appropriate shuttle virus vector. This pattern reflects the stage specific expression of heavy chain genes prior to light chain gene expression in B lymphocyte maturation. These data will be presented in combination with in vitro transcription data from purified light chain minichromatin in attempts to define and/or isolate light chain specific transcription factors.

In vitro generated mutations in mu heavy chain gene sequences involved in the regulated switch from membrane to secreted forms of IgM have been constructed. The expression of these mutants in developmentally staged lymphoctye lines will be presented, as well as their response to maturation factor stimulus.

1067 TISSUE SPECIFIC EXPRESSION OF RAT PANCREATIC ELASTASE GENES IN TRANSGENIC MICE.

Galvin H. Swift, Robert E. Hammer, Raymond J. MacDonald, and Ralph L. Brinster, The University of Texas Health Science Center at Dallas, Dallas, TX 75235 and The University of Pennsylvania, Philadelphia, PA 19103.

The gene for rat pancreatic elastase I has been introduced into mice to begin an analysis of tissue specific regulation of gene expression. A recombinant plasmid was constructed containing the entire rat elastase gene including 7 kb of 5' flanking sequence, 12 kb of transcribed region (8 exons), and 5 kb of 3' flanking sequences, as well as 4.4 kb of pBR322, and was linearized at ClaI and NruI sites within pBR322. Five of 24 mice arising from the first injection experiment contained the rat elastase gene in their genomes, with copy numbers ranging from a few to approximately 100. RNA has been prepared from the pancreas and seven other tissues of two of the transgenic mice. Northern blot and solution hybridization analyses of these RNAs using a hybridization probe specific for rat elastase I has shown rat pancreatic elastase mRNA to be present at high levels in the transgenic mouse pancreases (12-fold higher than rat in the mouse with approximately 100 copies and 2-fold higher than rat in the mouse with 10 copies) and not detectable ($<10^{-4}$ pancreatic levels) in any other tissues except spleen, where it is present at approximately 1% the level of pancreas. Four of the five mice transmit the rat elastase genes to approximately 50% of their progeny; the inheritance pattern of the other mouse (1 of 10 progeny receiving the rat gene) implies it may be a mosaic.

1068 EXPRESSION OF FOREIGN GENES IN UNDIFFERENTIATED TERATOCARCINOMA CELLS.

Cori Gorman, David Lane and Peter Rigby, Cancer Research Campaign, Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College, London, England.

We have used the transient assay system based on the pSV2cat vector to analyse the early events involved in expression of foreign genes in undifferentiated cells. F9, PCC4 and P19 teratocarcinoma cells have been successfully transfected and with the use of a highly sensitive antibody-peroxidase staining method we can visualize individual cells expressing the CAT gene.

We have tested two viral promoters, the SV40 early promoter and the Rous sarcoma virus LTR, as well as the mouse metallothionein promoter. Our data clearly show that viral promoters are efficiently expressed in the nullipotential cell line F9 as well as in the pluripotential cell lines PCC4 and P19 and that the metallothionein promoter can be induced with heavy metals. Transcription and regulation of foreign genes do occur in the early stages following transfection of these undifferentiated cells. The continued expression of these promoters following stable transformation is being analysed.

1069 REGULATION OF THE EXPRESSION OF THE αA-CRYSTALLIN GENE, Ana B. Chepelinsky, Charles R. King, Peggy S. Zelenka and Joram Piatigorsky, Laboratory of Molecular and Developmental Biology, National Eye Institute, National Institutes of Health, Bethesda, MD 20205

The crystallin genes (α, β, γ and δ) are a multigene family of structural proteins whose expressions are developmentally regulated in the differentiating vertebrate lens. There are at least two α-crystallin genes, αA and αB. We have characterized the αA-crystallin gene from mice. DNA sequencing has shown that the primary transcript of this gene is alternatively spliced to give either the αA2 mRNA or the αA^{ins} mRNA. The αA^{ins} mRNA encodes a protein identical to αA2 crystallin, except that it contains an additional 23 amino acid peptide inserted between amino acid 63 and 64 of the protein.S1 nuclease protection experiments using a DNA fragment from an αA^{ins} cDNA clone showed that the alternative splicing gives 5 to 10 times more αA2 than αA^{ins} mRNA when the αA-crystallin gene is expressed. We are examining structural and functional aspects of the 5' flanking region of the αA-crystallin gene. In addition to the TATA box, these sequences have direct and inverted repeats and regions of alternating purines and pyrimidines. Both features may have a regulatory function. To study further the regulated expression of this gene we have fused the 5' flanking sequences of the αA-crystallin gene to the prokariotic gene coding for chloramphenicol acetyltransferase (CAT). In order to study its expression we have developed a culture system consisting of primary explants of chicken and mouse lens epithelial cells which have the capacity to synthesize crystallins. At present we have driven the CAT gene with the αA-crystallin promoter and are in the process of identifying its regulatory elements.

1070 CHICKEN GROWTH HORMONE EXPRESSION IN VITRO AND IN VIVO USING A RETROVIRAL VECTOR. L. Souza, D. Murdock, R. Hsu, and B. Bosselman. Amgen, Thousand Oaks, CA 91320

A cDNA clone of the chicken growth hormone (cGH) gene has been cloned and expressed in a replication competent avian retrovirus. The cGH cDNA clone was cleaved with NciI which cleaves 8 bases 5' to the ATG initiation codon and NcoI which cleaves 17 bases 3' to the TGA termination codon. A recombinant plasmid 1090/29, containing PBr322 and a proviral copy of a transformation defective Rous Sarcoma Virus (tdSRA) lacking the src gene but retaining the src splice acceptor site was cleaved at a unique SstI site 3' to the src splice acceptor site. The NciI-NcoI cGH fragment was then modified with SstI conversion linkers and ligated into the unique SstI site in plasmid 1090/29. Two E. coli transformants (tdSRA cGH 4 and 9) containing the cGH gene in the proper orientation were used to transfect C/E chicken embryo fiberblast cultures. The cultures were maintained for 14 days and then tested for virus production using a reverse transcriptase (RT) assay and for cGH production using a competition radioimmunoassay (RIA). Cultures transfected with plasmid 1090/29 or either of the two tdSRA cGH plasmids were RT positive. RIA analysis of the supernatants from the tdSRA cGH cultures yielded approximately 25-70 ng/ml cGH in a 48 hour harvest of confluent plates, while similarly treated tdSRA or uninfected cultures contained no detectable cGH. The tdSRA cGH9 recombinant virus was harvested from tissue culture and used to infect 9 day old C/E Spafas White Leghorn embryos. Serum levels of cGH were analyzed seven days post hatch and weekly thereafter. Five tdSRA cGH9 infected birds were shown by RIA to contain persistant elevated (3-10 x normal) growth hormone levels. Studies are continuing to evaluate the biochemical and biological nature of the recombinant produced chicken growth hormone both in vitro and in vivo.

1071 EXPRESSION OF THE PRO-OPIOMELANOCORTIN GENE IN THE PITUITARY AND HYPOTHALAMUS J.Drouin, P.Burbach, M.Chamberland, J.Charron, J.P.Gagner, L.Jeannotte, M.Nemer Institut de Recherches Cliniques, 110 Ave des Pins O. MONTREAL, CANADA, H2W 1R7

Pro-opiomelanocortin (POMC) is the precursor to ACTH, β-lipotropin, β-endorphin and the melanotropins. We have isolated by molecular cloning from a rat genomic DNA library in bacteriophage λ1059 about 30 Kb of DNA containing the entire POMC gene. The three exons of the gene and about 600 bp of upstream sequences have been sequenced. Southern blot analysis suggest that there is only one POMC gene in the rat; this is supported by the fact that eight independant isolates of this gene contain the same genomic sequences. Northern blot analysis indicates that POMC mRNAs of similar size are found in the anterior and intermediate pituitary lobes but that the arcuate nucleus mRNA is larger; this size difference is due to different lenghts of polyadenylate tails. S1 mapping data indicate that the same exon sequences are present in POMC transcripts from these three tissues. The regulation of POMC gene expression and the processing of POMC are different in the two pituitary lobes. For example, glucocorticoids only decrease steady state POMC mRNA levels in the anterior pituitary and not in the intermediate lobe. We have measured the effect of dexamethasone treatment of adrenalectomized rats on POMC transcription rates by incorporation of ^{32}P-labelled UTP into transcripts of isolated pituitary cell nuclei. The labelled transcripts encoding POMC and actin (an internal control) were quantitated by hybridization. We found that dexamethasone inhibits by 10 to 20-fold the POMC transcription rate in parallel with its effect of plasma ACTH levels. This effect occurs within 30 min and is specific for the anterior pituitary. Actin transcription rates were not affected in these experiments. This glucocorticoid-dependent inhibition of POMC transcription is being investigated in transient and stable expression systems after transfection into fibroblasts and pituitary cells.

(Supported by the MRC and NCI of Canada)

1072 MICROINJECTION OF A METALLOTHIONEIN/GROWTH HORMONE GENE INTO MOUSE EMBRYOS
Richard F. Selden, Thomas E. Wagner*, and Howard M. Goodman Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114 *Departments of Chemistry Zoology-Microbiology, and Biomedical Sciences, Ohio University, Athens, OH 45701

We have constructed a fusion gene containing promotor sequences from the mouse metallothionein gene and structural sequences from a human growth hormone gene. Approximately 2000 copies of the fusion gene were microinjected into the male pronucleus of single cell mouse embryos, which were then implanted into pseudopregnant foster mothers. In one series of injections, 5 of 26 mice born contained 1-10 copies of the construct and were significantly larger than control mice (36 vs 18 gms at age 6 weeks). Serum growth hormone assays revealed human growth hormone at concentrations ranging from .135 ug/ml-100ug/ml. At present, we are studying the expression of the fusion gene with respect to tissue specificity and hormone responsiveness.

1073 GERMLINE TRANSFORMATION ANALYSIS OF DELETION MUTANTS OF THE DROSOPHILA ALCOHOL DEHYDROGENASE (ADH) GENE,
James W. Posakony, Victoria Corbin and Tom Maniatis, Harvard University, Cambridge, MA 02138

An 11.8 kilobase genomic DNA fragment containing the *Drosophila* Adh gene has been shown previously to be expressed in a correct developmental stage- and tissue-specific manner when introduced into the germline by P element mediated transformation. Recently we have analyzed transformants carrying smaller (4.8 and 3.2 kb) Adh gene fragments. The transformants were identified by selection for either ethanol resistance (conferred by the Adh gene) or *rosy*$^{+}$ eye color (conferred by a wild-type xanthine dehydrogenase gene). Adh expression was found to be normal in adult flies of these strains, but reduced an average of about 10-fold in larvae. This indicates a differential sensitivity of the larval and adult Adh promoters to the removal of certain 5' flanking DNA sequences. Analysis of additional 5' deletion mutants selected by the eye color marker has revealed that (1) sequences between -66 and -680 of the adult Adh promoter are necessary for its full activity and (2) deletion of the adult Adh promoter results in strong expression from the larval Adh promoter in adult flies.

1074 MOLECULAR GENETICS OF CLASSICAL PKU,
Savio L.C. Woo, Alan Lidsky, Fred Ledley, Kathryn Robson and Anthony DiLella, Howard Hughes Medical Institute, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

Classical Phenylketonuria (PKU) is a human genetic disorder caused by an inborn error in amino acid metabolism. The hereditary disease is characterized by absence of the hepatic enzyme phenylalanine hydroxylase (PAH) which catalyses the conversion of phenylalanine to tyrosine. The lack of this enzymatic activity causes severe mental retardation in untreated children. The disorder is transmitted as an autosomal recessive trait, and has a prevalence of about 1 in 10,000 neonates. It has been estimated that 1 in 50 Caucasians is a carrier of the trait. We have previously reported the construction of a 1.4 kb human PAH cDNA clone and the use of it to establish restriction fragment length polymorphisms in the human PAH locus. Additional polymorphic restriction sites had been discovered on the PAH locus using a recently constructed fullength human PAH cDNA probe. Haplotype analysis of the PAH locus has indicated that prenatal diagnosis and carrier detection of the genetic disorder can be performed for most of the PKU families in the Caucasian population. The chromosomal PAH genes from homozygous normal and PKU individuals had also been cloned using the cosmid vector pCV107 which contains the SV2-DHFR gene. These genes had been introduced into a DHFR^{-} hamster cell line and amplified by methotrexate selection. The capacity of the normal and mutant PAH genes to be expressed into mature mRNAs and functional enzymes in the heterologous cells is being analysed in order to establish the biological basis of the deficiency syndrome.

1075 CELL TYPE REGULATION OF GROWTH HORMONE GENES INTRODUCED INTO CULTURED CELLS, Peter Kushner, California Biotechnology Inc., Mountain View, CA 94043

The mammalian growth hormone gene is normally expressed in the somatotrophs of the anterior pituitary and in no other cell type. We have transferred the cloned human growth hormone gene into a mouse fibroblast line (L cells) and into a rat somatotroph line (GH3 cells) by co-transformation with selectable markers. Human growth hormone is produced by every co-transformant and the level of expression within each recipient cell type closely correlates with gene copy number. The rate of expression in GH3 cells, however, is at least 20 fold higher per gene template than in L cells. A hybrid gene consisting of the rat growth hormone 5' flanking region fused to the bacterial chloramphenicol acetlytransferase (CAT) coding sequence exhibits a similar propensity (high expression in GH3, low expression in L cells) when reintroduced and assayed for transient expression. L X GH3 hybrid cells allow only low level expression from the growth hormone-CAT construct. In contrast to the tissue specific preference of the growth hormone 5' regulatory region, the 5' regulatory region from the avian sarcoma virus long terminal repeat drives approximately equal expression in the three cell types. Hence, our observations suggest the presence of sequences in the 5' flanking region of the rat growth hormone gene important for its tissue specific pattern of expression.

1076 REGULATION OF Ig HEAVY CHAIN GENE EXPRESSION DURING B CELL DIFFERENTIATION, Rudolf Grosschedl and David Baltimore, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA 02139

A functionally rearranged mouse μ heavy chain gene was cloned into a transient expression vector containing the early region of polyoma virus and a modified mouse H4 histone gene. Transcription of the modified H4 gene serves as an internal control to quantitate the level of μ gene expression in transfected cells. Wild-type and mutant Ig genes were transfected into different mouse cell lines and their expression were studied by S1 nuclease analysis of total RNA. By deletion analysis a region in the large J/C intron could be identified which is required for high levels of μ gene expression in myeloma MPC11 cells. This functionally important DNA sequence can restore the activity of the deletion mutant when inserted in both orientations 5' to the V_H promoter, therefore having the properties of a transcriptional enhancer as also shown by others. To investigate whether the enhancer determines the cell type specificity of the μ gene we replaced the μ enhancer with the tissue-nonspecific MLV enhancer. The activity of the wild type and mutant μ genes was assayed in fibroblast 3T3 cells, in the lymphoid T cell line Yac, in the pre B cell line PD and in the myeloma line MPC 11. High levels of μ transcription can only be detected in myeloma cells when either the μ or MLV enhancer is present. Since the MLV enhancer activates the μ gene exclusively in myeloma cells, we conclude that a genetic element different from the enhancer regulates the cell type specificity of the μ gene in B cell differentiation. Experiments to define this DNA sequence are in progress.

1077 TRANSMISSION DISTORTION AND MOSAISISM IN TRANSGENIC MICE, Thomas Wilkie*, Ralph L. Brinster, and Richard D. Palmiter* *University of Washington, Seattle WA and University of Pennsylvania, Philadelphia PA

Transgenic mice contain chromosomal integrants of foreign DNA introduced by microinjection of fertilized eggs. Usually integration occurs as tandem repeats at a single chromosomal site; consequently the integrant DNA is transmitted to half of the offspring. Occasionally transgenic mice transmit the insert at a frequency less than 50%. We have mated 46 transgenic mice, each presumed to have a unique insertion site, and found that 4 mice transmit the integrant at an unexpectedly low frequency. These mice may have the foreign DNA integrated in only some of their cells; hence they are mosaic. One of these mice that has been analyzed in detail, MyK103♀, is uniformly mosaic in all tissues analyzed, including the germline. We suggest that integration of the microinjected plasmid in MyK103♀ occured after determination of the inner cell mass commenced but before these cells were committed to form the primary organ rudiments. Another group of 5 male transgenic mice fail to transmit the integrant DNA, yet they are all fertile. These males may exhibit either extreme mosaisism or transmission distortion. We have documented male transmission distortion in the MyK103 pedigree. Six hemizygous MyK103 males have failed to transmit the integrant DNA to any of their 83 offspring, whereas hemizygous females transmit the integrant DNA normally. We have observed that sperm DNA is deficient for the integrated plasmid relative to somatic tissues. We hypothysize that in males of the MyK103 pedigree, and possibly in other transgenic males, the plasmid insert prevents the normal function of a gene(s) which is essential for the formation of fertile spermatozoa.

1078 The LTR of Feline Leukemia Virus Promotes the Expression of the Bacterial Neo gene in Mouse and Human Cells. Nevis Fregien and Norman Davidson, California Institute of Technology, Pasadena, CA 91125

A variety of recombinant DNAs were constructed to put the bacterial Neomycin resistance gene under the control of the Feline Leukemia Virus(FeLV) long terminal repeat(LTR). These plasmids were tested for their ability to transform Mouse Ltk⁻ cells and Human RD(Rabdomyosarcoma) tk⁻ cells, and compared to HSV TK and SV40 promoters. The mouse cells transformed more efficiently then the Human cells. The efficiency of the promoters tested was equal in the mouse cells. In the Human cells the HSV TK promoter gave the lowest transformations. The SV40 promoter and a construction which put the Neo gene between the U3 and U5 of the FeLV LTR showed a ten-fold increase in the number of transformants. RNA blots and S1 analysis showed the transcripts of individual clones to be the expected sizes, and properly initiated and terminated. One construction was shown to be processed using the FeLV gag splice. Quantitative S1 experiments showed a large variation in the amount of RNA, as did enzyme assays. In general the constructions which transformed more efficiently had more RNA and more enzyme activity.

1079 TISSUE SPECIFIC EXPRESSION OF RAT α_{2u} GLOBULIN GENES: David T. Kurtz, William Addison, Janet MacInnes, Jin-Zhao Li, Debra Danna, Eva Nozik.

For the past several years, my laboratory has been studying the hormonal modulation of the synthesis of a rat protein called α_{2u} globulin. The major site of synthesis of this protein is the liver; however, we have recently found that the protein is synthesized in the salivary gland, the lachrymal gland, pregnant mammary gland, and possibly, the kidney. α_{2u} is encoded by a multigene family (20-30 genes per haploid complement) and it appears that different genes or gene sets are expresed in the different tissues. Furthermore, the genes expressed in the different tissues are under different hormonal control. Using gene transfer and cell fusion experiments with cloned tissue specific α_{2u} genes, we are attempting to determine the molecular basis for the tissue specificity of expression of the various α_{2u} genes, as well as the DNA sequences responsible for hormonal modulation.

1080 CELL-SPECIFIC EXPRESSION CONTROLLED BY THE 5'-FLANKING REGION OF INSULIN AND CHYMOTRYPSIN GENES, Michael D. Walker, Thomas Edlund, Anne M. Boulet and William J. Rutter, Hormone Research Laboratory, University of California, San Francisco, Ca 94143

DNA sequences containing the 5' flanking regions of the insulin and chymotrypsin genes were linked to the coding sequence of the chloramphenicol acetyltransferase (CAT) gene. The insulin gene recombinant elicits preferential expression of CAT activity when introduced into cells producing insulin; similarly, the chymotrypsin gene recombinant elicits preferential expression with chymotrypsin producing cells. Deletion mapping suggests that sequences located between 150 and 300 bp upstream are essential for efficient expression in both cases. Our results are consistent with the notion that these DNA sequences are part of a positive regulatory system which controls, at least in part, cell-specific gene expression.

1081 THE 5'-FLANKING DNA OF RAT AND HUMAN INSULIN GENES CONTAIN A CELL SPECIFIC ENHANCER-LIKE ELEMENT, Thomas Edlund, Michael D. Walker and William J. Rutter, Hormone Research Laboratory, University of California, San Francisco, Ca 94143

DNA sequences containing the 5' flanking regions of the human and rat insulin genes promote preferential expression of the chloramphenicol acetyltransferase (CAT) gene when introduced into cells producing insulin. The insulin 5' flanking regions can augment the activity of the Herpes virus thymidine kinase promoter in an orientation independent and to some extent position independent manner characteristic of enhancers. The effect with this insulin fragment is only observed in insulin producing cells. This element was mapped to -125 to -275 bp from the mRNA start site in the rat insulin I gene. A core enhancer sequence is present in the insulin 5' flanking region but could be deleted with no apparent effect on the enhancement activity. Preliminary experiments suggest the presence of another distinct element in the promoter region of the insulin gene which contributes to cell specific expression.

1082 EXPRESSION OF HUMAN GENES IN HUMAN/MOUSE TERATOCARCINOMA HYBRIDS F.J. Benham, M.V. Wiles, A. Tunnacliffe, P.N. Goodfellow. Imperial Cancer Research Fund, London W.C.2. U.K.

We have introduced single human chromosomes into the mouse embryonal carcinoma (E.C.) cell line PCC4 by 1) microcell transfer of a human X or X/autosome translocation into an HPRT-derivative of PCC4, or 2) introduction of the dominant selectable marker Ecogpt into a human genome followed by microcell transfer of the human chromosome which contains the gpt gene into PCC4. These lines retain the capacity to differentiate in vitro. The lines are being used to investigate regulation of human genes by the embryonic environment of the E.C. cell, for example, expression of HLA genes where results indicate that the E.C. cell does not regulate class I genes, but does regulate class II genes. Other human genes being studied include c-myb and collagen genes.

1083 THE EXPRESSION AND REGULATION OF CHICKEN ACTIN GENES INTRODUCED INTO MOUSE MYOGENIC AND NONMYOGENIC CELLS, Bruce M. Paterson, Anne Seiler-Tuyns, Juanita D. Eldridge, Laboratory of Biochemistry, National Cancer Institute, NIH, Bethesda, MD 20205

We have introduced the beta actin, alpha cardiac actin, and alpha skeletal actin genes from the chicken into the C2 murine myogenic cell line and into L-cells using the SV-40 derived vector, PSV2-gpt. Stable transformants represent between $1/10^4$ to $1/10^5$ of the starting cells. In each selection the entire transformed cell population was analyzed for the expression and regulation of the actin genes as compared to the expression of the vector marker, Eco-gpt. The chicken beta actin gene is expressed strongly in L-cells and down regulates in parallel with the mouse beta actin gene in C2 cells during differentiation. Eco-gpt expression in unaffected with differentiation. Both the alpha cardiac and skeletal actin genes of the chicken are expressed at low levels in L-cells. The alpha cardiac actin gene is expressed strongly in C2 cells but levels of expression do not change significantly with myogenesis. The alpha skeletal actin gene is expressed at low levels in pre and post fusion C2 cells, displaying no induction with differentiation. S1 nuclease and primer extension analyses demonstrate that the RNAs transcribed from the chicken actin genes initiate and terminate correctly in C2 cells, L-cells, and transiently treated C2 cells. Furthermore, these transcripts are processed since the 5' noncoding intron is each of the genes is spliced out. Factors other than the sequences flanking these genes are important in the regulation of gene expression during development. The down regulation of β-actin during C2 cell differentiation provides a model system in which to study gene repression during cell differentiation.

1084 POST-TRANSCRIPTIONAL CONTROL OF H-2 ANTIGEN EXPRESSION, John Weis and Jonathan Seidman, Dept. Genetics, Harvard Medical School, Boston, MA 02115

Hybrid genes in which two major histocompatability antigen genes, H-2D^d and H-2L^d, are fused to the metallothionein gene promoter have been constructed and introduced into mouse L-cells. Large amounts of H-2 mRNA are found in cells containing the hybrid gnes since the metallothionein gene promoter is more active than the H-2 promoter. More H-2 mRNA is expressed from the hybrid genes when cadmium is added to the culture media although the level of surface antigen expression does not change. Additonally, with or without induction, the level of the H-2 antigens (H-2K^k and H-2D^k) normally expressed on the surface of L-cells is greatly reduced. To test the possibility that the addition of beta-2 microglobulin (one subunit of the H-2 antigen) is a limiting step in the maturation of H-2 antigens, beta-2 microglobulin genes were introduced into L-cells already containing the hybrid H-2 genes. These cells express higher levels of the endogeneous H-2 antigens (H-2K^k and H-2D^k) indicating that competition among class I antigens for beta-2 microglobulin can influence the level of these antigens on the cell surface. Using the microinjection technique, we have produced transgenic mice containing these hybrid H-2 genes. We are currently examinq these mice for the expression of the foreign H-2 antigens.