MOLECULAR DETERMINANTS OF ANIMAL FORM

Gerald M. Edelman, Organizer

March 30 — April 4, 1985

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Determination of Embryonic Induction

1074 CYTOPLASMIC LOCALIZATION AND THE "ORGANIZER" OF THE FROG EMBRYO, Robert Gimlich, Department of Molecular Biology, University of California, Berkeley, CA 94720.

The precursors of the dorsal axial mesoderm of the amphibian embryo lie within a thick equatorial band of cells at the early gastrula stage. During subsequent development this group of cells undergoes a characteristic repacking, extends dramatically along the future cephalo-caudal axis, and forms the notochord and segmented somitic mesoderm. The central part of the mesodermal layer is thought to be responsible for organizing the surrounding mesodermal cell sheet and triggering neural plate formation by the overlying ectoderm. I have carried out experiments aimed at understanding how this important organization center of the embryo is established. By a combination of transplantation, deletion, re placment, and fate mapping of early cleavage stage blastomeres, I have addressed two questions: What is the state of developmental autonomy at various times throughout cleavage of blastomeres whose progeny normally form the dorsal mesoderm? To what extent do other blastomeres show a capacity for inducing dorsal mesoderm formation by the progeny of neighboring cells?

The results indicate flexibility in the process of early regional specification of cell fate. In some early cleavage stage embryos the dorsal equatorial cells have the ability to develop autonomously according to their normal fates when placed in abnormal embryonic surroundings. This autonomy encompasses the development of organizing capabilities as well as the formation of axial mesoderm. In some sibling embryos, however, dorsal equatorial cells show only limited autonomy in forming mesoderm. Instead, adjacent vegetal cells which form endoderm have the capacity to induce any neighboring equatorial cells to form abundant dorsal mesoderm. This vegetal-equatorial interaction is complete by the early gastrula stage, when each embryo has a fully autonomous organizer.

On the basis of such observations I suggest that the positioning of determinants which direct the development of the organizer is variable relative to the fate map. In some embryos cell lineages which contribute to the organizer gain their special properties largely by inheriting these determinants directly. In others the determinants are distributed primarily to presumptive endodermal regions, and contribute to axis formation by enabling vegetal cells to induce their neighbors to form the organizer.

A variety of approaches will be used to identify the specific mode of intercellular communication involved in this patterning system, to determine the biosynthetic requirements for inductive activity of vegetal cells, and to examine the spatial relationship between functionally defined determinants of organizer formation and morphologically defined cytoplasmic localizations which take place in the uncleaved fertilized egg.

see R.L. Gimlich and J.C. Gerhart (1984) Early Cellular Interactions Promote Embryonic Axis Formation in *Xenopus laevis*. Dev. Biol. 104: 117-130.

1075 TEMPORAL AND REGIONAL REGULATION OF ACTIN GENE TRANSCRIPTION IN *XENOPUS LAEVIS*, J.B. Gurdon, T.J. Mohun, S. Brennan, and S. Cascio, CRC Molecular Embryology Unit, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, England.

The genome of *Xenopus laevis* contains several actin genes, for some of which we have gene-specific probes. We have used these probes to determine the amounts of actin mRNA present at different stages and in different regions of early *Xenopus* embryos. Up till the end of gastrulation embryos contain a constant low amount of cytoskeletal actin mRNA (presumed to be of maternal origin), and no detectable cardiac or skeletal α-actin mRNA. Just after gastrulation there is a sharp rise in the level of all actin mRNAs (for which we have probes). The transcription of cytoskeletal actin genes takes place in all regions of early embryos, whereas cardiac and skeletal actin mRNA is found almost exclusively in the somite region of the mesoderm. Cardiac actin is a major component of axial muscle at this early stage of development.

When regions of blastulae are isolated and cultured until the normal time of actin gene activation, the equatorial region, but not the animal or vegetal region, undergoes cardiac gene transcription. Even in fertilized eggs at the 1-cell stage, the equatorial cytoplasm, but not the animal pole cytoplasm, contains all material required for cardiac gene activation, as shown by hair loop ligation experiments. Cell contact during cleavage is not required for some degree of cardiac actin gene transcription, since cardiac actin RNA appears at the usual time in cell aggregates which cleaved in $Ca^{++}Mg^{++}$-free medium. These and many other experiments indicate that the substances needed for cardiac gene activation are already localized in a subequatorial region. However, it is likely that induction is also required for some cells to undergo cardiac actin gene activation, since vegetal blastula cells can strongly induce animal pole cells to undergo cardiac actin gene activation. We are currently investigating this induction reaction, and are also characterizing genomic actin clones, with the aim of using these for gene injection experiments.

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1076 INDUCTIVE INTERACTIONS AND DETERMINATION, Pieter D. Nieuwkoop, Hubrecht Laboratory, Utrecht, The Netherlands

The embryonic development of multicellular organisms requires almost continuous interaction between their constituant cells. Messages released by cells may be recognised by adjacent cells as distinct signals to which they may react, provided the messages differ sufficiently from those they release themselves. The messages probably represent transient, ordinary products of cellular differentiation attendant upon the pathway of differentiation in progress in these cells. The released messages may vary from simple ion fluxes to high molecular membrane and extracellular matrix components.

An embryonic system can only develop when it has a minimal spatial heterogeneity. The majority of chordate eggs have a single polar axis and consist of only two different moieties. Chordate development is therefore almost completely epigenetic and development is based on the confrontation of different parts of the egg or embryo which come into contact during development, mainly as a consequence of morphogenetic movements.

In all interactions a distinction must be made between an action and a reaction system. Interaction implies reciprocity. Both action and reaction system show time-dependent processes occurring at particular phases of development; the reaction system showing well-defined periods of responsiveness or competence to certain messages from adjacent cells.

Only interactions which lead to entrance into a new pathway of differentiation should be called "inductive", as distinct from those supporting an already existing pathway. Inductive interactions are therefore restricted to pluripotential reaction systems open to a choice of alternative pathways.

It seems likely that the specificity of inductive interactions chiefly resides in the competence of the reaction system. Emphasis should be placed on the study of the physiology of the action and reaction system during their critical period of interaction, and particularly on the differences between the two systems, which are primarily responsible for their interaction.

Determination is the stepwise restriction of potential developmental pathways open to the reaction system. Although the mechanisms underlying induction and determination are still essentially unknown, there are some indications that competence may imply an incipient, low level of specific transcription.

Certain assumptions implicit in the title of this symposium will be criticised, particularly the putative specificity of cytoplasmic determinants (which may mostly represent differentiation products).

Fate Maps and Morphogenetic Movements

1077 A NEW MODEL FOR EPITHELIAL FOLDING: APPLICATION TO THE NEURAL PLATE

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We propose a model to account for the shaping and folding of epithelial tissues. The cells of epithelia may reduce their apical areas and tug on adjacent cells (1,2), and they may change neighbors, interdigitating between one another (1,3). Both these activities may shape the epithelium. The network of actomyosin in the cortex of cells is believed to drive both the changes of cell shape and the movements of cells. A "cortical tractor model" of cell movement of epithelial cells will be described. The boundaries between adjacent cellular domains with differing adhesive characteristics may organize cell movements in epithelia to produce some of the characteristic changes of shape of the tissue.

During neurulation, the neural plate elongates and narrows, the surface area of the plate progressively reduces, the plate cells get taller, neural folds arise, and the plate rolls into a tube. Boundaries between domains of cells are evident in the neural plate at the plate edges where the neural folds arise, and between a midline population of cells called the notoplate (3) and the rest of the neural plate. We will describe how cell movements that elongate these boundaries, together with the contractions of the apical surfaces of the plate cells may account for neurulation.

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1078 FATE MAPS AND MORPHOGENETIC CELL MOVEMENTS IN AMPHIBIAN EARLY DEVELOPMENT. R. Keller and Cathy Lundmark, Department of Zoology, and Mike Danilchik and Robert Gimlich, Department of Molecular Biology, University of California, Berkeley CA 94720.

The fate maps and morphogenetic cell movements of two amphibians, Xenopus laevis and Ambystoma mexicanum, have been investigated. In Xenopus, the superficial epithelium consists of prospective endoderm and ectoderm. The mesoderm lies in the deep region of the marginal zone, forming a torus just above the site of blastopore formation. Gastrulation is accomplished in two steps. Firstly, in the dorsal sector of the gastrula, the vegetal edge of the mesodermal torus is turned inward (involuted), probably as a result of bottle cell formation and perhaps traction of the leading mesodermal cells on the blastocoel roof. After their formation, bottle cells are not necessary for gastrulation. Secondly, involution of the marginal zone and closure of the blastopore are brought about principally by dual zones of dorsal convergence (narrowing) and extension (lengthening) of the circumblastoporal region. One zone lies in the postinvolution region and narrows and lengthens the prospective axial mesoderm and archenteron roof. The second zone lies outside the blastopore and supplies cells to the site of involution. Grafting experiments show that deep cells, rather than superficial cells, drive the convergent extension movement. Tracing of cells in the gastrula by labelling them with fluorescein-lysine-dextran cell lineage tracer and timelapse cine or videomicrography of cells in cultured explants show that convergent extension is accompanied by circumferential intercalation of deep cells to form a longer, narrower array. Intercalation involves breaking of cell contacts and motile, protrusive activity. In Ambystoma, mapping cells with lineage tracers shows that the notochord and somitic and ventral mesoderm do, in fact, lie in the superficial layer of the early gastrula. Cell marking studies and correlated scanning electron microscopy show that the movement of the somitic mesoderm from the superficial layer at the corners of the blastopore involves the formation of bottle-shaped cells, loss of their epithelial character, and their ingression into the deep region. Removal of the somitic mesoderm in zones lateral to the notochord may result in circumblastoporal tension that aids in the involution of the prospective notochord. The prospective notochord undergoes convergent extension, as in Xenopus, and is removed from the superficial layer in the neurula. Comparison of the cellular behavior and morphogenetic movements in Xenopus and Ambystoma suggests that various cellular motile processes are marshalled in bringing about a common system of mechanical stresses appropriate to produce involution and convergent extension of the dorsal tissues.

This work was supported by NSF Grant PCM81-10985 to R. Keller, PHS Grant GM 08738-03 to M. Danilchik, and PHS Grant GM19363 to Dr. John Gerhart.

1079 MORPHOGENETIC MOVEMENTS AND FATE MAPS IN THE AVIAN BLASTODERM, Lucien C. VAKAET, Laboratory of Anatomy and Embryology, Rijksuniversiteit Gent, Ledeganckstraat, 35, B-9000 GENT, Belgium

Using a combination of marking techniques (vital dyes, iron oxide grains and especially chick-quail grafts after N. Le Douarin (1)) the disposition of the Anlage Fields of the avian blastoderm has been investigated. Fate maps have been constructed of ten stages from the just laid blastoderm through the head fold stage (2). While the fate maps of stages 6 through 9 mainly confirm Nicolet's findings (3), a more precise description is given of the final placement of the gut endoderm by ingression through the anterior part of the primitive streak between stages 4 and 5. Early marking has made it possible to draw fate maps of just laid stage 0 blastoderms. A hypothetic fate map of a radially symmetrical avian blastoderm (about five hours before laying) is presented. The bilateral symmetrization of the blastoderm is discussed in relation with external (gravity) and internal (extracellular matrix) factors. The role of a band of extracellular fibers on the basal lamina of the upper layer (4) in the marginal zone of the young blastoderm is considered.

The description of the movements correlated with the evolution of the disposition of the Anlage Fields is updated using cinemicrographic data. Possible mechanisms for these movements are presented based on transmission electron microscopic studies.

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Primary Processes

1080 TWO CELL ADHESION MOLECULES: CHARACTERIZATION AND ROLE IN EARLY MOUSE EMBRYO DEVELOPMENT. Caroline H. Damsky, Margaret J. Wheelock, Jean Richa, Davor Solter and Clayton Buck, The Wistar Institute, Philadelphia, PA 19104.

Cells in tissues of adult organisms are firmly associated with one another and with their extracellular matrices by a variety of adhesive complexes. Blastomeres of the very early mammalian embryo are loosely associated. During subsequent development, particular populations of cells must recognize one another but must be able to change their associations migrate over considerable distances and establish new associations. Thus, a programmed display and regulation of cell-cell and cell-matrix interactions is essential for normal embryogenesis. We have identified and purified a cell-cell adhesion glycoprotein (cell CAM 120/80: 1) from cultured mammary tumor epithelium and a group of 120-160Kd cell-substratum adhesion glycoproteins (GP140: 2,3) from cultured epithelia or fibroblasts and examined their role in mediating adhesive interactions in early mouse development. The 80Kd fragment of cell CAM 120/80 was purified from the conditioned serum free medium from MCF-7 cultured human mammary tumor epithelium. Antiserum against the 80Kd glycopeptide (anti GP80) recognized a single 80Kd band in immunoblots of conditioned SFM and a single 120Kd band in blots of NP40 extracts of epithelial cells. The 120Kd material could be converted to 80Kd following trypsinization in the presence of calcium. Extraction studies suggest that the 120Kd parent molecule is an integral membrane glycoprotein. It has a pI of 4.8 and is highly protease sensitive. Localization studies show that cell CAM 120/80 is distributed diffusely along regions of cell-cell contact in epithelial cells but is not found in other cell types. When tested for its effects on early mouse development, anti GP80 inhibited compaction of 8-16 cell embryos and subsequent blastulation. The antibody had no effect on cell division or on the ability of individual cells to accumulate fluid. When exposed to isolated inner cell masses, anti GP80 caused decompaction and inhibited segregation of an endoderm layer (4). The GP140 integral membrane cell-substratum adhesion glycoproteins were purified from NP40 extracts of fibroblasts or epithelial cells. Anti GP140 detached cultured cells from diverse species and tissue origin. Anti GP140 inhibited attachment of both mouse blastocysts _in vitro_ and cultured parietal endoderm cells. Thus, both sets of antigens are expressed _very early_ in development. Cell CAM 120/80 is involved in cell-cell contacts required for proper cell lineage segregation while GP140 may be involved in implantation and parietal cell migration. Supported by CA32311, CA27909, CA07572, HD12487 and PCM-81-18801. (1) Damsky et al Cell _34_: 455 (1983), (2) Knudsen et al PNAS _78_: 6071 (1981), (3) Damsky et al J. Cell Biol. _89_: 173 (1981), (4) Richa et al Dev. Biol. _in press_.

1081 CELL ADHESION MOLECULES IN EMBRYOGENESIS AND HISTOGENESIS, Gerald M. Edelman, The Rockefeller University, New York, NY 10021

In the last decade, the specificities and binding mechanisms of several cell adhesion molecules have been characterized. These molecules include N-CAM (neural cell adhesion molecule, 180-250 Kd), L-CAM (liver cell adhesion molecule, 124 Kd), and Ng-CAM (neuron-glia adhesion molecule, 200 Kd, 135 Kd, and 80 Kd). CAMs are expressed in defined sequences during embryonic development beginning at very early times and appear in a definite topological order in fate maps. L-CAM is seen in derivatives of all 3 germ layers, N-CAM in ectodermal and mesodermal derivatives and Ng-CAM in neuroectodermal derivatives. These distributions persist in adult tissue derived from these layers.

Anti-L-CAM antibodies perturb liver cell colony formation _in vitro_. Monovalent fragments of serum antibodies and monoclonal antibodies against N-CAM _block_ neurite fasiculation, neurite-myotube interactions, and orderly retinal layering in tissue culture. Antibodies to N-CAM also disturb the order and precision of the retinotectal projection when introduced _in vivo_ into the tectum of _Xenopus laevis_. Antibodies to Ng-CAM inhibit migration of external granule cells in embryonic cerebellar slices _in vitro_.

Binding studies suggest that N-CAM and Ng-CAM can appear on the same neuron, mediating respectively neuron-neuron and neuron-glia adhesion in a calcium-independent fashion. N-CAM also mediates heterotypic neuron-myotube interactions by a homophilic (N-CAM to N-CAM) mechanism. The Ng-CAM interaction appears to be heterophilic to a putative molecule (Gn-CAM) on glial cell surfaces. L-CAM adhesion is Ca^{++} dependent and is suspected to be homophilic.

The carbohydrate moiety of N-CAM from embryos contains a large amount of sialic acid (30gm/100gm polypeptide) some of which appears to exist as polysialic acid. During development and in perinatal life this embryonic form (E form) is replaced by several adult (A) forms which have much less sialic acid. A model of N-CAM has now been correlated with kinetic studies of N-CAM binding after insertion in lipid vesicles. The results suggest that the order of binding rates is: E-E<E-A<A-A and that a two-fold increase in protein to lipid ratio leads to a 30-fold increase in binding rates. E-A conversion occurs in different sequences in different parts of the brain and is delayed in the mouse cerebellar mutant, _staggerer_. These results provide support for the idea that local cell surface modulation occurs _in vivo_ and suggest that neural patterns arise by kinetic constraints upon primary processes of development (division, movement, migration, death etc.)

We have proposed that CAM's act as regulators of morphogenetic movements and that the covariance of the timing of CAM gene expression and various morphogenetic movements are important factors in the control of embryonic induction. Several cDNA probes for CAM's have been recently isolated and are being used to test the onset of CAM gene expression. The combined data suggest that CAM's play major roles in the determination of animal form and tissue structure.

1082 AGGREGATION AND SEGREGATION OF CELLS CONTROLLED BY SPECIFIC CELL-CELL ADHESION MOLECULES, Masatoshi Takeichi, Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan

The Ca^{2+}-dependent cell-cell adhesion system is present in a wide variety of cell types and essential for tight binding of these cells. We have obtained two kinds of monoclonal antibodies, ECCD-1[1)] and NCD-1, both of which can block this adhesion system of mouse embryos. If these antibodies are given to monolayer culture of cells, their cell-cell adhesion is actively disrupted. An important difference between these antibodies is that they react with distinct cell types. ECCD-1 reacts with blastomeres of early embryos inducing decompaction[2)], and at the later develpmental stage it reacts specifically with epithelial cells of various organs such as skin, mammary gland, salivary gland, adenohypophysis, thyroid, lung, liver and pancreas[1, 3)]. On the other hand NCD-1 does not react with these epithelial cells, but it does with neuron and glia of various nervous tissues, myotube of skeletal muscles, cardiac muscle cell and lens epithelial cell. There are another class of cells that react with neither of ECCD-1 nor NCD-1, such as fibroblast and endothelial cell of blood vessels. These results indicate that cells in the body can be classified into three groups, the ECCD-1-reacting type, the NCD-1-reacting type and the third, non-reacting type.

When ontogeny of these Ca^{2+}-dependent adhesion molecules were studied, it was found that only the ECCD-1 type is expressed at the early developmental stage. Therefore, the other types become to be expressed at some later stages. Since a given cell expresses only one type of the Ca^{2+}-dependent adhesion molecule, it is assumed that many classes of cells in the body cease expression of the ECCD-1-reacting type at the certain stage of development and instead begin expression of another type. We suppose that such replacement of cell-type-specific Ca^{2+}-dependent adhesion molecules plays a crucial role in separation of cell populations with different phenotypes.

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1083 Mechanisms of embryonic cell migration and adhesion in the vertebrate embryo. J.P. Thiery. Institut d'Embryologie CNRS, 94130 Nogent-sur-Marne, FRANCE.

The extracellular matrix (ECM) associated with epithelial tissue basement membranes and with the mesenchyme plays a key role in tissue remodelling and cell migration. Fibronectin (FN) appears prior to or at the blastula stage in the vertebrate embryo. In amphibians, FN is assembled as a fibrillar network on the roof of the blastocoelic cavity preceding mesodermal cell migration. The latter do not migrate in regions of the roof where the ectoderm has been inverted, providing an ECM-free substrate. Microinjection of monovalent antibodies to FN in the blastocoelic cavity arrests gastrulation. The same effect is obtained after injection of a synthetic decapeptide containing the cell binding site sequence of FN. Neural crest cells migrate along or between FN-rich basement membranes. In birds, the pathways of migration have been traced using antibodies to FN and a monoclonal antibody which identifies crest cells. In most cases, crest cells maintain a high density in transient narrow pathways. Directionality of migration is in part given by population pressure and does not seem to require other mechanisms such as chemotaxis, haptotaxis or contact guidance. Crest cells adhere preferentially to FN both _in vitro_ and _in vivo_. Antibodies to FN and the cell binding peptide inhibit migration on FN substrates in culture and microinjection blocks cephalic crest cell migration in embryos. These data provide both circumstantial and direct evidence for the importance of FN in directed cell migration. The distribution of the two primary cell adhesion molecules N-CAM and L-CAM has been determined throughout embryogenesis. Both molecules are found in all cells of the blastoderm. A partial segregation is observed during gastrulation and neural induction. L-CAM disappears from the neural primordium but accumulates in the lateral ectoderm and in the endoderm. N-CAM becomes more abundant in the neuroectoderm and appears transiently in many mesodermal tissues. Ng-CAM, a secondary adhesive molecule mediating interactions between neurons and glia, was shown to appear exclusively on early differentiating CNS and PNS neurons.

In vitro and _in vivo_, the adhesive properties of neural crest cells and their neural derivatives are modulated by mechanisms including prevalence polarity and chemical modification. The role of cell migration and cell adhesion in the patterning of the nervous system will be discussed.

Systems for The Study of Morphogenesis

1084 PATTERNING OF NEURONAL MAPS. Scott E. Fraser and Nancy A. O'Rourke, Department of Physiology and Biophysics, and Dev. Biology Center, University of California, Irvine, CA 92717.

The interconnections between neural centers in the vertebrate brain show a great deal of order, and are usually arranged in a topographic fashion. The process of forming such ordered projections can be thought of as two nested developmental processes: i. the patterning and organization of each neural center, and ii. the patterning of the interconnections between the centers. The retinotectal projection of lower vertebrates offers an excellent system for studying both of these interwoven patterning processes. The projection from the eye is topographically arranged to produce a "map" of the retina onto the surface of the optic tectum. Experimental evidence from several laboratories indicates that the cells of the eye and the optic tectum possess some positional cues that help to guide the pattern of connections made by optic nerve fibers in the tectum. The major questions that must be answered are: i. how do the cells gain this positional information, and ii. how do the cells use this positional information to guide their connection pattern?

To explore the patterning of the eye during embyonic development of the frog, Xenopus laevis, we have been exploring the interaction between cells following a surgical rearrangement of the eyebud. Small pieces of the embryonic eye rudiment can be grafted together to form eyes made up of an unusual set of cells. For some combinations of fragments, the maps formed by these recombinant eyes do not faithfully reflect the origin of the tissues used to make-up the eyebud. Instead, the map shows that some form of repatterning or regulation takes place. By preloading the cells of one of the eyebud fragments with a fluorescent dye, it is possible to examine the position of the grafted cells in the eyebud and termination pattern formed by these recombinant eyes in a living larvae. This technique is now being used to examine when and where the regulation of positional cues in the eyebud occurs.

To investigate the patterning of the projection between the eye and tectum, we have employed a combination of computer modeling and experimental techniques. The computer simulations have been used to refine a model for the patterning of the projection, and to show that the model is consistent with the vast majority of the experimental literature. This model is based on a set of adhesive interactions among the optic nerve fibers and between the optic nerve fibers and the tectum. In order to test for the role of cell adhesion in the patterning of the projection, we have employed antibodies raised against Xenopus N-CAM, the neuronal adhesion molecule (in collaboration with G.M.Edelman and colleagues). The antibody causes a distortion in the projection pattern and an increase in the disorder of the projection when applied to the tectal neuropil. The results indicate a role for cell adhesion in the patterning of the retinotectal projection and implicate N-CAM in the process of neuronal map formation.

1085 POSITIONAL MAPS AND CELLULAR INTERACTIONS IN INSECT DEVELOPMENT, Vernon French, Zoology Department, University of Edinburgh, Scotland, U.K.

The "positional information" approach to the process of pattern formation proposes that cellular interaction generates a map of positional values and the cells then individually interpret their value and differentiate accordingly. The nature of a "positional map" and the cellular and molecular mechanisms by which it is generated and interpreted are the central problems of pattern formation. It is widely assumed that positional maps are relatively simple and repeated through the organism. Hence insects may develop through division of the embryo into a number of differently determined segments within each of which the same cellular interactions occur to build the segmental map, which is then interpreted in the various segment-specific ways. This idea has received some support from grafting experiments and homeotic transformations.

Numerous grafting experiments (particularly on bugs) have suggested that the abdominal segment epidermis contains a simple map consisting of a linear anterior-posterior sequence of positional values, while work on the thoracic appendages (particularly of cockroaches and Drosophila) has suggested a very different two-dimensional map of positional values, arranged along polar co-ordinates, down and around the appendage. In both systems the map seems to direct pattern, polarity and, to some extent, cell division. These experiments, however, have not really addressed the relationships between the appendage and the rest of the thoracic segment from which it arises, between the thoracic and abdominal segments and between the dorsal and ventral parts of the segment. These relationships will be discussed in the light of recent grafting and extirpation experiments performed on the beetle, Tenebrio.

1086 Retroviruses as Probes for Mammalian Development

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Experimental insertion of the Moloney leukemia virus into the germ line has resulted in an embryonic recessive lethal mutation in Mov13 mice. Integration of the proviral genome occured at the 5' end of the α1(I) collagen gene blocking formation of stable mRNA. 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.

To study the mechanism of how insertion of the provirus may interfere with gene transcription, the chromatin structures of the mutated and the wild-type alleles were compared. Limited digestions with DNase I revealed that a hypersensitive site located at 100-200 bp 5' of the cap site was strictly correlated with collagen α1(I) transcription. Integration of the virus into the first intron prevents the developmental appearance of this site, and this may interfere with proper activation of the gene during embryonic development.

Embryos homozygous at the Mov13 locus are arrested in development between days 12 and 14 of gestation. This is the time when abundant transcription of the α1(I) collage gene starts suggesting an essential role of type I collagen for midgestation development. Histological examination of day 12 embryos revealed a general cell necrosis without obvious malformation. Cell necrosis typically begins in erythropoietic cells of the liver. The embryos die, however, due to a sudden breakdown of the circulatory system. This ascribes to collagen I an important function in establishing, or in the maintenance of the circulatory systems.

Organ rudiments from day 12 embryos have been explanted in organ culture. No difference of in vitro development of organs from homozygous,heterozygous, or wild-type embryos was observed, suggesting that collagen type I has no essential role in early morphogenesis.

Genetically Defined Systems

1087 CELL SURFACE MOLECULES EXPRESSED WITH POSITIONAL SPECIFICITY IN DROSOPHILA IMAGINAL DISCS Danny L. Brower, Departments of Molecular and Cellular Biology and Biochemistry, University of Arizona, Tucson, AZ 85721.

Pattern formation in Drosophila imaginal discs appears to depend on continuous intercellular communication. Because the disc epithelia are composed of essentially one cell type, cell surface components that are distributed nonuniformly in imaginal discs are good candidates for molecules involved in patterning or morphogenesis. Using monoclonal antibodies, we have been searching for such molecules, and at least three of the antigens so identified appear to be related to one another, based on their structures and distributions. Groups of cells specified to differentiate identical structures, but located in different regions of the epithelium, may display very different patterns of expression of these antigens; this Positional Specificity has led us to designate the three antigenic specificities PS 1-3. For example, in the epithelium that will produce the adult wing, the PS1 antigen primarily is found on the ventral cells, and the PS2 antigen on the dorsal cells; the PS3 antigen is found on both dorsal and ventral cells, but there is a band cells separating these domains that appears to contain reduced levels of the antigen. Generally, the distributions of the PS antigens appear to correlate with morphogenetic events in the disc epithelia. More specifically, morphogenetic events are observed where there are discontinuities in PS antigen expression. Large, sharp differences in antigen concentration are characterized by distinct morphological entities (e.g. the epithelial grooves along the nascent wing margin) and small or gradual variations are characteristic of more subtle or smoother morphological features (e.g. the forming wing pouch). An extensive analysis of the distributions of the PS antigens leads us to hypothesize that they are involved in cell-cell recognition and/or adhesion processes. Also, examination of binding patterns in flies of various genotypes has shown that the PS antibodies can be very useful as probes for developmental events in the undifferentiated imaginal discs.

Structurally, the PS antigens are oligomeric complexes of large (92-125 KD) glycoproteins. One or more members of this complex seem to be common to all of the PS groups; these common components are probably recognized by the PS3 antibodies. Other components of the complex appear to be modified to give the more localized PS1 and PS2 specificities. Work is currently underway to clone the structural genes for these glycoproteins, in order to proceed with a genetic analysis of their functions.

1088 MOLECULAR ANALYSIS OF *engrailed*; A GENE INVOLVED IN EARLY *DROSOPHILA* DEVELOPMENT, Patrick H. O'Farrell, Jerry M. Kuner, Tom Kornberg, Jim F. Theis, Judy A. Kassis, University of California, San Francisco, CA 94143

We are studying an early developmental step that divides the *Drosophila* embryo into the dominant pattern elements, the segments. Segments appear to be an obvious morphological manifestation of subdivision into smaller units - the developmental compartments. Each segment is composed of anterior and posterior compartments. these compartments are important because they behave as units of specification of developmental fate and units of growth control. We have cloned and are characterizing a gene, the *engrailed* gene, that is essential to the processes that establish a developmental compartment. The *engrailed* gene is thought to encode a regulator that is expressed in a precise spatial pattern that contributes to the establishment of the repeating array of compartments and segments.

Mutations defective in *engrailed* function have been physically mapped; disruptions throughout a 70 kb sequence give rise to the *engrailed* phenotypes (Kuner, Nakinishi, Ali, Drees, Gustavson, Theis, Kauvar, Kornberg, and O'Farrell, submitted). Despite the large size of the *engrailed* complementation group no large transcription units have been defined. Early *engrailed* function appears to be encoded in 2.7 kb RNA that is derived from a relatively small, 4.5 kb, genomic region (Drees, O'Farrell and Kornberg, in prep.). We have proposed that proper *engrailed* function requires extensive flanking sequence because these areas encode regulatory RNA's that act in cis to direct the processing of the nascent coding sequence (O'Farrell, in prep.). The coding region contains a long (1700 bp) open reading frame and includes a sequence resembling the conserved "homeobox" sequence that is found at other key developmental loci (Pool and Kornberg, submitted). *In situ* hybridization to RNA in tissue sections reveals that transcripts from the region are localized to narrow bands of cells corresponding to the posterior parts of the embryonic segments (Kornberg, Ali, O'Farrell and Siden-Kiamos, submitted). Comparison of the *engrailed* region of *D. melanogaster* to clones of the *engrailed* region of *D. virilis* reveals that conserved sequences are present throughout the 70 kb region (Kassis and O'Farrell, in prep.) and that the pattern of sequence conservation in the proposed coding sequence is unusual. We have constructed hybrid genes that express portions of the *engrailed* sequence as protein in *E. coli*. Antibodies raised against two of these fusion proteins detect a number of proteins in extracts of *Drosophila* analyzed by protein blotting. The probes we have developed should allow us to characterize the *engrailed* gene product, examine the basis for regulation of its expression and to investigate its interaction with the other developmental loci.

Histogenesis

1089 MATRIX REGULATION OF EPITHELIAL BEHAVIOR INVOLVES HEPARAN SULFATE-RICH PROTEOGLYCAN AS A CELL SURFACE RECEPTOR, M. Bernfield, A. Rapraeger, M.Jalkanen, J. Koda, and S. Banerjee, Pediatrics Department, Stanford University, Stanford, California, 94305.

The substratum of adherent cells, which in vivo is the extracellular matrix, modifies their shape and their ability to exhibit specific cell behaviors. Cells recognize this substratum with matrix receptors that may vary in type or amount depending on the nature of the cell substratum, cell type, and developmental stage. During branching epithelial morphogenesis, tissue form becomes stable where interstitial matrix accumulates and changes continuously where the matrix turns over most rapidly. Various epithelia form stable branched structures that resemble their in vivo configuration when cultured within type I collagen gels. Thus, morphologic stability may involve linkage of cell surface matrix receptors to insoluble matrix components.

We have studied the role of cell surface heparan sulfate-rich proteoglycan (PG) as a matrix receptor because heparan sulfates bind several matrix components. The cell surface PG on mouse mammary epithelial cells consists of at least two functional domains: (i) a membrane-intercalated domain which anchors the PG to the cell, and (ii) an ectodomain which bears both heparan sulfate and chondroitin sulfate chains. The PG behaves as an authentic integral membrane protein: it intercalates into liposomes, is displaced from the cell surface only by cleavage from its membrane domain, and is mobile in the plane of the membrane as shown by its aggregation into clusters by cross-linking antibodies. The ectodomain is released from the cell surface by mild protease treatment, yielding a soluble PG which bears all the glycosaminoglycan of the parent molecule and which binds interstitial collagen fibrils at a high affinity site that is specific for highly sulfated polyanions. Cell surface PG behaves identically because intact mammary epithelial cells duplicate the binding specificity shown by the ectodomain, type I collagen fibrils can immobilize the PG in clusters at the cell surface, and polyanions which inhibit the interaction prevent the cells from forming duct-like structures when cultured within collagen gels. The turnover of the PG, in turn, may depend on this type of interaction: in vitro, the PG is rapidly shed when the cells are rounded, but its degradation is markedly slowed when the cells are cultured on top of collagen gels. Thus, the matrix may immobilize the PG which anchors the cells to the matrix, thus stabilizing the morphology of the epithelial sheet. Cell surface heparan sulfate PG's, ubiquitous on adherent cells, may serve as cell-matrix anchors which transmit changes in the matrix into cell behavior.

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1090 THE BASEMENT MEMBRANE IN HISTOGENESIS, Peter Ekblom, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstrasse 37 D-7400 Federal Republic of Germany

Kidney differentiation is driven by interactions between three cell lineages, the nephrogenic mesenchyme, the epithelial ureter bud, and the endothelium. The mesenchyme is induced to differentiate by the ureter bud, and this in turn stimulates the migration of the endothelium into the developing tissue. Immunocytochemistry suggests that the mesenchyme in response to induction switches the matrix composition. Interstitial collagens are lost, whereas basement membrane(BM) components appear. That change preceeds morphogenesis. The stimulation of the mesenchyme also leads to an increase in cell proliferation, and to an acquisition of transferrin responsiveness. Thus, the matrix on which the cells differentiate is the BM, while the growth factor is transferrin.

BM components are also involved in the migration of the endothelium. This has been demonstrated in interspecies hybrid kidneys, which were constructed from endothelium of the quail, and from the epithelium of the mouse. Both these cells produce a BM, as judged by staining with species-specific antibodies against either mouse or quail type IV collagen. Staining with species-specific antibodies against fibronectin shows that epithelial podocytes do not produce any detectable fibronectin. It is noteworthy that the migrating endothelium at all stages produces a rather linear BM. Thus, the migrating endothelial cells do not apparently use the matrix of the target organ for migration.

1091 MORPHOGENESIS OF SKIN AND CUTANEOUS APPENDAGES: ROLE OF EXTRACELLULAR MATRIX, Philippe Sengel, Annick Mauger and Madeleine Kieny, UA CNRS 682, Department of Biology, Université scientifique & médicale de Grenoble, 38402 Saint-Martin-d'Hères, France

During the development of avian and mammalian skin and cutaneous appendages, several extracellular matrix (ECM) macromolecules, like interstitial collagen types I and III, fibronectin and glycosaminoglycans, exhibit a changing heterogeneous distribution (1, 2). Their synthesis, deposition and degradation are related to skin morphogenesis. Indeed, in zones of high morphogenetic activity, like outgrowing feather or scale buds, or ingrowing feather follicles, collagens are sparse or absent, while fibronectin is abundant. Contrariwise in histogenetically stabilized zones, such as inter-appendage or glabrous skin, fibronectin is rare, while collagen is dense.

In order to test whether the morphogenetic performance of skin cells is influenced by their environment, the effect of several ECM components on _in vitro_ cultured 7-day chick embryonic dermal cells was analyzed using homogeneous or heterogeneous substrates of type I collagen or fibronectin, as compared to polystyrene (3). For quantification of cell behavior a series of ten arbitrary stages of cell patterning was defined. Fibronectin had no effect on the rate of cell patterning, but collagen significantly retarded it. When fibronectin was deposited on the collagen substrate, the retarding effect of collagen was suppressed. Also, when a heterogeneous substrate was used, consisting of fibronectin coated areas surrounded by either collagen or polystyrene, the cells tended to align along the edge of the fibronectin spot. Cell spreading was greater on collagen than on either fibronectin or polystyrene, indicating that cell-substrate adhesion was strongest on collagen.

These preliminary results indicate that the cells' behavior is influenced in a significant way by the presence of ECM components in their environment. Collagen slows down the rate of cell patterning, which is in agreement with the hypothesis that interstitial collagens play a stabilizing role in morphogenesis. The fact that fibronectin is able to restore a "normal" rate of cell patterning and that the cells are able to sense its presence is likewise in agreement with the idea that fibronectin facilitates morphogenetic movements. Thus the microheterogeneous distribution of ECM macromolecules in skin might constitute at least part of the morphogenetic message that the dermis is known to transmit to the epidermis during the development of cutaneous appendages.

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The Nervous System

1092 RULES OF RETINOTECTAL MAPMAKING. Stephen S. Easter, Jr., Division of Biological Sciences, University of Michigan, Ann Arbor, MI 48109.

The highly ordered topographic map that relates the retinal positions of ganglion cell bodies and the tectal positions of their terminal arbors has been studied, mainly in fish and amphibia, for several decades. The vast majority of these terminals are added over a period of months to years after the initial establishment of contact by a relatively few pioneers. In the goldfish, the rules followed by the later axons are the following. A) In the retina, new ganglion cells originate only in the periphery, and send their axons along the most superficial route, directly beneath, and in contact with, the basal lamina of the inner limiting membrane (1). B) In the optic nerve, the new axons from the entire retinal periphery cluster together, such that their neighbors are from the same general retinal region (2,3). C) In the tract, the fibers rearrange to form two brachia, one from ventral hemiretina to dorsal hemitectum, the other from dorsal hemiretina to ventral hemitectum (2,4). D) In the tectum, the new axons grow along the outer edge and leave the bundle sequentially, temporal axons first, nasal last, to terminate retinotopically on the tectal periphery (5). E) As the tectum enlarges by adding new cells along its dorso-caudo-ventral edge, new generations of axons follow the same rules, but in an enlarged tectum, and the connections previously formed shift to new locations (6). The ordered and stereotyped pathway provides an efficient route for the axons to their proper termination sites, but axonal guidance by itself cannot account for the ability of regenerated axons to find their proper locations, since the regenerated nerve and tract are disordered, but the map is restored nonetheless. Relatively permanent markers must exist on the tectal surface, but it remains an open question whether or not these markers exist prior to the arrival of the axons.

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1093 NEURAL CELL ADHESION MOLECULE (N-CAM) IS PRESENT AT THE SITES OF NERVE-MUSCLE CONTACT IN THE DEVELOPING AND THE ADULT VERTEBRATE NEUROMUSCULAR SYSTEM. François Rieger, *Martin Grumet, Martine Pinçon-Raymond, and *Gerald M. Edelman, *The Rockefeller University, 1230 York Avenue, New York, New York, 10021; Groupe de Biologie & Pathologie Neuromusculaires INSERM U.153, 17, rue du Fer-á-Moulin 75005 Paris.

Immunoreactivity with specific monoclonal and polyclonal antibodies to the neural cell adhesion molecule (N-CAM) has been detected on adult mouse diaphragm muscle fibers at the nerve-muscle contacts by indirect immunofluorescent techniques. Specific N-CAM staining was mostly coextensive with α-bungarotoxin (α-BgTX), which stains specifically the acetylcholine receptor (AChR) accumulated at the motor endplate. In double label experiments using fluorescein-αBgTX and an indirect rhodamine label for anti-N-CAM IgG, only the gutter-like structures of the motor endplate stained with α-BgTX, whereas both these structures and the nerve terminal arborizations showed strong staining for N-CAM. Similar experiments were performed on frog and chick adult muscles. In the developing mouse muscle, just before or after birth, a similar staining pattern for N-CAM and AChR was also found at sites of nerve-muscle contacts, with additional N-CAM staining on axons, axon terminals, and myofiber surfaces. Qualitative and quantitative biochemical studies using SDS gel electrophoresis followed by immunoblotting with anti-N-CAM IgG showed that detergent extracts of muscle contained a major immunoreactive component of Mr 140,000. This form of N-CAM was present even in the embryonic muscle, in contrast to N-CAM in nerve which displays a heterogeneous embryonic form (Mr 180,000 to 250,000) and three adult forms (Mr 180,000, 140,000 and 120,000). The Mr 140,000 component from muscle, unlike N-CAM from nerve, was not detected by monoclonal antibody anti-N-CAM No. 5 which recognizes N-linked oligosaccharide determinants. These results suggest differences between N-CAM in nerve and muscle, particularly in their carbohydrate structures. We also found a significant decrease of N-CAM concentration in muscle during postnatal development. In the mouse, a spontaneous recessive mutation, muscular dysgenesis (mdg), affects the nerve-muscle interactions in mutant embryo resulting in nerve overgrowth, multifocal polyinnervation and immature myotubes. Comparison of normal and mutant embryo muscles for their content of N-CAM and its localization in nerve and muscle showed that N-CAM is significantly increased in mdg/mdg diaphragms with increased muscle surface staining and multiple intense spots, demonstrating N-CAM accumulations all over the mutant muscle. Thus, muscular dysgenesis offers a natural genetic perturbation of N-CAM localization and content in muscle during the early development of the neuromuscular system.

1094 SELECTIVE STABILIZATION OF RETINOTECTAL SYNAPSES BY AN ACTIVITY DEPENDENT MECHANISM,
John T. Schmidt, Dept. Biological Sci., State Univ. of New York at Albany, NY 12222.

During regeneration of the optic nerve in adult goldfish, the ingrowing retinal fibers successfully seek out their correct places in the overall retinotopic projection on the tectum, as assessed by electrophysiological recording. Chemospecific cell surface interactions (1) alone are sufficient to organize only a diffusely retinotopic map; the precise retinotopic ordering is achieved via an activity dependent stabilization of appropriate synapses. Four treatments block this sharpening process: A) Blocking activity of the ganglion cells with intraocular tetrodotoxin (TTX, 2), B) Rearing in total darkness (3), C) Correlated activation of all ganglion cells via stroboscopic illumination (3), D) Block of retinotectal synaptic transmission with alpha-Bungarotoxin (unpub. data). These experiments show that it is not just activity per se, but locally correlated activity in neighboring ganglion cells of the same type (4) that drives the sharpening of the diffuse projection, and further suggest that the correlated activity interacts within the postsynaptic cells, probably through the summation of EPSP's and the stabilization of the most effective (retinotopic) synapses.

In order to study the morphological events during the sharpening, regenerated and normal optic arbors were stained via anterograde transport of HRP and viewed in tectal whole mounts. Normal optic arbors range in size from 100 to 400 μm across. Individual regenerated arbors at early stages (2 to 4 weeks postcrush) were up to 5 times larger than normal in extent although more sparsely branched. Many were more than 2 mm across and covered more than 2/3 of the tectal extent. These could account for the early diffuse projection. By 2 months, few of the enlarged arbors were found, and by 6 months, all were of normal size again. Thus, many, if not all, optic fibers initially form enlarged arbors. The elimination of retinotopically inappropriate branches of these arbors may be the method by which the map is sharpened using activity.

Intraocular TTX experiments suggest a similar mechanism for the formation of ocular dominance patches in fish tectum (5) and kitten visual cortex (6), and for the segregation of different receptive field types in the lateral geniculate nucleus (7). Activity dependent stabilization may therefore be a general mechanism whereby the diffuse projections of early development are brought to the precise, mature level of organization.

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Theory, Evolutionary Implications and Summation

1095 MOTILITY AND SHAPE CHANGE IN EMBRYONIC CELLS, **George F. Oster, Department of Biophysics, University of California, Berkeley, CA 94720. Garrett M. Odell, Department of Mathematics, Rensellaer Polytechnic Institute, Troy, NY 12181.**

Embryonic cells are motile, changing their shapes and/or crawling about and aggregating in patterns that establish subsequent tissue geometries. While it is clear that the cellular cytoskeleton is the 'motor' for cell motility, the exact mechanism by which cells propel themselves has remained obscure. We shall present a mechanochemical model for cell motion based on the physical chemistry of actomyosin gels. The model accounts for a variety of motility phenomena, and suggests a general mechanism for shaping tissue geometries in both mesenchymal and epithelial cell populations.

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1096 PATTERN FORMATION AND POSITIONAL INFORMATION. Lewis Wolpert, Department of Anatomy and Biology as Applied to Medicine, The Middlesex Hospital Medical School, Cleveland Street, London W1P 6DB, UK.

Positional information can provide the basis for generating patterns of cellular differentiation. This will be assessed with respect to limb morphogenesis, and particularly in relation to mechanisms that might generate a prepattern such as reaction-diffusion. The concept of positional value will be considered as a cell parameter and related to both limb morphogenesis and pigment pattern formation in birds. It will be suggested that there is quite a fine grained assignment of positional value. The problem of how this might be implemented and interpreted will be discussed, in relation to recent advances in the understanding of homeotic genes in insects.

Poster Session

1097 TRANSIENT EXPRESSION OF TISSUE-SPECIFIC ANTIGENS IN THE XENOPUS LAEVIS EMBRYO, Rebecca M. Akers, Carey R. Phillips, and Norman K. Wessells, Stanford University, Stanford, CA 94305 and University of California, Berkeley, CA 94720

The distribution of tissue-specific antigens was studied in *Xenopus laevis* embryos with monoclonal antibodies. Antibodies were generated against a membrane preparation isolated from late neurula stage embryos (Nieuwkoop and Faber, st. 19-22). Several antibodies were found which recognize antigens expressed during restricted periods of embryonic development. Two of these antibodies demonstrated interesting patterns of tissue specificity. ENDO 1 recognizes a subset of large yolk platelets localized in the vegetal hemisphere of blastula stage embryos; after gastrulation this antigen is restricted to platelets found in the trunk endoderm. This antigen is associated with the yolk platelet membrane and disappears from the endoderm during the late tailbud stages. A second antibody, DORSAL 1, selectively stains dorsal axial structures (notochord, somites, and neural tube) in the neurula stage embryo. The DORSAL 1 antigen first appears in the embryo at the end of gastrulation (st. 13) and persists in the dorsal structures through the completion of neural tube closure. After that time, it progressively disappears from the neural tube and notochord but is maintained in the somites through the tailbud stages; during this time it is selectively localized at the boundaries of individual myotomes. There is no evidence for expression of this antigen in mature muscle or nervous system tissue. We are currently investigating the molecular identity, subcellular localization, and functional roles of these transiently-expressed antigens. Supported by NIH grant HD 04708 and NSF grant PCM 83-092621.

1098 MANY MUSCLE CELL TYPES EXPRESS SOME FORMS OF N-CAM, Richard A. Akeson, Children's Hospital Research Foundation, Cincinnati, OH 45229

N-CAM's are a family of glycoproteins previously reported to be on the surfaces of vertebrate nerve and skeletal muscle cells (Edelman, G.M., Ann. Rev. Neurosci. 7:339-377, 1984). We have examined clonal muscle cell lines to determine which muscle phenotypes express N-CAM's. Both rabbit and monoclonal anti-N-CAM's bound to rat L6 skeletal myoblasts, A7r5 and A10 smooth muscle cells and H9c2 cardiac myocytes. Rabbit anti-N-CAM also bound to mouse BC_3H1 smooth muscle cells. These lines all expressed more than $2x10^5$ monoclonal anti-N-CAM binding sites per cell indicating N-CAM is a relatively high abundance surface protein. To determine the molecular forms of N-CAM expressed by muscle cells, cultures were lactoperoxidase iodinated, solubilized, and N-CAM's immunoprecipitated. L6, A7r5, BC_3H1 and H9c2 cells all expressed only the 140 and 120 Kd forms. Little if any 180 Kd or higher molecular weight forms were immunoprecipitated. No N-CAM was found on fibroblast cells, another mesodermal derivative. Immunofluorescence analysis indicated both L6 myoblasts and postfusion myotubes expressed N-CAM. There was no obvious differential distribution of N-CAM on the myotube surface. These results further indicate that individual cell types express only selected molecular weight forms of N-CAM.

1099 ANALYSIS OF FIBRONECTIN RECEPTOR FUNCTION USING A DIRECT BINDING ASSAY AND FIBRONECTIN FRAGMENTS, Steven K. Akiyama and Kenneth M. Yamada, Membrane Biochemistry Section, Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205

Fibronectin is an adhesive glycoprotein which plays crucial roles in developmental and biological processes. In order to understand fibronectin-mediated events on a molecular level, and to identify possible candidates for cell-surface fibronectin receptors, we developed a direct binding assay to measure the parameters which define the interaction of soluble fibronectin with cells using BHK cells adapted for growth in suspension as a model system. The binding of intact fibronectin is of moderate affinity (K_d=0.8 μM), consistent with a mechanism involving the repeated making and breaking of contacts as presumably would be the case during cell migration on a fibronectin-coated substrate. A purified 75 kDa cell-binding domain of fibronectin binds with slightly higher affinity (K_d=0.4 μM) suggesting a slight proteolytic activation of this fragment. A smaller 11.5 kDa fragment of fibronectin which interacts with cells has been identified and isolated using a monoclonal antibody. This fragment binds with low affinity, as judged by inhibition assays. We have begun to characterize the cell-surface fibronectin receptor(s) using these fragments and our binding assay. Our results apparently rule out proteoglycans as possible fibronectin receptors and suggest that the receptor function is dependent on a protein component which is sensitive to physiological concentrations of divalent cations.

S.K.A. was partially supported by N.I.H. grant #CA 06782, PH.S., D.H.H.S.

1100 AGGREGATION CHIMAERAS IN CAPILLARY TUBES.AN EFFICIENT NEW TECHNIQUE FOR CLONAL ANALYSIS OF DEVELOPMENT IN MAMMALS.Juan Arechaga,Robert Wells and G.Barry Pierce.University of Granada (SPAIN) and University of Colorado Health Sciences Center,Denver,CO 80262(USA)

The introduction of chimaeric technology has made possible the study of the fate and differentiation of cell populations of different genotypes into mammalian embryos.The methods currently available are: cell injection by micromanipulation and aggregation,gently placing cells and/or embryos together in a microdrop of culture medium under paraffin oil,with or without special chambers and using phytohaemagglutinin as an aggregation agent.However,all these procedures are complicated,tedious and typically of low yield.We have developed a very simple technique that permits the production of hundreds of chimaeric aggregations per week by a single person.The method comprise:(a)digestion of "zona pellucida" with 0.2% pronase in 0.5% PVP-40 in PBS,(b)Aggregation and culture in a few microliters of MEM plus 10% of FCS supplemented with Sodium Piruvate and Glutamine,utilizing only the force of gravity as the cohesive agent.Experiments in progress include capillary aggregation cultures of fluorescent labeled teratocarcinoma cells and normal mouse embryos by this technique.(picture: aggregation between CD-1 and C57BJ/6 two days mouse embryos-vaginal plug=day 0)

1101 LOCALIZATION OF NERVE TERMINAL-SPECIFIC ANTIGENS AT NEUROMUSCULAR SYNAPSES *IN VITRO*, John L. Bixby and Louis F. Reichardt, U.C.S.F., San Francisco, CA 94143

The biochemical events underlying the induction of differentiation in motor nerve terminals are still largely a mystery. In order to examine these events, we have developed an assay system employing antibodies which recognize two molecules specific to nerve terminals, synapsin I and p65. These molecules are associated with synaptic vesicles at mature synapses (De Camilli *et al.*, J. Cell Biol. 96, 1983; Matthew *et al.*, J. Cell Biol. 91, 1981). When dissociated chick ciliary ganglia are plated onto cultures of embryonic chick myotubes, both of these molecules become concentrated at sites of nerve-muscle contact over a period of 4-5 days. By 5 days *in vitro*, clusters of these molecules are found apposed to patches of Ach receptors at ~25% of nerve-muscle contacts. This is in the range of the fraction of contacts which result in functional synapses in such cultures (Betz, J. Physiol. 254, 1976). Since N-CAM has been reported to be involved in initial neuromuscular interactions (Rutishauser *et al.*, J. Cell Biol. 97, 1983), we have begun to examine the role of N-CAM in this system. Preliminary results indicate that Fab fragments of polyclonal antibodies to N-CAM disrupt the formation of early nerve-muscle contacts, and slow down subsequent co-localization of vesicle antigens and Ach receptors, but do not prevent this differentiation entirely.

1102 THALIDOMIDE METABOLITE INTERFERES WITH LECTIN MEDIATED ATTACHMENT, Andrew G. Braun, Fiona A. Harding and Michael Collins, Massachusetts Institute of Technology, Cambridge, MA 02139

A large class of chemical teratogens inhibits acites tumor cell attachment to lectin coated plastic surfaces. To assay for inhibitory activity radiolabeled cells are treated with a test chemical, poured over concanavalin A coated polyethylene disks and allowed to attach for 20 minutes. The extent of attachment is determined by measuring the radioactivity adherent to the disks. Incubation with thalidomide does not inhibit attachment. However inclusion of rabbit hepatic microsomes, an NADPH generating system and FMN results in a dose dependent, time dependent and temperature dependent inhibition. Microsomes from human placenta are also active in the system. Omission of microsomes, NADPH or thalidomide eliminates attachment inhibition. FMN (0.2mM) increases the degree of inhibition. CO, metyrapone, N-octylamine, SKF 525A and alpha-naphthaoflavone prevent activation. These results suggest a thalidomide metabolite inhibits attachment. It is, therefore, possible this metabolite also interferes with normal embryonic cell-cell interactions and is the cause of thalidomide mediated teratogenesis.

Supported by Department of Energy Contract DEACO 282-ER-60070.

1103 TRANS-SPECIES EXPRESSION OF CLONED GENES, Francois C. Bregegere, Stanford University, Stanford, CA 94305

Genetic transformation of Drosophila melanogaster by injecting DNA into preblastoderm embryos provides new means to assay for functional divergence between genes from different insect species. Not only can one try to complement Drosophila mutations by grafting foreign DNA sequences in Drosophila genome, but one can also determine which structural features of the genes are involved in their possibly different phenotypic expression.

We have constructed a gene library of Calliphora erythrocephala (European flesh fly) and have isolated a DNA segment homologous to the coding sequence of the rosy (ry) gene of Drosophila melanogaster, which codes for the xanthine-dehydrogenase (XDH) and is involved in the eye pigment synthesis. Inserting the Calliphora XDH gene in ry^- Drosophila genome would provide a good model system for this kind of studies on the functional evolution of genes.

1104 REVERSAL OF A DEVELOPMENTAL RESTRICTION IN NEURAL CREST-DERIVED CELLS OF AVIAN EMBRYOS BY THE TUMOR PROMOTER 12-O-TETRADECANOYL PHORBOL-13-ACETATE (TPA), Gary Ciment and James A. Weston, University of Oregon, Eugene, Oregon 97403

The neural crest of early vertebrate embryos gives rise to a variety of different cell types in the adult, including neurons and glial cells of the dorsal root ganglia (DRG) and pigment cells. Earlier studies of Nichols and Weston (1977) showed that 5 day embryonic chicken DRG contain cells which undergo adventitious pigmentation in culture, but that DRG from older embryos do not, suggesting that the developmental restriction of melanogenesis in this early crest derivative occurs after the 5th day. We have reexamined this phenomenon in quail DRG cultures using the tumor promoter TPA. We have found that this drug dramatically extends the developmental period in which adventitious pigmentation in DRG cultures can be produced. This drug effect is dose- and stage-dependent and also occurs with other crest derivatives. In addition, when TPA-treated DRG explants from quail embryos are grafted into 4-day old chicken embryos, the grafted cells not only give rise to pigment cells, but display other appropriate morphogenetic behaviors of normal pigment cells. We interpret these results to mean that TPA can reverse the developmental restriction of melanogenesis that occurs in at least some of the crest-derived cells of the early DRG.

1105 INFLUENCE OF SYMPATHETIC NERVES ON DEVELOPMENT OF β-ADRENERGIC RECEPTORS IN SALIVARY GLANDS, Leslie S. Cutler, University of Connecticut Health Center, Farmington, CT 06032

During the development of the rat submandibular gland (SMG) there is a well defined sequence in the morphologic and functional maturation of the acinar secretory cells. There is a temporal correlation with regard to the ingrowth of catecholamine containing nerves, the appearance of β-adrenergic receptors on the secretory cell surface and the functional coupling of the stimulus-secretion system, all of which occur at 6 days after birth. This temporal correlation suggested the possibility of a cause and effect relationship between the appearance of the catecholamine containing nerves in the gland and the maturational increase in the number and the coupling of β-adrenergic receptors. This study utilized chemical sympathectomy and chronic isoproterenol treatment to investigate the role of the nerves on the development of the β-adrenergic receptors in the neonatal rat SMG. Chronic isoproterenol treatment resulted in accelerated maturation of the gland with a concomitant premature appearance of the beta receptors. However, chemical sympathectomy did not effect the time of appearance or the number of β-adrenergic receptors seen in the developing gland. Taken together, these data suggest that the increase in the number of β-adrenergic receptors which normally occurs in the developing gland at 6 days after birth is a specifically programmed step which is closely associated with the degree of maturation attained by the cells and is independent from the ingrowth of catecholamine containing nerve processes.

1106 USE OF GENE FUSION TO ANALYZE "ENGRAILED" GENE PRODUCTS.
Claude DESPLAN,Jerry KUNER,Jim THEIS,Judy KASSIS,Liz SHER,Emily LIM,Patrick O'FARRELL.

Segmentation and compartimentalisation in Drosophila are controlled by genetically defined genes,a number of which have already been cloned.Several of these genes contain an evolutiommary conserved coding sequence,the"Homeobox".
The "Engrailed" gene is involved in the development of the posterior part of each segment. This gene has been cloned and appears to express a major transcript of 2.7kb.A 2.4kb cDNA has been isolated and could encode a 566 aminoacid "Engrailed" protein.This putative proteir contains several special features:it contains a distantly related "Homeobox" sequence at the C-terminus;the N-terminal region contains a 13 *Alanine* repeat as well as a 11 *Glutamine* repeat.
In order to analyze this protein biochemichally and developmentally,we produced LacZ fusion proteins from expression vectors in E.Coli(pUR 290).Fusion proteins containing the full lenght putative "Engrailed" product and specific sub-domains of the protein(i.e."Homeobox", repeated sequences.)have been produced to raise antibodies.
Since the cDNA also contains an other long open reading frame on the other strand (1354bp) we also produced a fusion protein from this frame to test for the presence of this hypothetical protein in Drosophila.
These fusion protein and the antibodies raised against them will be a powerfull tool to analyse the "Engrailed" gene products during embryogenesis.

1107 **DETERMINATION OF IDENTIFIED NEURONAL PRECURSOR CELLS IN INSECT EMBRYOS**
Chris Q. Doe and Corey S. Goodman, Stanford Univ., Stanford, CA 94305
The insect central nervous system (CNS) is composed of a brain and a chain of segmental ganglia; each hemi-ganglion contains about 1000 individually identifiable neurons. The neurons of a hemi-ganglion develop from a stereotyped pattern of 30 unique neuronal precursor cells, called neuroblasts (NBs). Neurogenesis involves two major steps: first, an undifferentiated ectodermal cell sheet produces the stereotyped pattern of NBs; second, each NB contributes a specific family of neuronal progeny to the developing CNS. We have used a laser microbeam to ablate individual cells in the grasshopper embyro in order to study the early events of neuronal determination: how does a layer of ectodermal cells produce a highly stereotyped pattern of unique NBs? Our results suggest the following mechanism for NB determination. (1) Cell interactions between the ~150 equivalent ectodermal cells of a hemi-segment allow 30 cells to enlarge into NBs. (2) As these young NBs enlarge they inhibit the adjacent ectodermal cells from becoming NBs; the adjacent cells differentiate into non-neuronal support cells. (3) Each NB is assigned a unique identity due to its position of enlargement within the neuroepithelium. (4) The NB then generates its characteristic family of neurons by an invariant cell lineage. Development of the insect CNS depends on cell interactions and positional cues to create a pattern of NBs, and then on cell lineage to restrict the fate of the NB progeny.

1108 RESTRICTION OF COLONY FORMING POTENTIAL OF EARLY CHICK EMBRYONIC CELLS AS DEVELOPMENT PROGRESSES. Lena Einhorn and Eduardo Mitrani The Hebrew University of Jerusalem, Embryology section, Department of Zoology, Jerusalem 91904, Israel.

Early chick embryonic cells, prior to the formation of the primitive streak, have been shown to form colonies when cultured in a two layer soft-agarose system (Mitrani 1984). The present work is an attempt to determine at which stages of early chick embryonic development this ability is expressed, and also which areas of the chick embryo harbour the colony forming cells. We found that the capacity of early chick embryonic cells to form colonies in agarose decreases as development progresses. For a given developmental stage the capacity to form colonies, if found, is concentrated in the peripheral areas of the embryo and decreases towards the centre. With the onset of hypoblast formation only cells from area opaca and,to a lesser degree, the marginal zone, can form colonies in agarose. At post-primitive streak stages only extra-embryonic cells can form colonies in agarose. By stage 10 (48 hrs incubation) all cells of the chick blastoderm seem to have lost the capacity to form colonies in agarose.

1109 STRUCTURE OF THE MOUSE EGG'S EXTRACELLULAR COAT, Jeffrey M. Greve and Paul M. Wassarman, Dept. Biol. Chem., Harvard Medical School, Boston, MA 02115
The zona pellucida is a thick extracellular coat that surrounds mammalian eggs and embryos and plays important roles during both fertilization and preimplantation development. The mouse egg's zona pellucida is composed of three different glycoproteins, designated ZP1 (200 kD; a disulfide-linked dimer), ZP2 (120 kD), and ZP3 (83 kD; the "sperm receptor"), that together account for virtually all of its mass. The three glycoproteins are synthesized by oocytes, secreted, and assembled into the extracellular coat during oocyte growth. Biochemical and electron microscopic analyses of solubilized material have revealed that the mouse egg's zona pellucida consists of a three-dimensional array of interconnected glycoprotein filaments. Each filament resembles "beads-on-a-string", with beads (approx. 9 nm in diameter) spaced at regular intervals (approx. 17 nm) along the filament axis. Proteolysis of ZP1 or reduction of intermolecular disulfides of ZP1 results in both solubilization of zonae pellucidae and disruption of interconnections between individual zona pellucida filaments. These observations strongly suggest that ZP1 is involved in crosslinking of filaments. Furthermore, results of both chemical crosslinking and immuno-electron microscopic studies (using monoclonal antibodies directed against each of the zona pellucida glycoproteins) have provided additional information about the relative arrangement of ZP1, ZP2, and ZP3 in the matrix. All of this information will be incorporated into a three-dimensional model of the mouse egg's extracellular coat.
Supported in part by the National Institute of Child Health and Human Development (HD-12275).

1110 CLONAL ORIGINS OF CELLS IN THE PIGMENTED RETINA OF THE ZEBRAFISH EYE, David Jonah Grunwald, George Streisinger, and Charline Walker, University of Oregon, Eugene, OR 97403
Mosaic analysis has been used to study the clonal origins of cells of the pigmented retina of zebrafish. Zebrafish embryos heterozygous for a recessive mutation at the gol-1 locus were exposed to irradiation at various developmental stages to create mosaic individuals consisting of pigmented cells and a clone of pigmentless (golden) cells in the eye. The number of cells that give rise to progeny that participate in the pigmented retina increases exponentially during very early cleavage indicating that until the 32-cell stage almost every blastomere will have descendants that contribute to the formation of the pigmented retina of the 76 hr postfertilization zebrafish larva. During subsequent cell divisions up to the several thousand cell stage a constant number of cells are present, on the order of 35 to 70, that will give rise to progeny in the pigmented retina. These experiments indicate that there is extensive intermixing between families of clonally distinct cells during zebrafish embryogenesis. Our experiments do not reveal at what developmental time the approximately 50 cells or their descendants are instructed to form the pigmented retina.

1111 AXON PATTERN FORMATION IN THE DEVELOPING AVIAN RETINA Willi Halfer, Max-Plank-Institut fur Entwicklungsbiologie Spemannstr 35/11, D 7400 Tubingen F.R.G.
In order to gain more information about the rules governing the navigation of nerve fibers in the embryonic central nervous system, I studied the growth of axons in the avian embryonic retina. Silver staining of retinae from chick, quail, and pigeon embryos of successive stages of development shows that the first ganglion cells appear at stage 16 (H+H) in the dorso-central part of the retina. The optic fiber layer expands with time and eventually covers the entire retinal surfaceat E6 (stage 29) in chick and pigeon and at E5 (stage 28) in quail. Due to an uneven dorso-to-ventral polarized growth of the entire retina, the optic disc (which is the convergence point of all axons) shifts during development from an initially ventral position coming to lie close to the geometrical center of the retina. The expansion of the optic fiber layer also proceeds asymmetrically from dorsal to ventral and from temporal to nasal. The asymmetry of the growth of the optic fiber layer in combination with the changing relative position of the optic disc results inaxons from different retinal segments having different growth directions toward the optic disc. Quantitative measurements show that the initial polarity of axons is not random, rather from the very beginning axons extend in the direction of thed optic disc. This early polarity of axons was found even on cells distant from the next ganglion cell or axons, thereby excluding axon-axon interaction or axon-ganglion cell contact as being essential for initial fiber direction.

1112 STRUCTURALLY ALTERED CELL ADHESION MOLECULE ON HEPATOCELLULAR CARCINOMA CELLS,
Douglas C. Hixson & Kerry D. McEntire, U.T. Science Park-Research Div., Smithville, Texas, 78957.

In an effort to determine the molecular basis for the aberrant behavior of malignant cells, we have used polyclonal and monoclonal antibodies to delineate changes in the expression of cell adhesion molecules during hepatocarcinogenesis. To date, we have demonstrated by immunoprecipitation analysis that the expression of two 105 kd acidic glycoproteins involved in cell-cell adhesion of hepatocytes (cell-CAM 105) has been altered on 12 different transplantable hepatocellular carcinomas (THC). This was suggested by the absence of these glycoproteins in material immunoprecipitated from extracts of ^{125}I-labeled THC cells with anti gp105-2, a heteroantisera known to be reactive with cell-CAM 105 and capable of inhibiting reaggregation of rat hepatocytes. Further analysis with monoclonal antibodies (MAb) specific for cell-CAM 105 revealed that on some THC, cell-CAM 105 was no longer expressed while on others, it was still present but had been structurally altered in a manner which significantly changed its mobility (pI and/or Mr) on two-dimensional gels. Comparison of peptide maps prepared following digestion with V-8 protease showed that MAb reactive components from normal hepatocytes and THC cells displayed strong structural homology, thus suggesting that the altered mobility of cell-CAM 105 on THC cells does not involve major differences in the peptide moieties. Supported by NIH grant CA 31103.

1113 ROLE OF EMBRYONIC GLYCANS IN CELLULAR RECOGNITION,
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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 that are transient in nature. We are exploring the role played by these carbohydrates in cellular recognition during early embryogenesis. Embryonal carcinoma cells in culture demonstrate the ability to adsorb and recognize carbohydrates prepared from other embryonal carcinoma cells. We have characterized these carbohydrates and on the basis of these studies, we have selected embryonal carcinoma variants with altered carbohydrate expression. Variant embryonal carcinoma cells with a decreased level of expression reaggregate from single cell dispersion slowly and the cellular aggregates are very easily disrupted. Other variants with a very high level of expression reaggregate rapidly and have unusually tight cellular interactions. We are currently exploring the cellular interactions that regulate the expression of these carbohydrates, and, in complementary studies, we are exploring the roles played by these carbohydrates during normal development.

1114 FIBROUS ECM ON THE INNER ROOF OF THE BLASTOCOEL OF FROG GASTRULAE.
Kurt E. Johnson, George Washington University Medical Center, Washington, DC 20037

Reciprocal exchange transplants were performed using the roof of the blastocoel of Stage 10 (early gastrula) R. pipiens normal embryos and arrested hybrid embryos (R. pipiens X R. catesbeiana). After a 5 h period of subsequent development, recombinant embryos were fixed and dissected. Specimens were then prepared for scanning electron microscopy. In normal hosts receiving a hybrid roof of the blastocoel graft, gastrulation appeared to be unaltered. The hybrid grafts had large amounts of fibrous extracellular matrix (F-ECM) and normal host migrating mesodermal cells attached to the inner surface of the roof of the blastocoel. In hybrid hosts receiving a normal roof of the blastocoel graft, gastrulation did not occur. The normal grafts had little or no F-ECM and no attached hybrid host migrating mesodermal cells on the inner surface of the roof of the blastocoel. In sham operated normal controls, gastrulation happened normally and the inner surface of the roof of the blastocoel had a large amount of F-ECM and many migrating mesodermal cells attached. Hybrid sham operated controls showed no gastrulation and had neither F-ECM nor migrating mesodermal cells present on the inner surface of the roof of the blastocoel. These results suggest that the F-ECM components are not synthesized by the roof of the blastocoel epithelium but elsewhere in the embryo and that hybrid cells have the ability to bind F-ECM components synthesized elsewhere in normal host embryos. This research was supported by National Science Foundation Grant PCM-8400256 to Dr. Kurt E. Johnson.

1115 METASTATIC PATTERNS OF EMBRYONAL CARCINOMA CELLS, Brenda W. Kahan, University of Wisconsin, Madison, WI 53706

Embryonal carcinoma cells from an ovarian teratocarcinoma produce highly specifically localized tumors in mice in unusual sites. The distribution of these sites depends on the route of injection. Following intraperitoneal injection of the cells, nearly exclusively ovarian tumors are formed. Cells adhere preferentially to the surface of the whole target organ in vitro and then invade the germinal epithelium. After intracardiac injection, the cells distribute themselves throughout the body in numbers closely proportional to the percent cardiac output to each organ. Following their general arrest, however, tumors develop exclusively in the gonads of both sexes and in the adrenals, eyes and whisker area. This localization pattern suggests that factor(s) determining embryonal cell tumor localization are related to those involved in normal germ cell and selected neuronal cell migrations and/or survival. It is possible to obtain embryonal cell migration in vitro in response to tissue from these sites.

1116 EMBRYONIC LINEAGES AND MIGRATIONS OF CELLS DURING EPIBOLY AND AXIS FORMATION IN THE ZEBRAFISH, Charles B. Kimmel and Rachel Warga, University of Oregon, Eugene, OR 97403. We have microinjected single cells at blastula stages with a fluorescent lineage tracer molecule, and subsequently tracked in vivo the lineages and morphogenetic movements of the labeled cells during the time the primary organs form. Clonally related cells originating from a single blastomere are later dispersed in the embryo, and contribute to several tissues. The dispersion is a consequence of a complex pattern of cell migrations that begin during epiboly. The cells appear to move actively, as individuals, and neighboring cells often spread apart from one another. We observe restricted cell fates, usually to a single tissue, within individual sublineages that originate after the spreading movements have begun. These restrictions could mean, but do not show, that cellular determinations have occurred at this early stage of development.

1117 MECHANISMS THAT RESTRICT THE MINGLING OF XENOPUS BLASTOMERES, Steven L. Klein, Univ. Utah Sch. Med., Salt Lake City, UT 84112.
At the 512-cell stage, *Xenopus* blastomeres are segregated into groups (Ancestral Cell Groups; ACG's) that are each ancestral to a specific body region (Jacobson, '83. J. Neurosci.3:1019). Before and during gastrulation the progeny derived from a single ACG usually intermingle but, progeny derived from different ACG's do not mingle. The orientation of cell movements during gastrulation may result, in part, from the absence of mingling between the cells of different ACG's. Although a variety of mechanisms that guide cells to their destination probably occur only in the presence of the concerted movements of cells in the intact embryo, the mechanism that restricts the mingling of cells of different ACG's may involve only interactions between different ACG's. To test this hypothesis the interactions between cells of different ACG's were examined in explants. An HRP labeled ACG was placed in contact with either the same or a different unlabeled ACG. In these combinations, as in vivo, the progeny of different ACG's did not mingle whereas the progeny of the same ACG usually intermixed. Thus, the mingling restriction observed in vivo may result from an interaction between the cells of different ACG's that does not require the gastrulation movements of the intact embryo. To identify the nature of this interaction, the behavior of an individual labeled cell was studied following its implantation into an unlabeled ACG. The individual cell produced fewer progeny when it was among cells of a different ACG than when it was among cells of its own ACG, suggesting that, in vitro, cell division may be inhibited by cells of a different ACG. Thus, the lack of mixing between ACG's during gastrulation may result from an interaction between ACG's that is manifested in vitro as an inhibition of cell division.

1118 NEURONAL MEMBRANE FRAGMENTS DIRECTING NEURITE GROWTH, Alphonse Krystosek, Univ. Colorado Health Sciences Center, Denver, CO 80262

Differentiation of the neural hybrid cell line NG108 was used to study the cues directing neurite growth. NG108 cells stochastically exhibit neurite formation or proliferation upon culture in a defined (N2) medium (Krystosek, submitted for publication). Some cells sequentially formed neurites and later divided. Neurite retraction occurred during this process and the daughter cells had a high probability to re-extend neurites. Cell divisions for which neurite retraction left growth cone fragments were followed photomicroscopically. Forty-seven per cent of such divisions yielded a progeny cell which initially re-extended a neurite projecting within 15° of the parental neurite track. Furthermore, elongating neurites had a high probability to correct the angle of their growth toward the source of the membrane material, often resulting in contact with such growth cones. Directed regrowth occurred both when the division plane was parallel or perpendicular to the parental fiber path. Cells which divided and did not leave behind neuritic material visible at the light microscopic level also exhibited neurite regrowth along the parental pathway. These observations cannot be explained solely by endogenous determinants of neurite growth. Rather they suggest that adhesive or recognition molecules deposited on the substratum during growth cone movement may be detected at later times by other cells and have a directive influence. Daughter cells can exhibit divergent behavior based on their micro-environment, mimicking cellular decision making during normal neurogenesis.

1119 SUBSTRATE BONDED HYALURONIC ACID ACTS AS AN INFORMATIONAL MOLECULE TO CONTROL THE DIFFERENTIATION OF MUSCLE AND CARTILAGE *Mary J. Kujawa and Arnold I. Caplan*, Biology Dept. Case Western Reserve University, Cleveland, Ohio 44106

Extracellular matrix molecules have been implicated in several differentiative and morphological processes. To examine the effect of one such molecule, hyaluronic acid (HA), on the differentiation of chick limb mesenchymal cells, we have plated cells isolated from two stages of development onto HA bonded to the culture substrate. Cultures of stage 24 (day 4 1/2) chick limb mesenchyme and day 11 leg muscle myoblasts have been examined. With early limb mesenchymal cells, HA stimulates chondrogenic expression. Exposure to substrate bound HA causes cells in the myogenic lineage to continue to cycle and bars their entrance into the terminal myogenic pathway; these cells do not fuse or initiate the muscle macromolecular biosynthetic program until they are removed from the HA substrate. These effects appear to be specific to substrate bound HA; other glycosaminoglycans are ineffective, digestion of the HA substrate with hyaluronidase abolishes the response, and the responses are dependent upon the concentration of HA bound to the culture surface. Cell attachment and replication do not appear to be affected. It is interesting that the same molecule, HA, can both stimulate chondrogenesis and inhibit myogenesis. These experiments suggest that the developmental state and the decisional history of embryonic cells are important in determining whether signals such as HA will elicit permissive or non-permissive states for specific phenotypic expression. (Supported by MDA and NIH).

1120 HYBRID DYSGENESIS IN THE NEMATODE PANAGRELLUS REDIVIVUS? Christopher D. Link and William B. Wood, University of Colorado, Boulder, Colorado 80309

Matings between certain Panagrellus strains lead to an increased mutation frequency and a unidirectional decrease in brood size of approximately 95%. This decrease in brood size results from embryonic lethality: affected embryos fail to complete morphogenesis and eventually degenerate. We have isolated from the Panagrellus genome a repetitive element which is a candidate for a putative dysgenic element. This repetitive element differs in copy number between interactive strains, and appears to be in different chromosomal locations in different strains. A morphological mutant recovered from the anomolous cross has at least one copy of the element in a new location (based on Southern blot experiments). We are presently attempting to directly implicate this (or some other) element in the mutagenic effect by "catching" the element in a gene which we have recently cloned.

1121 A SUBPOPULATON OF DORSAL ROOT GANGLION NEURONS, IDENTIFIED BY MONOCLONAL ANTIBODY SN1, IS AFFECTED BY ALTERATION OF PERIPHERAL TARGETS. M. F. Marusich, K. Pourmehr, and J. A. Weston, Dept. of Biology, Univ. of Oregon, Eugene, OR 97403.
We have developed a monoclonal antibody (designated SN1) that binds to the cell surface of a subpopulation of avian sensory neurons. Indirect immunofluorescence revealed that the distribution of SN1(+) neurons in 12-16 day embryos is correlated with axial level. Thus, neurons within lower thoracic DRGs are almost exclusively SN1(+), whereas brachial and lumbo-sacral DRGs, which innervate the limbs, have significant numbers of SN1(-) neurons. To test the hypothesis that the proportion of SN1(+) neurons within a ganglion is regulated by interactions between the developing neurons and their peripheral targets, we performed unilateral wing-bud amputations at 3 days of incubation (prior to sensory innervation). The embryos were sacrificed on day 15 and serial sections were made through all brachial DRGs. DRGs on the operated and contralateral (control) sides displayed strikingly different patterns of SN1 immunoreactivity. Approximately 60% of the neurons in control DRGs were SN1(+), whereas SN1(+) neurons were dramatically reduced in DRGs supplying the experimentally ablated regions. These results support the hypothesis that peripheral targets promote the appearance of SN1(+) neurons. The identity of the peripheral targets of these neurons, and the functional significance of the SN1(+) phenotype is presently unknown. Likewise, it remains to be determined if regulation is accomplished by the selective survival of precommitted neurons or through the modulation of phenotypically plastic neurons. Supported by NSF Grant PCM-8218899, NIH Grant DE-04316 and NIH Postdoctoral Fellowship HD-06292 to M.M.

1122 Differential expression of sea urchin early and late histone genes in Xenopus oocytes in response to a trans-acting factor isolated from late-stage sea urchin embryos.

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The sea urchin histone gene family comprises two subfamilies, "early" and "late", each of which is expressed during a unique developmental interval. Early histone genes are active from fertilization to the blastula stage and late histone genes are expressed from the blastula stage onwards. To search for trans-acting factors that play a role in this developmental switch, we have microinjected sea urchin histone genes and fractions prepared from sea urchin embryos into the nuclei of Xenopus laevis oocytes. Using S1 nuclease protection of early and late H2b histone DNA probes to monitor histone gene transcripts in recipient oocytes, we have found that a chromatin fraction prepared from gastrula-stage embryos selectively stimulates late H2b histone gene expression. We observe a similar selective stimulation of late H2b transcript levels when we inject gestrula-stage whole cell RNA along with early and late histone genes. These results provide evidence for sea urchin factors whose ability to differentially affect transcription of early and late histone genes in Xenopus oocytes may provide part of the explanation for the switch from early to late histone subtypes in vivo.

1123 EXTRA-SEX-COMBS AND TAILLESS MUTANT COMBINATIONS. John Merriam and Teresa R. Strecker, Department of Biology, University of California, Los Angeles, CA 90024.--Obtaining Drosophila embryos simultaneously carrying mutants at esc (extra-sex-combs, a homeotic gene) and tll (tailless, a zygotic lethal gene) is an approach to determine if both mutants affect the same step(s) in development. In esc mutants the normal pattern for all of the trunk segments and some of the head segments is replaced by repeated differentiation of the terminal eighth abdominal segment (Struhl, 1981). The tll mutants lack the eighth abdominal segment as well as the earlier appearing ninth and tenth abdominal segments, and have reduced procephalic lobes anterior to the gnatocephalic forming segments (Strecker, Kongsuwan and Merriam, submitted).--We wish to explore the following issues: (1) is the repeating pattern in the double mutant something other than the expected A8? (2) Does esc affect all three gnathocephalic forming segments and are the esc and tll mutant patterns reciprocal around a line on the head? (3) What is the effect of esc on the normal ninth and tenth abdominal segments and does esc differentiate an extra abdominal segment as suggested by Struhl?--Our preliminary results with mature embryos indicate that the eighth abdominal pattern is repeated in the double mutant; the esc and tll phenotypes appear to be additive. An analysis of younger stages by means of the SEM will distinguish the numbers and locations of the segments altered by the esc mutations. We suggest that early in development regions at the anterior and posterior tips of the pattern are normally set aside for further specialization apart from the trunk portion of the embryo. The normal gene function is required for the anterior and posterior specialization (Strecker, Kongsuwan and Merriam); we suggest the normal esc function is required for proper development of the central trunk region.

1124 REGIONAL CONTROL OF ACTIN GENE EXPRESSION IN FROG EMBRYOS. Tim Mohun, S. Brennan, J.B. Gurdon, CRC Molecular Embryology Research Group, Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK.

Cells in the early frog embryo destined to contribute to somitic mesoderm tissue (and hence embryonic muscle) are able to activate the muscle-specific actin genes at the appropriate time after egg fertilisation irrespective of whether the normal embryonic cell interactions are maintained.[1,2] Indeed, all the requirements for activation of muscle actin genes in later development appear to be localised in a sub-equatorial region of the fertilised frog egg.[3]

In addition, the muscle-specific actin genes can be activated in ectodermal cells, derived from the animal half of the egg even though such cells do not contribute to embryonic muscle during normal development. Activation results from an inductive interaction between ectodermal cells and vegetal endodermal cells of the early embryo.

To study the molecular mechanisms of such gene regulation we have isolated five fragments of genomic frog DNA each containing a different actin gene. These have been characterised by DNA sequencing and examined for putative regulatory sites that determine the differing patterns of actin gene expression. Microinjection of cloned genes into frog eggs has been used to study the regulatory activity of these sites.

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1125 GASTRULATION MOVEMENTS OF PROGENY DERIVED FROM 16-CELL STAGE XENOPUS BLASTOMERES, Sally A. Moody, Dept. Anatomy, Univ. Virginia, Charlottesville, VA, 22908

Marking clones of individual Xenopus blastomeres by intracellular injection of horseradish peroxidase has demonstrated that cleavage stage blastomeres are ancestral to constituents of each of the primary germ layers. Single blastomeres were marked at the 16-cell stage in order to define the pathway their progeny follow during gastrulation to populate the developing organ systems. At the 512-cell stage each clone remained coherent and in the original position of the marked cell. The progeny of the animal hemisphere blastomeres predominantly gave rise to ectodermal and axial mesodermal derivatives that followed the classically described gastrulation movements. Superficial progeny of these blastomeres rapidly expanded over the surface of the embryo, constituting epiboly. These cells freely mixed with unlabeled cells derived from adjacent blastomeres, whereas all other progeny moved as relatively coherent clones. Although the surface cells from the equatorial region of the clones derived from vegetal hemisphere blastomeres followed classical gastrulation movements, most of their progeny moved very little. The deep cells in the equatorial region at the caudal edge of the blastocoele did not roll over the blastoporal lip, but moved directly into the head mesoderm. The surface layers near the vegetal pole invaginated as bottle cells, which eventually populated the head, and the rostral archenteron roof and floor. The large cells of the yolk plug formed a coherent clone continuous with internal central cells that extended to the floor of the blastocoele. These cells virtually did not move during gastrulation and did not intermix with the progeny of adjacent blastomeres. This technique allows us to follow clones through gastrulation and to study lineage separation at these times. (Supported by NS-20604)

1126 CELL MOVEMENTS IN AN INSECT WING: PARADIGMS FOR EMBRYOGENESIS, James B. Nardi, University of Illinois, Urbana, IL 61801

An asymmetry exists along the proximodistal (PD) axis of the Manduca wing that is manifested in the folding movements of transposed epithelial populations as well as the movements of sensory neurons and tracheole cells. Following transposition of epithelial monolayers along the PD axis, grafts can either invaginate or evaginate. Invagination only occurs following a P → D exchange; evagination only occurs following a D → P exchange. Evidence exists for a gradation of cell adhesive properties along the PD axis, and the folding movements of grafts can be attributed to the presence of an adhesive disparity between folding populations of cells and surrounding epithelia. Consistent with this interpretation is the observation that frequency of invagination and evagination is a function of graft size as well as distance of graft transposition. Movements of individual neurons and tracheoles occur within the space between the two epithelial monolayers of the wing. Sensory neurons migrate in a D → P direction whereas tracheole cells move in a P → D direction. Cues directing the active migration of sensory neurons reside on the basal surface of the dorsal epithelial layer. By contrast, movement of tracheoles is apparently a process whose motive force resides in adjacent epithelial cells. These epithelial cells extend basal processes that form intimate contacts with tracheoles and apparently control tracheole distribution by exerting traction on these air-filled cells.

1127 INTERACTIONS OF EMBRYONIC MESENCHYMAL CELL SURFACE PROTEINS WITH COLLAGEN, Roy C. Ogle and Charles D. Little, University of Virginia, Charlottesville, VA 22908
Polypeptides specifically binding native collagen were isolated from detergent extracts of membranes purified from cultured chicken embryonic fibroblasts and mesenchyme by repeated collagen affinity chromatography. These collagen binding proteins were not extractable in urea or NaCl containing buffers. The proteins were labeled by lactoperoxidase catalyzed iodination of intact cells released non-enzymatically from the substratum. These biochemical properties suggest that the collagen binding proteins are integrally associated with the mesenchymal cell surface. Interactions between these purified molecules and native collagen were studied in vitro. The rate and extent of collagen fibrillogenesis was inhibited by the addition of these proteins. The degradation of collagens by proteolytic enzymes was altered in the presence of collagen binding proteins. These results suggest that collagen binding proteins may affect the native conformation of collagen molecules. If these proteins function on the cell surface as in vitro, they may serve as extracellular matrix adhesion molecules functioning to maintain cellular morphology and promote cellular locomotion through matrices by reducing pericellular viscosity. Furthermore these collagen binding proteins may direct both matrix assembly by regulating collagen fibrillogenesis and disassembly by conformationally altering collagen such that proteolysis occurs. The data suggest the collagen binding proteins are candidates for collagen "receptors" mediating cell-ECM interactions important to differentiative and morphogenetic processes.

1128 DEVELOPMENTAL REGULATION OF THE GENE FOR LIPOPHILIN, THE MAJOR INTEGRAL PROTEIN OF MYELIN, J.R. Riordan, A.L. Naismith, E. Hoffmann-Chudzik and L.-C. Tsui, Research Institute, The Hospital for Sick Children, Toronto, Canada M5G 1X8.
Myelination in both central and peripheral nervous systems of higher animals is regulated both temporally and positionally. Thus as neuronal development occurs myelination is required for the establishment of motor function. This occurs primarily either pre- or perinatally depending on the animal species. With respect to positional regulation, Schwann cells synthesize myelin only when they are in contact with appropriate axons. The molecular signals and their targets are unknown. The myelin membrane has two major proteins, the peripheral basic protein and an intrinsic protein, lipophilin. Biochemical studies of the membrane structure and biogenesis imply that lipophilin, being an integral protein in the membrane, might be expected to be involved in initial biosynthetic events. The basic protein, on the other hand, may participate in the apposition of successive myelin layers and, therefore, be required only secondarily. To study the molecular control of myelination we have cloned cDNAs for bovine lipophilin which enable identification of restriction enzyme fragments of the lipophilin gene in several animal species including rodents and man. They also recognize an mRNA of approximately 2.5 Kb. This mRNA as detected by blot hybridization analysis in developing rat brain was not present prior to 10 days after birth, was maximal at 20 days and minimal again by 25 days. This provides an apparently ideal precursor-product relationship between the production of lipophilin mRNA and myelination. Studies of the structure and organization of the lipophilin gene are in progress so that interactions of putative temporal and axonal signals can be studied. (Supported by MRC of Canada)

1129 EXPRESSION OF THE BETA NERVE GROWTH FACTOR GENE IN SYMPATHETIC EFFECTOR ORGANS, D.L. Shelton and L.F. Reichardt, Div. of Neuroscience, UCSF. San Francisco, CA. 94143
Beta Nerve Growth Factor (NGF) is a protein necessary for normal development and maintenance of sympathetic and sensory neurons in vivo and in vitro. Evidence has accumulated which indicates that NGF is required at growing tips of axons and when present there, is bound, internalized, and transported retrogradely to the cell body. This has led to the hypothesis that NGF is produced by target tissues of the responsive neurons, but so far this has been impossible to demonstrate. Using an assay capable of detecting 10 fg of mRNA encoding NGF (NGFmRNA), we have surveyed tissues with varying densities of sympathetic innervation for their content of NGFmRNA as an indication of their levels of synthesis. Using norepinephrine content as a measure of the density of sympathetic innervation, we have found a strong positive correlation between the NGFmRNA level and innervation density. An iris denervated either in vivo or by growth in vitro rapidly accumulates NGF. This seems to at least partly reflect an increase in synthesis as we have found a large, rapid (< 1 hour) and sustained (> 3 days) increase in NGFmRNA content when irides are placed into culture. We are investigating the magnitude and time course of changes after denervation in vivo. NGF has recently been shown to have effects on some cholinergic neurons of the central nervous system (CNS) of mammals. We find levels of NGFmRNA in the CNS higher than can be accounted for by the sparse sympathetic innervation of CNS vasculature. There are up to 5-fold differences in NGFmRNA content between different regions of the CNS, further arguing against vasculature as the only source. The septal projection to the hippocampus is one system which has been shown to be affected by NGF and we find high levels of NGFmRNA in the hippocampus.

1130 MOLECULAR DETERMINANTS OF NEURAL SPECIFICITY ARE SHARED IN INVERTEBRATES AND VERTEBRATES, Shahid Siddiqui & Joseph Culotti, Dept of Biochemistry, Molecular Biology & Cell Biology, Northwestern University, Evanston, IL 60201

Antibodies that recognize specific molecular determinants within the nervous system are excellent probes to study neural development at the molecular level. We have characterized a battery of monoclonal and polyclonal antibodies to study the morphology of identified neurons, in wild type and mutants of the simple nematode Caenorhabditis elegans, at the level of light microscopy. Nerve specific antibodies have been identified using indirect immunofluorescence on wholemunt squashes of nematodes. These include: monoclonal antibodies raised against leach(B. Zipser, S. Siddiqui, & J. Culotti), D. melanogaster(K. White, A. Perira, S. Siddiqui & J. Culotti), and rat(R. Akeson , S. Siddiqui & J. Culotti) nervous tissue. Significant cross reactivity is observed between the monoclonal antibodies raised against leach, Drosophila, rat and the nematode neural tissue, at the cytochemical and molecular level. Similarly, polyclonal antibodies raised against mouse neural cell adhesion molecule (mouse N-CAM) label a specific subset of neurons in C. elegans nervous system (U. Rutishauser, S. Siddiqui & J. Culotti). Our results suggest that the simple metazoan C. elegans, shares common neural antigenic determinants with other invertebrates and vertebrates. These antibodies are being used to identify their target molecules, and eventually the genes involved in the expression of the neural antigens.

1131 INVOLVEMENT OF THE CONTACT SITE A GLYCOPROTEIN IN CELL-CELL ADHESION DURING DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM. C.-H. Siu and A.H.C. Choi. Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario.

A membrane glycoprotein of Mr 80,000 (gp80) has been implicated in mediating the EDTA-resistant type of contact sites (A) during development of Dictyostelium discoideum. However, the evidence has been circumstantial and it remains to be determined whether gp80 is directly involved in cell cohesion. To resolve this, we have prepared monoclonal antibodies (mAb) directed against gp80. One of them is monospecific and blocks the EDTA-resistant sites. Its inhibitory activity can be neutralized by purified gp80. Inhibition of cell reassociation by this mAb is only effective at the aggregation stage, suggesting that gp80 has only a transient function and additional surface components are required for cell-cell binding at the post-aggregation stages. To further investigate the role of gp80 in intercellular adhesion, we have examined its topographic distribution on the cell surface using mAb and protein A-gold conjugates. Quantitative analysis shows that gp80 has a biased distribution and is preferentially localized at contact areas and on filopodia. These results suggest that gp80 may play an important role in mediating the initial recognition and contact formation via filopodia during cell aggregation. (Supported by MRC of Canada).

1132 EXPRESSION OF MICROINJECTED GENES IN XENOPUS LAEVIS OOCYTES AND EMBRYOS. David N. Standring and William J. Rutter, Hormone Research Institute, University of California, San Francisco, Ca 94143

We have used microinjection to introduce into the germinal vesicle of Xenopus laevis oocytes supercoiled plasmids containing eucaryotic promoters linked to the gene encoding the chloramphenicol acetyltransferase (CAT) reporter function. Assays of CAT activity in injected oocytes have revealed that a range of promoter activity spanning greater than 1000 fold can be assayed in this system, with viral promoters (e.g. SV40 early) showing great activity while the rat insulin promoter shows no detectable activity. The state of these genes and their expression are also being studied in transgenic tadpoles and frogs.

1134 PROLIFERATION OF DEVELOPMENTAL NEURONS IN A CHEMICALLY DEFINED MEDIUM, Doris K. Wu and Jean de Vellis, UCLA School of Medicine, Los Angeles, CA 90024

Previous studies have shown that cultured neurons from chick telencephalons of various embryonic ages have different requirements for survival in a serum-free environment (Aizenman, *et al*., 1984, submitted). Some of the neurons (40%) from 6 day-old chick embryos (6 ED) can survive in DMEM/Ham's F-12 medium, without any addition, whereas the remaining neuronal population require insulin for survival. In contrast, all neurons from 9 ED are totally dependent on both insulin and transferrin. Here, we report that neuronal cultures from 6 ED embryos incorporate [^{3}H]-thymidine. [^{3}H]-Thymidine uptake occurred up to the 4th day of culture. Addition of insulin at anytime during this period triggered a transient rise in DNA synthesis. Autoradiographic studies showed that 40% of the cells in culture incorporated the radioactive DNA precursor into their nuclei. Currently, we are using this system to screen for neuronal mitogens among known hormones and factors, as well as tissue extracts. (Supported by NIH Grant HD 06576 and DOE Contract DE AM03-76-SF00012.

1135 Uvomorulin, a mouse embryo cell adhesion molecule, Nadine Peyriéras, Service de Génétique cellulaire, Institut Pasteur, 25 rue Dr. Roux, Paris

Uvomorulin is a cell surface glycoprotein involved in compaction of early mouse embryo. With the aid of a rat monoclonal antibody (DE1) raised against a tryptic fragment of uvomorulin, the detergent solubilized forms of uvomorulin as well as its biosynthesis have been examined. DE1 recognizes, in a detergent lysate of continuously labeled embryonal carcinoma cells, at least three molecules (120, 100 and 88 kDa) that are not related by peptide mapping. Only the 120 kDa product is structurally related to the tryptic fragment of uvomorulin. Although all three products are expressed at the cell surface, only the 120 kDa form is glycosylated. The 120 kDa molecule shares this property with its presumed intracellular precursor (135 kDa product). Interference with N-linked oligosaccharide processing using glucosidase and mannosidase inhibitors (1-deoxynojirimycin and 1-deoxymannojirimycin) does not inhibit surface expression of uvomorulin nor does it affect compaction and early differentiation of embryonal carcinoma cells in culture induced by retinoïc acid.