MOLECULAR BIOLOGY OF INVERTEBRATE DEVELOPMENT

J. Dennis O'Connor, Organizer

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Keynote Address

K 001 SPATIAL SPECIFICATION OF DIFFERENTIAL GENE FUNCTION IN EARLY EMBRYOGENESIS, Eric H. Davidson, Division of Biology, California Institute of Technology, Pasadena, California 91125

Early embryonic development is carried out in very different ways in different groups of organism. Among the parameters that distinguish different modes of building an embryo are the extent of reliance on ***cell lineage*** in embryonic cell specification, as opposed to ***regional*** specification of sheets of undifferentiated cells; the stage, with respect to cell number, when spatially differential patterns of gene expression appear; and the degree of reliance on intercell, inductive interactions, as opposed to specification according to inheritance of maternal cytoplasmic factors. These differences may imply fundamental, rather than superficial, diversity in the genomic regulatory architecture of embryogenesis. I shall attempt to discuss the nature of cell specification, so far as it is beginning to be understood at the molecular level, in several systems that provide diverse paradigms, *viz. C. elegans*, *Drosophila*, sea urchin, and *Xenopus*.

Vitellogenesis/Oogenesis

K 002 EVOLUTIONARY CONSERVATION OF POTENTIAL REGULATORY SEQUENCES AT THE 5' ENDS OF C. ELEGANS VITELLOGENIN GENES, Erin Zucker, John Spieth, Jerome Cane and Tom Blumenthal, Department of Biology, Indiana University, Bloomington IN 47405.

In C. elegans the vitellogenins are encoded by a diverse family of six genes. These genes are expressed exclusively in the intestine of the adult hermaphrodite. By comparing the sequences of the promoter regions of all six genes we identified two repeated heptameric sequence elements. One of these, Box 1, has the sequence TGTCAAT, and is always present just 5' of the TATA box, again at position -180 (with respect to the CAP site) and at least once in between. The Box 2 sequence, CTGATAA, is always present as a perfect match to the consensus between -90 and -150. In order to obtain evidence for or against the functional importance of these heptamers we sequenced the promoters from the five homologous genes of another Caenorhabditis species, C. briggsae. In general we found the heptamers have been very highly conserved, while the sequences between them have diverged considerably. Since neither Box 1 nor Box 2 is found in other sequenced C. elegans promoter regions, we believe they may be specifically involved with the developmental regulation of the vitellogenin genes. In support of this idea, we have noted the presence of Box 2 as a perfect match to the consensus in both chicken and frog vitellogenin gene promoters, and with a one base pair mismatch in the Drosophila yolk protein gene promoters. Recently Hitpass et al. (Cell 46, 1053) have tested the idea that this heptamer plays a role in Xenopus vitellogenin gene induction by estrogen in a transient expression assay using a human mammary cell line. They present evidence that it is required for promoter activation and estrogen stimulation. These results indicate a remarkable degree of conservation of sequences involved in vitellogenin gene induction and also suggest that proteins that recognize the Box 2 sequence are present in nematode intestine and human mammary cells.

In addition, there are sequences in the 5' untranslated and coding regions of the mRNA that may influence mRNA stability and translatability. The first 70 bases of each of the mRNAs can be folded into a series of stem-loop structures with predicted free energies of about -24 Kcal/mole. Two lines of evidence suggest that these structures have been selected for and therefore perform some function. First, most base pair changes between the two Caenorhabditis species occur in unpaired regions: Although the sequences differ by about 15%, there are no changes that result in an altered stem stability. Second, the asymmetric codon usage characteristic of most of the length of the vitellogenin genes is sacrificed within the first 70 bases of each of the mRNAs in such a way as to allow stem formation. We hypothesize that the stems might function to modulate translation of these abundant mRNAs, perhaps as a response to environmental conditions.

K 003 REGULATION OF VITELLOGENESIS IN DROSOPHILA, John Postlethwait, Joel Parker, Denita Widders, and Yi-Lin Yan, Department of Biology, University of Oregon, Eugene OR 97403.

Yolk polypeptides (YPs) are normally made by fat body cells in Drosophila females but not males. 20-hydroxyecdysone stimulates the accumulation of YP transcript in fat body cells of both males and females. However, a sex difference in hormones is not responsible for sex-specific gene expression since we found that in situ hybridization to YP transcripts in sex mosaics (gynandromorphs) revealled two populations of cells -- some with male and some with female levels of transcript. Sexually ambiguous genotypes were investigated by in situ hybridization to YP transcript to determine if individual cells expressed YP genes exclusively in either male or female amounts, or alternatively if all cells were intermediate in expression. Finally, the gene for YP3 was sequenced and conserved elements identified that may regulate gene expression.

Molecular Sequelae of Fertilization

K 004 THE ROLE OF EGG ACTIVATION IN THE INITIATION OF CA-CHANNEL FUNCTION, Gary Freeman, Department of Zoology, University of Texas, Austin, TX 78712.

The eggs of the hydrozoan Phialidium gregarium contain a calcium specific photoprotein. When oocytes, unfertilized eggs, or eggs which have just been fertilized are depolarized, there is no action potential and no light is produced. The action potential and light production will occur in Na^+ substituted seawater. The calcium blockers, cobalt and nifedipine, block the action potential and light production. The magnitude of the action potential and light production is related to the calcium concentration of the seawater.

Treatment of unfertilized eggs with the ionophore A23187 artificially activates them. One hour after activation, calcium channel function appears in these eggs. Treatment of oocytes with A23187 does not induce calcium channel function. When maturing oocytes are cut in half prior to germinal vesicle (GV) breakdown, to give a half with a GV and a half that lacks a GV, and both halves are subsequently activated, calcium channels only appear in the half which inherits the GV. If the same cutting experiment is done just after GV breakdown, calcium channels appear in both halves after activation. One or more components which are necessary for calcium channel function appear to be sequestered in the GV of the oocyte, moved to the cytoplasm at GV breakdown, and made to function at one appropriate developmental period as a consequence of egg activation. Supported by N.I.H. Grant GM20024.

K 005 RECRUITMENT OF STORED mRNAs AND ACTIVATION OF THE CELL CYCLE IN EARLY EMBRYOS, Joan Ruderman, Tim Hunt*, Katherine Swenson and Joanne Westendorf. Zoology Department, Duke University, Durham NC 27706 and *Biochemistry Department, University of Cambridge, England, CB2 1QW. Oocytes contain enormous stockpiles of mitotic proteins that enable early embryos to proceed through very rapid cell division cycles. Despite this, the oocyte is arrested and cannot enter the cell cycle until fertilization activates the translatio of several stored maternal mRNAs which then direct the synthesis of several proteins that are essential for progess through the cell cycle. In clams and sea urchins, two of these proteins called cyclins (no relationship to another "cyclin" described in mammalian cells) appear to be key regulatory proteins. Cyclin levels rise across the cell cycle and are rapidly destroyed near the end of each mitosis. Cyclin A acts as an inducer of entry into M-phase: when clam cyclin A is introduced into frog oocytes, it activates meiosis, namely nuclear envelope breakdown, chromosome condensation and spindle formation. The disappearance of cyclin B appears to allow exit from M-phase, namely anaphase, reassembly of the nuclear envelope and decondensation of the chromosomes. Despite extensive homology between the two cyclin protein sequences, they show very different patterns of intracellular localization and behavior in arrested cell cycles. We are now trying to figure out exactly what these proteins do in the cell cycles of early embryos.

K 006 CELLULAR MESSENGERS AND SEA URCHIN EGG ACTIVATION. Michael Whitaker, Department of Physiology, University College London, London WC1E 6BT, UK. The transition from quiescence to proliferation in sea urchin eggs is triggered and controlled by cellular messengers. Sperm binding leads to the production of inositol phosphates and diacylglycerol, two cellular messengers produced by the polyphosphoinositide messenger system. These molecules in turn cause alterations in intracellular free calcium intracellular pH. Calcium ions and hydrogen ions are the proximate messengers responsible for the rapid onset of protein and DNA synthesis and cell division. I shall discuss the mechanisms involved in the generation of these messengers and the part they play in the explosive activation wave at fertilisation. I shall also consider how good a model the fertilisation response in sea urchin eggs is for growth transitions in other cell types.

K 007 REGULATION OF PROTEIN SYNTHESIS DURING EARLY DEVELOPMENT IN SEA URCHIN EGGS, Matthew M. Winkler and James L. Grainger, Center for Developmental Biology, Department of Zoology, University of Texas, Austin, TX 78712.

A long-standing regulatory problem in early development concerns the molecular mechanisms by which protein synthesis is activated following fertilization of the sea urchin egg. This 20-40 fold increase in the rate of protein synthesis is mediated by a 2-3 fold increase in the peptide elongation rate and a 10-15 fold increase in the amount of mRNA being translated. All of the components of the protein synthetic machinery, such as ribosomes, initiation factors, tRNAs, etc. and mRNA are apparently stored in the unfertilized egg. Therefore, the lack of activity of one or more of these components limits protein synthesis until fertilization occurs.

Protein synthesis in the egg is limited in part by a low capacity of the translational machinery to translate RNA. Using sea urchin egg cell-free translation systems we have shown that the capacity of the protein synthetic machinery to translate mRNA is limiting in the unfertilized egg. This has been confirmed by microinjection of mRNA into eggs. In addition we have shown that the activity of egg mRNAs (actually messenger ribonucleoprotein particles or mRNPs) are also limiting. The "masked message hypothesis" suggests that mRNA in the egg is inactive because it is bound to repressor molecules forming a nontranslatable mRNP. In this model, fertilization results in the alteration of the mRNP so that it can be translated. To test this hypothesis, several groups have isolated mRNPs from eggs and assayed their translational activity in cell-free translation systems. However, their conclusions conflicted as to the egg mRNP translational activity.

Recently, we have assayed the translational activity of egg mRNPs in crude homogenates and demonstrated that they are largely inactive. When these mRNPs are partially purified by gel filtration chromatography, they remain inactive. However, the interaction of the "masking factor" with the mRNPs appears to be quite labile since manipulation of the mRNPs results in progressively more active mRNPs. The lability of this binding probably explains previous findings in which egg mRNPs were found to be active. Treatment of inactive mRNPs with 0.5 M NaCl, 10 mM EDTA results in almost complete activation of the mRNP. Following rechromatography of the activated mRNPs, one of the included fractions contains an inhibitor of translation. We have made antibodies to the inhibitory fraction and identified on Western blots a candidate for an mRNP masking factor. Our results indicate that protein synthesis is regulated at the level of activity of mRNPs and several other steps in the pathway of protein synthesis.

This work was supported by a grant from N.I.H., HD17722-04 to M.W.

Early Molecular Events in Cell Lineage

K 008 STRUCTURE AND EXPRESSION OF THE SEA URCHIN SPEC GENES, William Klein, Mark Kozlowski, Susan Hardin and Paul Hardin, Dept. of Biochemistry and Molecular Biology, M. D. Anderson Hospital, University of Texas System Cancer Center, Houston, Texas 77030.

The sea urchin Spec gene family encodes 10 to 12 calcium binding proteins belonging to the troponin C superfamily. The Spec mRNAs accumulate exclusively in aboral ectoderm cells and the Spec proteins are among the most actively synthesized proteins in the late stage embryo and larva. The aboral ectoderm cells stop dividing during the blastula stage and differentiate to become the epidermis that covers the larva. Four Spec genes have been studied: Spec1, which encodes a 1.5 kb mRNA that accumulates to its maximal level by the gastrula stage, and Spec2a, 2c and 2d, which encode 2.2 kb mRNAs that accumulate to their maximal levels during post-gastrula stage development. The Spec gene family appears to have arisen in sea urchins via a duplication of the calmodulin gene. The Spec genes are unusual in that the 5' and 3' untranslated regions are more conserved than the protein coding region. The Spec genes are closely associated with a repetitive sequence element approximately 600 base-pairs in length. There are a few dozen of these elements in the sea urchin genome and most are a few kb upstream or downstream from Spec genes. In the Spec2a gene, the element abuts what would normally be exon 1 and is actually part of the Spec2a transcription unit, with the initiation of transcription mapping to the middle of the element. To determine whether these repetitive elements contain sequences important for proper Spec gene expression, microinjection experiments with sea urchin eggs are being performed. DNA fragments containing sequences upstream from the Spec1 gene, with and without the repetitive element, are fused to the bacterial CAT gene and levels of CAT activity monitored as a function of developmental time.

K 009 "DNA Synthesis and Embryonic Gene Expression in C. elegans" by James D. McGhee, Kenichi, Ito and Lois G. Edgar, Department of Medical Biochemistry, University of Calgary, Calgary, Alberta, CANADA.

We have previously described a non-specific esterase that is localized to the intestine of both embryonic and adult C. elegans. Esterase expression is cell autonomous, is independent of cytokinesis and depends on zygotic transcription at a stage when the embryonic gut has 4-8 cells (Dev. Biol., 114, 109-118 (1986). We have now investigated how esterase expression depends on DNA synthesis, using the DNA polymerase inhibitor aphidicolin. If DNA synthesis is inhibited when the embryo has two or four cells, esterase expression is completely inhibited, even though embryos remain viable. However, once the 4-to-8 cell division has occurred and the next round of DNA synthesis has begun, esterase will now be expressed with high efficiency. This crucial period of DNA synthesis occurs immediately after the division that clonally establishes the gut, but two cell cycles before the esterase gene is transcribed (as inferred from α-amanitin sensitivity). The appearance of a second intestinal specific marker, autofluorescent rhabditin granules, shows an identical dependence on DNA synthesis but has a different time of sensitivity to α-amanitin. Thus it appears that more than one gut-specific genes are potentially activated by this particular period of DNA synthesis. Esterase transcription is not the result of attaining the normal nuclear: cytoplasmic ratio nor is it the result of counting the normal number of rounds of DNA synthesis, since the two rounds of DNA synthesis that normally take place between clonal establishment of the gut and esterase transcription can be completely inhibited without lowering the extent of esterase expression. We will also discuss experiments showing that segregation of parental DNA strands during development appears to be completely random.

K 010 THE CYTOSKELETON AND ASYMMETRIC CYTOPLASMIC MOVEMENTS IN C. ELEGANS ZYGOTES, Susan Strome and David Hill, Department of Biology, Indiana University, Bloomington, IN 47405.

We are investigating how asymmetry is generated and cell fates specified in early embryos of the nematode C. elegans. Our approach is to monitor several motility events that normally occur asymmetrically, such as pseudocleavage, pronuclear migration, P-granule segregation, spindle placement, and cytokinesis, in zygotes that have been treated with cytoskeleton inhibitors. We have found that the asymmetric aspect of all of these movements is dependent on microfilaments (MFs) and independent of microtubules; when zygotes are treated with the MF inhibitor cytochalasin D (CD), they behave "symmetrically". Furthermore, by "pulsing" zygotes with CD, we have defined the periods of zygote development during which MFs are required for each motility event. MFs do not appear to be required prior to the onset of the asymmetric movements, approximately midway through the first cell cycle. Then, during the period when the pronuclei are migrating and P granules are being segregated, MFs are required to provide asymmetry to the movements; when MF arrays are disrupted, these movements occur "symmetrically". Once P granules are segregated posteriorly, MFs are not required to hold them there; thus MFs appear to participate in localizing but not anchoring P granules. In order for the zygote to undergo an asymmetric cleavage, MFs are required from midway through the cell cycle until cytokinesis; when zygotes are pulsed with MF inhibitor at any time during this period, they divide symmetrically.

How do MFs mediate the asymmetric motility events discussed above? Visualization of the distribution of MFs has demonstrated that, concomitant with the motility events that require MFs, MFs themselves become asymmetrically reorganized. The MF pattern is consistent with certain models and inconsistent with other models of MF mediation of cytoplasmic motility. The two most likely models are currently being tested.

K 011 THE MICROMERE-SPICULE LINEAGE OF SEA URCHIN EMBRYOS. Fred Wilt and Steve Benson, Dept. Zoology, University of California, Berkeley, 94720. The goal of our research is to understand the molecular and cellular mechanisms governing determination and differentiation of the lineage established by micromeres in the developing sea urchin embryo. Micromeres are formed at the fourth cell division at the vegetal pole, and they thereafter undergo cell division, lose adhesion to other epithelial cells of the blastula; just before gastrulation they emigrate into the blastocoel, where they become very motile, eventually settling in characteristic locations and fusing to one another. Vacuoles form in the cytoplasm of the syncytium, and within these vacuoles the endoskeletal calcite rods, spicules, are laid down. Micromeres may be isolated and cultured, and they recapitulate many aspects of this differentiation including formation of spicules. In earlier work we isolated the calcareous spicules and characterized their integral matrix proteins. There are at least 9 soluble glycoproteins in the matrix, 4 of which make up 70% of the mass. A polyspecific antibody raised against the whole matrix was used to isolate a cDNA and gene that encode one of the matrix proteins (MW, 50 kd) termed SM50. The SM50 gene is expressed by mid-cleavage at low levels; when primary mesenchyme appears, the accumulation of SM50 transcript increases over 100 fold as the spicule forms. The transcript appears only in primary mesenchyme cells. We have used the expression of this lineage specific gene as a tool to investigate the regulation of differentiation of the lineage. Amplification of the SM50 gene does not appear to occur. The differentiation of the mesenchyme was modified in a number of ways. First, treatment of embryos with sulfate free sea water, which interferes with sulfation of GAG in the blastocoel, prevents spicule formation. Nonetheless, SM50 transcripts accumulate normally. Embryos have been raised in conditions where cells no longer adhere to one another, thus totally eliminating any close range cell-cell interactions; nonetheless, some SM50 gene expression occurs. Results of experiments in which embryos have been treated with Li^{+} or Zn^{++} will also be discussed. (Supported by NIH grant HD15043)

Segmentation and Early Pattern Events

K 012 SEGMENTATION AND NEURAL DEVELOPMENT IN VERTEBRATE EMBRYOS, Roger J. Keynes, Department of Anatomy, Downing Street, Cambridge CB2 3DY, U.K.

The study of early neural development in the chick embryo has revealed some interesting, albeit superficial, similarities between vertebrate and invertebrate segmentation. First, like the Drosophila epidermal segment, the mesodermal somite of higher vertebrate embryos is divided into anterior (rostral) and posterior halves. In particular, when motor and sensory axons first grow out from the neural tube region they are confined to the anterior half of each successive somite-derived sclerotome. The basis for this phenomenon, which also applies to migrating neural crest cells, lies in the cells of the sclerotome itself: molecular differences must exist between anterior and posterior sclerotome halves, to which both growth cones and crest cells are responsive. One such difference, at the phenotypic level, concerns the distribution of a peanut lectin receptor.

A second point for discussion is whether, as in many invertebrates, the vertebrate nervous system is intrinsically segmented. The question is relevant both to understanding how axons are guided to their targets during later stages of development, and to interpreting the patterns of expression of homeo-box genes that have been described recently in mouse embryos.

K 013 SPATIAL REGULATION OF HOMEO BOX GENE EXPRESSION IN DROSOPHILA, Michael Levine, Manfred Frasch, Christine Rushlow, Timothy Hoey, Helen Doyle, and Katherine Harding, Department of Biological Sciences, Columbia University, New York, NY 10027.

In Drosophila, embryonic cells select different pathways of morphogenesis based on their physical locations within the developing embryo. Elaboration of this positional information involves early zygotic gene functions that are expressed in response to maternal cues present in the unfertilized egg. Many of the zygotic genes that are required for the specification of positional identity along the anterior-posterior axis have been described in detail, and include the pair-rule genes and homeotic genes. Pair-rule genes divide the embryo into a repeating series of homologous segment primordia, whereas homeotic genes establish the diverse pathways by which each embryonic segment develops a distinct adult phenotype. Molecular analyses of pair-rule and homeotic gene activity have been facilitated by the demonstration that a number of these genes are evolutionarily related and share a similar 180 bp protein coding sequence, called the homeo box. There are at least 20 homeo box genes in Drosophila. Thus far, 14 of these genes have been isolated and characterized. Twelve of the genes are involved in the specification of anterior-posterior positional information, and include eight homeotic genes as well as four pair-rule genes. The two remaining genes (called z1 and z2) both derive from the zerknullt (zen) region of the Antennapedia gene complex and appear to be involved in the differentiation of the dorsal-ventral pattern. Each of these 14 genes shows a unique pattern of expression during early embryonic development. Selective patterns of homeo box gene expression appear to involve cross-regulatory interactions among these genes. That is, the expression of a given homeo box gene can influence the expression patterns of others. Here we show that the pair-rule gene even-skipped (eve) plays a key role in a hierarchy of regulatory interactions among homeo box genes, and that zen might influence the patterns of pair-rule and homeotic gene expression.

K 014 SEGMENTATION AND EARLY PATTERN EVENTS IN LEECHES, David A. Weisblat and Shirley T. Bissen, Department of Zoology, University of California, Berkeley CA 94720.

Segments in annelids, such as leeches, are presumably homologous to those of arthropods, such as insects, but arise by cellular pathways that seem quite different. Leech segmentation has been studied using cell lineage tracers, cell ablations, and radionucleotide incorporation. Cleavage generates five bilateral pairs of stem cells, the M (mesodermal) and N, O/P, O/P and Q (ectodermal) teloblasts. Each teloblast undergoes several dozen highly unequal divisions, generating a column of blast cells. The columns of blast cells (designated m, n, o, p and q bandlets) come together in an ordered, parallel array along the future ventral midline of the embryo. From this array, designated the germinal plate, the segments arise. Each bandlet contributes a distinct, segmentally iterated set of cells (designated as M, N, O, P or Q kinship groups) to the ipsilateral half of the embryo.

Each blast cell in an m, o or p bandlet makes one segment's worth of M, O or P progeny in a spatially stereotyped clone homologous to those of the other blast cells in its bandlet. Serially adjacent clones interdigitate, however, so that each morphologically defined segment contains progeny of more than one m, o and p blast cell per side. This suggests that leech segmentation does not proceed according to a strict compartment model (1).

In the n and q bandlets, two blast cells are required to make one segment's worth of progeny; these bandlets each contain two different classes of blast cells (nf and ns; qf and qs) in exact alternation. The two classes can be determined early in development by the timing and symmetry of their first mitoses (2). TTP incorporation studies show that the difference in cell cycle length between nf and ns blast cells is due to a difference in the length of the G2 phase in these cells. Various data suggest that nf and ns blast cells are determined to be different at the time they arise from the teloblast. A model is proposed in which these differences are established by a mechanism entailing syncytial feedback from nascent blast cells onto the parent teloblast. This model suggests an area of homology between the segmentation processes in leeches and insects.

1. D.A. Weisblat and M. Shankland (1985) Phil. Trans. R. Soc. Lond. B 312:39-56.
2. S.L. Zackson (1984) Dev. Biol. 104:143-160.

Cell Lineages and Commitment

K 015 AXIAL DETERMINANTS IN ASCIDIAN EGGS, William R. Jeffery and William R. Bates, Center for Developmental Biology, University of Texas, Austin, TX 78712.

The determination of cell types and patterns during ascidian embryogenesis has been attributed to cytoplasmic determinants which are localized in the egg and partitioned to specific regions of the embryo. Despite extensive investigation, the identity and mechanism of action of these cytoplasmic determinants are unknown. We review here our current progress in elucidating the identity and mode of action of determinants involved in establishing the dorsal-ventral axis in ascidian embryos. In fertilized eggs of the ascidian Styela, a yellow cortical cytoplasm segregates first to the region centered around the vegetal pole (VP), then to the subequatorial region of the egg, and subsequently is partitioned to the muscle cell lineage. The position of the VP determines the future dorsal side of the larva. We have developed a microsurgical method in which selected regions of the unfertilized egg or uncleaved zygote are excised by extruding a portion of the cytoplasm and severing the extruded fragment from the remaining nucleated portion of the egg. When cytoplasm was extruded from a point centered at the VP of the zygote during the period when yellow cytoplasm is positioned in this region, the VP-deficient zygotes cleaved normally, but did not gastrulate and form a dorsal-ventral axis. The removal of as little as 5% of the egg volume from the VP region of zygotes was sufficient to produce radialized embryos. Removal of similar volumes of cytoplasm from the VP region of unfertilized eggs and the animal pole or equatorial regions of fertilized eggs did not affect gastrulation or normal development. Using chalk granules applied to the VP, the VP region was followed into several vegetal cells of the blastula which were the first to invaginate during gastrulation. The results suggest that determinants that specify the position of gastrulation and the dorsal-ventral axis are segregated into the VP region with the yellow cortical cytoplasm. SEM analysis of Triton X-100 extracted eggs and uncleaved zygotes showed that the yellow cortical cytoplasm contained a cytoskeletal domain which was segregated into the VP region of eggs after fertilization. Based on its sensitivity to DNase I and its ability to be stained with phallacidin, this cytoskeletal domain was shown to contain actin filaments. Contractile activities involving actin filaments appear to be necessary for vegetal cell invagination since gastrulation can be blocked by cytochalasin treatment. It is proposed that an axial determinant system is present in the VP region of fertilized eggs and embryos which endows the vegetal cells with the potential to establish the site of gastrulation. The actin cytoskeleton localized in the VP region may be an important constituent of the axial determinant system.

Supported by NSF grant PCM-8416768

K 016 SPATIAL PATTERNS OF CELL COMMITMENT IN THE EMBRYO OF THE LEECH, Marty Shankland, Dept. of Anatomy and Cellular Biology, Harvard Medical School, Boston MA 02115. The leech embryo develops via stereotyped cell lineages, and each lineally identified embryonic precursor gives rise to a precise set of descendants in the mature leech. While certain of these precursors may have intrinsic properties that determine their fate, there are also instances in which a group of seemingly equivalent precursors become committed to divergent pathways as the result of positionally determined cell interactions. One case involves the determination of segment number. The mature leech has 32 segments, and these segments arise from an iterated array of embryonic blast cells that are laid out in five parallel rows called bandlets. Early on each bandlet contains 5-10 supernumerary blast cells at its posterior end, but these supernumeraries degenerate and the bandlet is thereby reduced to the appropriate length, i.e. to that number of blast cells which are sufficient to generate the mature segments. Normally one can predict a blast cell's segmental destiny on the basis of its ancestral lineage. However, altering the segmental register of one or two bandlets with respect to the rest will cause normally viable blast cells which have come to lie posterior to the first 32 segments to degenerate at the same time as the supernumeraries. This finding indicates that final segment number is not determined by the number of viable blast cells produced, and suggests that each bandlet is reduced to the appropriate length by a process of positionally determined cell death. A second instance of positional determination involves the blast cells which comprise the o and p bandlets. These two bandlets are defined by position, and are in contact along their length. Normally the o and p blast cells follow divergent O and P developmental pathways, but if either bandlet is ablated, cells in the remaining bandlet will follow the P pathway regardless of their original fate. Moreover, there is evidence which suggests that a p bandlet which has been transposed into the o bandlet position follows the O pathway. Taken together, these findings imply that o and p blast cells are equipotent, and that an o/p blast cell chooses the P pathway in the absence of bandlet interactions. However, adjacent o/p bandlets interact in such a way that blast cells in the o bandlet position are diverted into the O pathway. The commitment of o blast cells to the O pathway has been examined by lesioning the neighboring p blast cells following interactions of varying duration. The results indicate that an o blast cell clone becomes committed to the O pathway in a series of three discrete steps. Each of these commitment events seems to determine the O or P fate of a different blast cell sublineage, and two of these events occur just prior to cell divisions which segregate the committed sublineage from the remainder of the clone. Thus, it would appear that there may be some mitotic event which determines the state of commitment of only one of the two daughter cells produced at these divisions.

K 017 EARLY DEVELOPMENTAL DECISIONS IN EMBRYOS *C. ELEGANS*, W. B. Wood, C. Trent J. Manser, S. Burgess, P. Schedin, and I. Schauer, Deptartment of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO 80309. Early events in the *C. elegans* embryo are influenced by both maternally and embryonically acting genes, identified by mutations that result in abnormal or inviable embryos. General embryonic transcription in *C. elegans* appears to begin at about the time of gastrulation. We have obtained evidence for some transcription before this time, and have begun characterizing probable early transcripts.

Determination of the sex of the embryo in response to the ratio of X chromosomes to autosomes is a major early decision, in which the *her-1* gene plays a central role: high expression of *her-1* leads to male development and low expression to hermaphrodite development, based on earlier genetic work of J. Hodgkin and co-workers. Analysis of intersex mosaics indicates that this decision can be made independently in several of the major embryonic cell lineages. We have taken both genetic and molecular approaches to analyzing *her-1* expression and its control. Several loci that influence *her-1* expression have been identified as suppressors of a *her-1* dominant mutation. A transposon-induced *her-1* mutation has been used as the starting point to obtain genomic clones covering about 70 kb of DNA in the region of the *her-1* locus. Analyses using these clones to identify and characterize the *her-1* gene and its transcripts are in progress.

Molecular Neurobiology

K 018 MOLECULAR BIOLOGY OF DROSOPHILA NEUROGENESIS, S. Artavanis-Tsakonas, R. Fehon, D. Hartley, K. Johansen, A. Preis, R. Ramos and T. Xu, Yale University, New Haven, CT 06511-8112.

Neurogenesis in Drosophila appears to be under the zygotic control of a small number of genes, collectively known as the zygotic neurogenic loci. We are studying these genes in order to gain insight into the mechanisms responsible for the decision of undifferentiated ectodermal cells to follow the neural or the epidermal developmental pathway. The molecular analysis of the Notch locus, which phenotypically and genetically is the best characterized neurogenic locus, has provided important clues regarding the nature of the mechanism involved in these developmental events. We found that Notch codes for a large transmembrane protein with the extracellular domain showing homology to the epidermal growth factor and the intracellular part containing sequences reminiscent of nucleotide phosphate binding sites.

Based on these findings, we proposed that Notch and perhaps one or all of the other neurogenic loci may be involved in a mechanism that mediates and interprets cell interactions, which control the correct differentiation of the ectoderm (Cell 1985, 40:55). We are seeking experimental evidence in favor or against this working hypothesis on one hand by extending the analysis of the Notch locus and on the other by initiating a detailed analysis of Enhancer of split, another neurogenic locus known to phenotypically interact with Notch. The study of the Notch locus continues with the examination of point mutants, in vitro mutagenesis, germ line transformation experiments and cell biological studies with the aid of antibodies. We have cytogenetically localized the E(spl) locus on the 96F11-97A1 region and have isolated genomic and cDNA clones complementary to this chromosomal region. I will summarize our latest results in regard to the molecular analysis of these genes and discuss the evidence we have so far gathered implicating a cell-cell interaction mechanism in the control of developmental switches during early ectodermal differentiation.

Genetic and Cellular Mechanisms in Immaginal Disc Development

K 019 THE CELLULAR BASIS OF EPITHELIAL MORPHOGENESIS. Dianne Fristrom. Genetics Dept., University of California, Berkeley, CA 94720.

Cells of developing organisms can be classified into epithelia (cell sheets) or mesenchyme (single cells or cell aggregates). There are only a few basic cellular processes responsible for converting embryonic epithelia into the vast array of shapes represented in the animal kingdom. Cell rearrangements and cell shape changes are the most pervasive of the processes that lead to an overall change in shape of the epithelial sheet while maintaining its cohesive integrity. Invertebrates provide excellent systems for focusing on the capacity of epithelial cells to change shape and rearrange because, in contrast to vertebrates, epithelial morphogenesis often occurs independently of interactions with mesenchyme. The cellular basis of epithelial morphogenesis in some representative invertebrates will be reviewed.

Imaginal discs of Drosophila are particularly useful for investigating the molecular basis and genetic regulation of epithelial morphogenesis. These simple epithelial organelles arise as invaginations of the embryonic hypoderm, grow by division throughout larval life and then at the onset of metamorphosis undergo a dramatic change in shape to give rise to the appendages, head and thorax of the adult. Prepupal morphogenesis of leg discs takes place in vivo and in vitro in response to 20-hydroxyecdysone. Because this process occurs in the absence of cell division it provides a convenient system to study the molecular and cellular mechanisms of cell rearrangement and cell shape changes. Furthermore, because morphogenesis takes place in hormone regimens that suppress other developmental processes (such as cuticle synthesis), the possibility of identifying and cloning genes encoding the so far elusive morphogenetic molecules can been explored. Finally, complex genetic loci (eg. 2B5 and dpp) that appear to regulate different aspects of imaginal disc morphogenesis provide further tools with which to study the genetic regulation of morphogenetic processes.

K 020 PATTERNING EFFECTS OF THE DECAPENTAPLEGIC GENE COMPLEX IN DROSOPHILA, William M. Gelbart, Leila M. Posakony, Richard W. Padgett, R. Daniel St. Johnston, Ronald K. Blackman, M. Macy D. Koehler, Holly A. Irick, Vivian F. Irish and Raymond Grimaila, Dept. of Cellular and Developmental Biology, Harvard University, 16 Divinity Ave., Cambridge, MA 02138. The decapentaplegic gene complex (DPP-C) is a large genetic unit divided into three regions: shortvein (shv), Haplo-insufficiency near decapentaplegic (Hin-d) and decapentaplegic (dpp). We will focus on the effects of two of the regions: dpp and Hin-d. dpp mutations lead to the production of distally incomplete appendages due to the loss of pattern elements derived from the centers of all imaginal disks. Hin-d mutations can be show to produce the same effects on imaginal disk development as do dpp lesions, but in addition, homozygous Hin-d mutations are embryonic lethals, causing complete ventralization of the embryonic epidermis. Our present model is that ~12 kb of the shv - Hin-d region encodes several transcripts which overlap except at their 5' ends, generating the same (or similar) polypeptide(s); the polypeptide-coding region is entirely within Hin-d. This polypeptide is homologous to the members of the transforming growth factor-ß gene family, which includes TGF-ß, inhibins and Müllerian inhibiting substance. The dpp region appears to be a >25 kb array of 3' cis-regulatory elements. We have found two dpp^{+} isoalleles containing insertions of a copy of the 'hobo' mobile element in the dpp region. We have found that the hobo element can be mobilized in response to hybrid dysgenesis-like crosses. Employing this system, we have been able to generate internal deletions of the dpp region. The phenotypes produced by these deletions indicate that specific sites within the dpp region control a subset of the imaginal disk expression of the shv - Hin-d transcripts. Tissue in situ hybridization studies have revealed a developmentally evolving spatial pattern of expression of the shv - Hin-d transcripts. This pattern can be related to the phenotypes of mutations affecting embryonic and disk structures, and to the inferred location of a dpp focus of imaginal disk expression based upon clonal analyses of mosaic wings.

K 021 MOLECULAR ORGANIZATION OF THE 2B5 ECDYSTERONE-RESPONSIVE PUFF IN DROSOPHILA

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University of Pennsylvania, Philadelphia PA 19104-6017

Gene expression in *Drosophila melanogaster* can be observed by monitoring polytene chromosome puffing in the larval salivary gland genome. Analysis of puffing patterns displayed in developing third instar animals indicates that three puff sets are activated in a program of gene expression controlled, in part, by the steroid molting hormone ecdysterone. The sequential nature of this activation has been studied in several ways and indicates that the "early" puffs play a key role. Genetic and developmental analyses of the genes in the 2B5 early puff confirm this pivotal role in the regulation of this puffing pathway. More specifically, 2B5 gene products are necessary for the transcriptional activation of other puff sets and the absence of 2B5 gene products leads to the death of the animal.

We have used the techniques of transposon tagging, chromosomal walking, and breakpoint analysis to collect the genomic DNA corresponding to the 2B5 puff. Hybridization of labeled cDNA populations to this cloned and mapped region allowed for the recognition of at least nine regions that encode hormone stimulated transcripts or exons. Additional experiments including the analysis of several cDNA clones has revealed at least one 35 kb transcription unit which encodes a family of RNA species in the salivary gland and other tissues. The size heterogeneity among the members of this family is probably due to RNA processing.

K 022 GENE ACTIVITY DURING IMAGINAL DISC MORPHOGENESIS IN DROSOPHILA. Jeanette E. Natzle*, Ann S. Hammonds, John Moore, David Osterbur, Stephenie Paine-Saunders, James W. Fristrom, Department of Genetics, University of California, Berkeley, CA 94720, *Department of Zoology, University of California, Davis, CA 95616.

The Drosophila imaginal disc system is well-suited for analysis of the molecular events that contribute to epithelial morphogenesis. The processes that transform the single-layer disc epithelium into the adult appendages are initiated at pupariation by an increase in the level of the insect steroid hormone, 20-hydroxyecdysone (20-HOE). Utilization of a culture system that supports morphogenesis of imaginal discs in vitro in response to exogenously added 20-HOE has allowed us to investigate molecular aspects of this complex developmental process. Because rearrangement of cells within the epithelium is a central feature of disc morphogenesis, we have focused our attention on identification of hormone-dependent genes whose products might alter cell-cell and/or cell-substratum interactions during morphogenesis. We have used a differential hybridization screen to isolate six different Drosophila genes that encode hormone-inducible imaginal disc transcripts found on membrane-bound polysomes.[1] These genes are differentially expressed during imaginal disc morphogenesis in vitro, with three genes (IMP-E1, E2, and E3) active very soon (within 1 hour) after addition of hormone to the culture medium and three genes (IMP-L1, L2 and L3) primarily active later (after 8 hours). Analysis of the spatial distribution of transcripts has defined specific aspects of morphogenesis in which the gene products might function. Transcripts of one of the early genes, IMP-E1, are found in the epithelium in all imaginal disc types except the eye disc, in which morphogenesis occurs earlier and in a different manner. The transcript is also observed in specific cells in the prepupal brain, another organ that undergoes dramatic restructuring during metamorphosis. In these tissues IMP-E1 may then be involved in early alterations of cell associations during morphogenesis. In contrast, the transcript of one gene that is expressed primarily later during disc development (IMP-L2) has been localized to the peripodial membrane of imaginal discs and may therefore function during a second aspect of prepupal disc development, eversion of the disc to the exterior of the animal and fusion of the periphery of adjacent discs to form a continuous thoracic epithelium. Further analysis of the identity and localization of the gene products, as well as application of genetic techniques will provide a clearer understanding of the regulation and function of these genes during morphogenesis.

[1]Natzle, J.E., Hammonds, A.S., and Fristrom, J.W. (1986) J. Biol. Chem. 261: 5575-5583.

Juvenile Hormone, Gene Expression and Metamorphosis

K 023 STRUCTURE OF JUVENILE HORMONE-INDUCED COCKROACH OOTHECIN GENES, Richard N. Pau, Agricultural and Food Research Council, Insect Chemistry and Physiology Group, University of Sussex, Brighton BN1 9RQ, England.

Oothecins are structural proteins which form the egg cases of cockroaches and mantids. They are synthesised by an accessory sex gland: the left colleterial gland. Synthesis of eleven glycine-rich oothecins in the cockroach Periplaneta americana is induced coordinately by juvenile hormone during reproductive maturation of the adult female. The glycine-rich oothecins are encoded by a multigene family. The primary structure of 16 kDalton oothecins, derived from cDNA sequences, shows that the oothecin has a tripartite structure, consisting of a short central domain, flanked by arms of similar structure. The principal feature of the arms is the presence of extensive tandem repeats of the peptide Gly.Tyr.Gly.Gly.Leu. There are striking similarities between 16 kDalton oothecins and silkmoth chorion (egg shell) proteins. Genes for 16 kDalton oothecins have been cloned and the structure of the transcription units determined. Comparison of the sequences of the 5'-flanking DNA of juvenile hormone-regulated genes allows the identification of conserved features of possible regulatory significance.

K 024 RADIOLIGANDS FOR IDENTIFICATION OF RECEPTORS FOR JUVENILE HORMONES AND JH ANALOGS, Glenn D. Prestwich, Department of Chemistry, State University of New York, Stony Brook, New York 11794-3400.

High specific activity radioligands are required for the detection and characterization of macromolecular receptors for insect hormones and hormone analogs. Recently, we have made available five new classes of JH and JHA receptor probes labeled at >2000 Ci/mmol for no-carrier-added ^{125}I or up to 58 Ci/mmol for carrier-free reductions with $^{3}H_2$ gas.

1. Enantiomerically pure JH I and JH II in both the naturally occurring (10*R*, 11*S*) and the unnatural (10*S*, 11*R*) forms have been prepared at 58 Ci/mmol and used to demonstrate homolog and enantiomer specificity in larval lepidopteran hemolymph and epidermal JHBP.

2. Photoaffinity labels analogous to JH I and JH II possess diazoacetate groups and have been synthesized at 58 Ci/mmol in optically pure form. The racemic JH III analog, [^{3}H]-EFDA, is prepared at 11 Ci/mmol and has been used to photolabel JHBP from various tissues of caterpillars, flies, roaches, grasshoppers, termites, and beetles.

3. Hydroprene has been prepared at >55 Ci/mmol in a three-step sequence from (7*S*)-methoprene. The key step is protection of the readily reduced dienoate as an iron complex, followed by homogeneous tritiation.

4. Iodinated analogs of JH I (12-iodo-JH I) and of methoprene (iodovinylmethoprenol, IVMA) have been prepared and have *in vivo* JH activity in *Manduca*. Moreover, the [^{125}I]-labeled compounds are useful for receptor characterization in *Chironomus* and *Manduca*, respectively.

5. An [^{125}I]-labeled phenoxyphenyl ether IGR containing a 5-iodo-4-pentenyl group has been prepared by hydrostannylation and then electrophilic iodination of the pentynyl ether.

K 025 JUVENILE HORMONE AND THE CONTROL OF SEQUENTIAL GENE EXPRESSION IN INSECT EPIDERMIS, Lynn M. Riddiford, Department of Zoology, University of Washington, Seattle Wa 98195.

The insect epidermis produces a series of different cuticles as it grows, then metamorphoses. The production of this new cuticle is initiated by 20-hydroxyecdysone (20HE). When juvenile hormone (JH) is present, the same genes will be expressed; when it is absent, new genes can be expressed. In the tobacco hornworm, Manduca sexta, we have identified two classes of larval cuticle genes: I) Those expressed during the larval intermolt growth phase; II) Those expressed just before the onset of metamorphosis that cause a change in cuticular structure. Expression of 4 Class I genes is transiently turned off by 20HE acting in the presence of JH during each larval molt. When 20HE acts in the absence of JH to cause pupal commitment, 3 of these genes are permanently repressed. A fourth Class I gene is permanently turned off in the intrasegmental region but is expressed again in the flexible intersegmental regions in the pupa and adult just before ecdysis. The Class II cuticle genes are first activated by a small rise of ecdysteroid that occurs after the drop of JH in preparation for metamorphosis and appear within 3 hrs exposure to 20HE in vitro. These genes are also permanently repressed by the commitment rise of ecdysteroid. JH binds to epidermal nuclei with high affinity and presumably somehow prevents alterations in chromatin structure that are necessary for the permanent repression of larval genes and the activation of new genes. The structure of the larval cuticle genes and their regulation by JH will be discussed.

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K 026 REGULATION OF JH PRODUCTION BY CORPORA ALLATA, Barbara Stay and Charles R. Paulson, Department of Biology, University of Iowa, Iowa City, IA 52242.

The course of metamorphosis in insects is influenced by the interaction of ecdysteroids and juvenile hormone. In the viviparous cockroach, Diploptera punctata, the final larval stadium is characterized by a decline in juvenile hormone synthesis by the corpora allata (Paulson and Stay, 1985) and an increase in hemolymph concentration of ecdysteroids. We have shown by cutting nerves between the corpora allata and the brain that the decline in juvenile hormone synthesis is in large part elicited by way of the intact nerves between the corpora allata and the brain (Paulson and Stay, 1985). There are also humoral factors which inhibit the corpora allata. Ecdysteroid titer is high when corpora allata are inhibited and administration of ecdysteroids inhibits JH production by the corpora allata in vivo. In addition, a factor(s) from the brain inhibits the corpora allata through a humoral pathway (Paulson and Stay, 1987). This factor, which can be extracted from the brain of animals from a large range of developmental stages (Paulson, Stay, Kikukawa, and Tobe, 1987; Rankin and Stay, 1987), including embryos, inhibits the corpora allata in vitro.

Paulson, C.R. and Stay, B. (1985) J. Insect Physiol. 31: 625-630.
Paulson, C.R. and Stay, B. (1987) J. Insect Physiol. (in press)
Paulson, C.R., Stay, B., Kikukawa, S. and Tobe, S.S. (1987) J. Insect Physiol. (in press)
Rankin, S.M. and Stay, B. (1987) J. Insect Biochem. (in press)

K 027 JUVENILE HORMONE CONTROL OF GENE EXPRESSION IN LOCUST FAT BODY, Gerard R. Wyatt, Kathryn E. Cook and Michael R. Kanost, Dept. of Biology, Queen's University, Kingston, Ontario K7L 3N6, Canada.

In order to understand how juvenile hormone (JH) modulates gene expression in an insect tissue, we have been studying the effects of methoprene, an active, stable JH analog, on fat body of Locusta migratoria. In adult female fat body, two vitellogenin (Vg) genes are stimulated from total silence to initiate transcription. Transcripts from the two Vg genes are first detected about 24 hours after primary application of methoprene and about 12 hours after secondary stimulation, and then accumulate coordinately to become the major mRNAs in the cell. The two Vg genes show no cross-hybridization between most of their protein coding regions, but have apparent homology at the 5'-end and several blocks of sequence similarity in the 5'-flanking DNA which may be involved in regulation of transcription. In adult male fat body, the Vg genes are not expresssed, even under high doses of methoprene. Fat body of both sexes, however, responds to JH or methoprene by DNA replication, proliferation of rough endoplasmic reticulum, and moderate stimulation of general protein synthesis. Immunoassay with antisera for two major secreted proteins, apolipophorin III and arylphorin (storage protein), show that, though they are produced in fat body under JH deprivation, synthesis is stimulated several fold by methoprene, and the effect of the JH analog is observed at least by 6 hours, much earlier than the induced synthesis of Vgs. mRNA assays with cloned probes show elevation of apoLpIII mRNA earlier than the induced appearance of Vg mRNA. It is suggested that JH may have two distinct modes of action in locust fat body cells. The time-course and possible specificity of the early effect is being studied, and early events are being sought which might help to explain the time lag in activation of the Vg genes.

Vitellogenesis, Oogenesis and Fertilization

K 100 THE CHEMICAL PROPERTIES OF THE PERIVITELLINE FLUID OF THE FOURTH EMBRYONIC MOLT EMBRYO AND HATCHING STAGE TRILOBITE LARVA OF LIMULUS POLYPHEMUS. Brenda Doris, North Carolina A&T State University, Greensboro, NC 27411.

An electrophoretic, column chromatographic, and UV-spectroscopy study has been undertaken to determine what the chemical properties are that make up the perivitelline fluid of the American horseshoe crab, Limulus polyphemus, during the fourth embryonic molt and the hatching stage. Studies conducted using these techniques indicate that stage-specific: acid and basic proteins, carbohydrates, phospholipids, nucleic acids, acid glycoproteins, nuclear particles, respiratory proteins, and sulfated and nonsulfated mucopolysaccharides make up the egg fluid. Their role during this in not known. But, they may function in: (1) maintaining homeostasis, (2) serving as a source of food for the developing embryo, and (3) as a media that prevents dessication, infection and freezing.

K 101 IDENTIFICATION AND CHARACTERIZATION OF THE LOCUST VITELLOGENIN RECEPTOR, Hans J. Ferenz and Axel Roehrkasten, Dept. Biology, University, 2900 Oldenburg, F.R.G.

In the insect Locusta migratoria the yolk protein vitellogenin is the principal hemolymph protein selectively accumulated by maturing oocytes. The incorporation process has all characteristics of a receptor-mediated endocytosis. In membranes isolated from locust oocytes specific and saturable binding sites for vitellogenin can be demonstrated (Kd = $1x10^{-7}$ M). These binding sites can be solubilized from the membranes with the non-ionic detergent octyl-ß-D-glucoside. The solubilized receptor has a similar high affinity for vitellogenin (Kd = $4.2x10^{-8}$ M). The membrane-bound as well as the solubilized vitellogenin receptor is a heat and protease sensitive protein. It is unable to bind vitellogenin chemically modified at lysine and arginine residues. All results are in support of the hypothesis that the locust vitellogenin receptor exposes some negative charges and recognizes certain positive charges at the vitellogenin molecule. Studies to purify the vitellogenin for further characterization are in progress.

K 102 STRUCTURE AND EXPRESSION OF AN EGGSHELL GENE IN DROSOPHILA MELANOGASTER Franco Graziani, Carla Malva and Andrea Manzi, International Institute of Genetics and Biophysics, CNR, Naples, Italy

We have isolated two cDNA clones, one (140 bp long) from an egg chamber and another (570 bp long) from an adult female Drosophila cDNA library using as probe a phage from a 200 kb walk in the 32 region of the second chromosome. The 140 bp of the first clone were identical to the clone originating from the 34C bands of the second chromosome isolated by Mindrinos et al. (The EMBO J. 4: 147-153, 1985) and considered to correspond to a vitelline membrane protein gene. The 570 bp clone in Northern blots identifies a 470 base transcript expressed in a tissue-specific and stage-specific manner, stage 10 egg chambers. By sequence analysis we found that 60 bases have 90% homology with the Mindrinos clone. In situ hybridization of the 570 bp clone confirms localization of the gene that we isolated in the 32 region (32E) and stresses the finding that the vitelline membrane protein genes constitute a family whose members are located in the left arm of the second chromosome. In fact, Minoo et al. (Genetics 113: s52, 7.15, 1986) have isolated from the 26A region both genomic and cDNA clones which they report to code for the 23 and 17 kd eggshell proteins. Analysis of the molecular structure and expression of these genes could lead to a complete understanding of the follicle cell function during oogenesis.

K 103 Regulation of Maternal mRNA Utilization during Early Cleavage Stages of S. purpuratus, Rosemary Jagus, Wun-Ing Huang and Linnea J. Hansen, University of Pittsburgh, Pittsburgh, PA 15261.

Our previous studies, using cell-free translation systems from S. purpuratus eggs and embryos, have demonstrated an inhibitor of initiation in the unfertilized egg, that reduces 60S subunit interaction with the 48S, mRNA-containing, initiation intermediate. This inhibitor, found in the 100,000g supernatant, reduces mRNA utilization in cell-free translation systems from sea urchin embryos and from rabbit reticulocytes. The effects of this inhibitor can be prevented in cell-free translation systems from embryos and reticulocytes by the addition of purified eIF-4F, the factor that mediates the interaction of mRNA with the small ribosomal subunit. Other known initiation factors are without effect in preventing inhibition by the inhibitor or in potentiating the restoring effects of eIF-4F. The eIF-4F inhibitory activity is higher in unfertilized eggs than early embryos. The activity decreases gradually over the first 2-3 hr post-fertilization with a $T_{1/2}$ of 40 min. These data suggest that the low rates of mRNA utilization in the unfertilized egg may be attributable to low eIF-4F activity, and that the slow rise in translational activity post-fertilization may represent the gradual activation of eIF-4F. However, when eIF-4F is added to translation systems from unfertilized eggs, little stimulation is observed; the rate of protein synthesis remains at least 10 fold lower than in translation systems from embryos. This suggests that although the inhibitor of eIF-4F may be partially responsible for the low level of translation prior to fertilization, it is only one of the factors involved in the regulation of maternal mRNA utilization.

K 104 ANALYSIS OF DROSOPHILA MELANOGASTER FOLLICLE CELL PROTEIN VARIANT FORMS AND FEMALE STERILE MUTATIONS AFFECTING THEIR SYNTHESIS, Katrin Lineruth and Andrew Lambertsson, Department of Genetics, University of Umeå, S-901 87 Umeå, Sweden.

When analysing the ovarian protein synthesis pattern in a temperature sensitive *su(f)* allele, a set of three previously undescribed proteins was noted. These proteins were shown to be synthesized in the follicle cells of stage 10 egg chambers, and is therefore tentatively called Fc (follicle cell) proteins. Although the Fc proteins appear to be egg shell components, they differ from the vitelline membrane and chorion proteins in that they incorporate labelled methionine at a substantial level. Results from in vitro culture experiments suggest that the proteins are processed as the egg chamber develops. When screening geographic wild-type strains three variant forms has been found. The variation involves all three proteins, and as heterozygous females display both parental forms, we conclude that the variation is due to an altered structural gene. In the three variant forms all the three proteins in a set are smaller compared to the standard form. These variant forms made it possible by recombination analysis and cytogenetical mapping to localize a locus involved in the production of Fc proteins to 7C1-9 on the X-chromosome. Female sterile mutants, mapping in the same region as the Fc locus were analysed with respect to Fc synthesis. Two of these, *fs(1)384* and *fs(1)1501*, were shown to be involved in the production of Fc proteins. In the *fs(1)384*, no synthesis of Fc proteins could be detected. The *fs(1)1501* produce a set of proteins that is much larger than the standard Fc form. Our hypothesis regarding the nature of the variation, the nature of the sterile mutations, the processing of the proteins, and the function of the proteins will be discussed.

K 105 THE USE OF PROMOTER-FUSIONS TO STUDY THE REGULATION OF THE VITELLOGENIN GENES IN D. MELANOGASTER, David Osterbur, Barry Aprison, and J. Jose Bonner. Indiana University.

We have constructed a p-element transformation vector that fuses a portion of the 5′ end of the YP1 vitellogrnin gene with the coding region of the alcohol dehdrogenase (Adh) gene such that when the YP1 promoter is used, a functional Adh protein is produced. The 5′ region used in the fusion construct contains the portion of the promoter responsible for specific expression of YP1 in the fat body of adult female flies (Garabedian et al.; Cell 45:859; 1986). Several transformants show correct expression of Adh in the fat body adult female flies. When tested with a mutation in a known regulator of vitellogenins in Drosophila, the temperature-sensitive transformer (tra^{ts}) locus, the YP1-Adh fusion functioned as predicted. Flies that lack Adh activity die when exposed to ethanol; flies that have Adh activity die when exposed to 1-pentyn-3-ol. We have used this Adh selection systems to recover putative mutations in trans-acting regulators of the YP1 promoter.

K 106 ISOLATION AND CHARACTERIZATION OF cDNA AND GENOMIC CLONES ENCODING SEA URCHIN EGG DERIVED SPERM ACTIVATING PEPTIDES, E. William Radany, Marine Research Laboratory, Battelle, Pacific Northwest Laboratories, Sequim, WA 98382.

Eggs and/or secretions of the female reproductive tract from many animals appear to stimulate spermatozoan metabolism and motility. Recently, a series of peptides have been isolated from the eggs of several species of sea urchins and shown to have pronounced effects on homologous sperm. In addtion to stimulating respiration and motility, the interaction of peptides with their receptors causes a net H+ efflux leading to intracellular alkalinization, elevated cellular cyclic AMP and cyclic GMP concentrations, and a rapid decrease in guanylate cyclose activity.

We have used synthetic oligonucleotide probes, based on the amino acid sequence of the peptide speract, to screen a λgt10 cDNA library prepared from Strongylocentrotus purpuratus female gonads. Several clones were isolated and appear to represent divergent members of a multigene family. One clone, pSp1, was found to contain an insert of 145 bp and consist of of a single open reading frame which contained the coding region for the peptide speract. This clone was subsequently used to probe a S. purparatus λL47 genomic library. The genes coding for speract in the sea urchin S. purpuratus appear to be encoded in a tandemly repeated unit approximately 1500 bases in length. The clone, pSp1, was then used to screen Arbacia punctalata, Strongylocentrotus franciscanus, Strongylocentrotus droebachiensis, and Lytechinus pictus genomic libaries. Positive clones were identified in libraries from S. franciscanus, S. droebachiensis, and L. pictus and preliminary sequence analysis indicates that they contain genes highly homologous to the genes encoding spearact.

K 107 PROPERTIES OF THE MOSQUITO YOLK PROTEIN; A STUDY USING MONOCLONAL ANTIBODIES, Alexander Raikhel, Department of Entomology, Michigan State University, East Lansing, MI 48824

Yolk protein of the mosquito Aedes aegypti was analysed with radiolabeling experiments and monoclonal antibodies (mABs). Labeling with 35_S-methionine as well as immunoblot analysis with mABs demonstrated that during vitellogenesis two polypeptides (195 ± 5 kDa and 65 ± 5 kDa) were synthesized and secreted by the fat body and were subsequently accumulated by developing oocytes. Further analysis utilized yolk proteins from the fat body and ovaries which were radiolabeled either by 125_I in situ or by 35_S in vivo. Immunoprecipitation experiments with mABs demonstrated that these two polypeptides were the subunits of the mosquito yolk protein. Both vitellogenin (VG), secreted by the fat body, and vitellin (VT), accumulated by oocytes, consisted of these two subunits. The yolk protein subunits were not covalently associated since they behaved similarly after reduced and non-reduced SDS PAGE. Non-denaturing PAGE indicated that electrophoretic mobilities of the native VG was the same as that of solubilized VT either from developing oocyte or from newly-laid eggs. The native molecular weight was estimated at 480 ± 20 kDA. Labeling with 32_p-orthophosphate revealed that both subunits of VG were phosphorylated within 15 min of the synthesis and they remained phosphorylated after their processing into VT by the oocyte. Similarly, both subunits of VG and VT were glycosylated. Their carbohydrate moeities were composed of mannose and N-acetylglucosamine.

K 108 EVOLUTIONARY CHANGES IN SILKMOTH CHORION GENE EXPRESSION, Jerome C. Regier, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60201.

Discrete changes in silkmoth choriogenesis have occurred during evolution, as exemplified in *Antheraea polyphemus* and *Hyalophora cecropia*. At the level of morphology, the chorion of *A. polyphemus* has surface structures, called aeropyle crowns, that are absent from *H. cecropia*. Aeropyle crowns form during the very late period of choriogenesis and consist of two substructures -- lamellae and filler. Filler is present in *H. cecropia* in greatly reduced amounts. At the level of protein synthesis, overall similarities in the two species are maintained until the very late period of choriogenesis, when synthesis of aeropyle crown components is maximal. In *H. cecropia*, very late period-specific proteins are reduced in number and abundance. Several of these minor proteins are candidates for E1 and E2, the components of filler. E1 and E2 RNAs are about 35 times more abundant in *A. polyphemus*, despite very similar gene copy numbers and times of expression in the two species. These results support two hypotheses: 1) that evolutionary changes in chorion morphology have resulted from regulatory changes in the expression of chorion genes, either at the level of transcription or mRNA decay, and 2) that evolutionary changes in chorion morphology are based on addition onto a preexisting developmental program.

Cell Lineages and Pattern Development

K 200 GENETICS AND MOLECULAR BIOLOGY OF POLYHOMEOTIC, A GENE REQUIRED FOR SEGMENT DETERMINATION IN DROSOPHILA MELANOGASTER, Hugh W. Brock, Sally Freeman, Joani McKeon, Mark DeCamillis, J-M. Dura, N. Randsholt, J. Deatrick, P. Santamaria, U. British Columbia, Vancouver, Canada V6T 2A9, and CGM, CNRS, Gif-sur-Yvette, France.

Polyhomeotic (ph) is a complex locus in Drosophila that is required for normal segment determination, and for development of cuticle. Genetic analysis shows that there are two mutable regions at the ph locus, each of which is required for normal function of the gene. Molecular analysis of the ph locus reveals the presence of two large, tandem, highly conserved regions, both of which are transcribed. Analysis of transcripts shows a complex pattern of transcription that changes during development. We are currently cloning and mapping cDNAs from the region, and have begun sequence analysis of the gene. These studies should allow us to correlate the genetic data with the gene structure, and may provide information about the function of the ph product in segment determination and cuticle development. ph differs from other loci that are thought to regulate the Bithorax Complex because it has a strong maternal and zygotic effect, and because it affects development of the cuticle. It could be that the absence of the ph product has only a secondary effect on segment determination and cuticle formation, or that the ph product has a general role in the maintenance of determination and the disturbance is most readily detected in segments and cuticle, or that the ph product has a specific role in development.

K 201 TRANSPOSON RESCUE OF MUTATIONS AT DECAPENTAPLEGIC IN DROSOPHILA, F. Michael Hoffmann and William Goodman, University of Wisconsin, Madison, Wisconsin 53706. The Drosophila decapentaplegic gene (dpp) is required for proper dorsal/ventral pattern formation in the embryo and for proper growth of the imaginal disks. It encodes a protein with homology to the TGF-β family of peptide hormones. To define what portions of the 50 kb dpp gene are required for function, we are assaying molecularly defined portions of dpp for their ability to rescue dpp mutant phenotypes in transgenic animals. An 8 kb EcoRI fragment from the center of the dpp DNA was inserted into a P-element transposon. Eight independent lines of the transposon were tested for rescue of a variety of dpp mutant phenotypes. The transposon is able to rescue flies with haplo-insufficient, embryonic lethal deletions of dpp to wild-type adults. As determined by rescue of mutant phenotypes the 8 kb of dpp on the transposon is sufficient for embryonic dorsal/ventral pattern formation, but is not sufficient for the other functions of dpp in the larvae and the imaginal disks.

K 202 IDENTIFICATION OF GENES REQUIRED FOR CYTOPLASMIC LOCALIZATION IN C. ELEGANS. Kenneth J. Kemphues, James Priess*, Niansheng Cheng, and Diane Morton. Section of Genetics and Development, Cornell University, Ithaca NY 14853. *MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH England.

Pattern formation in the development of many invertebrates is dependent upon proper localization and partitioning of maternally-provided cytoplasmic factors. We have isolated eleven strict maternal-effect lethal mutations identifying four genes that are required for proper cytoplasmic localization and partitioning in the nematode C. elegans. Mutations at these four partitioning loci (par-1 through 4) are remarkably similar in their affect on early cleavages. Mutations at all loci lead to defects in: 1) localization of germ-line-specific P granules, 2) timing of cell divisions, and 3) positioning and orientation of mitotic spindles. The exact nature of the defects and the extent to which they are expressed are characteristic of the mutant allele and the locus. Late embryonic phenotypes of the mutants also vary. All affected embryos produce large numbers of differentiated euploid cells. Embryos from par-1 and par-4 mutant mothers do not undergo morphogenesis, arresting as clusters of differentiated cells, but are specifically lacking in gut cells. Some embryos from mutants at par-2 and par-3 exhibit phenotypes identical to those of par-1 and par-4 but others survive to adulthood. The survivors are sterile, apparently lacking germ line cells. The phenotypes of the mutants suggest that the action of these genes is required for the functioning of a maternally-derived system for cytoplasmic localization and partitioning during early cleavage, and that proper cytoplasmic partitioning is required for specification or maintenance of the gut and germ-line cell lineages.

K 203 CHARACTERIZATION OF MONOCLONAL ANTIBODIES WHICH RECOGNIZE EMBRYONIC NUCLEI OF THE SEA URCHIN, S. PURPURATUS, Patricia Kuwabara and Eric Weinberg, University of Pennsylvania, Philadelphia, PA 19104

Embryonic nuclei from blastula stage sea urchin embryos were used as a source of antigen to prepare monoclonal antibodies. Two MAbs, 5F6-11 and 5B9-5, generated by this procedure are discussed. MAb 5F6-11 recognizes a protein doublet with molecular weight of approximately 68kD as determined by Western blot analysis. Indirect immunofluorescence using paraffin embedded embryo sections shows that the protein detected by MAb 5F6-11 is not present during early cleavage and accumulates in the nucleus at the morula/early blastula stage. This pattern is also reflected by Western blots as an increase in the amount of this protein doublet from the morula stage onward. Consistent with this finding, immunoprecipitation results indicate that synthesis of the proteins begins during late cleavage. The major nuclear protein recognized by MAb 5B9-5 has a molecular weight of 65kD. Indirect immunofluorescence studies indicate that the protein is present in all nuclei at every stage of development. Western blots also indicate that the 65kD protein, although present at all stages, decreases in amount per nucleus as development procedes. These results show that quite different temporal programs of appearance of nuclear proteins can be revealed using MAb probes. Such probes will also be useful in identifying nuclear proteins with spatial localization in the embryo.

K 204 ARE THE DROSOPHILA POSITION-SPECIFIC ANTIGENS RELATED TO FIBRONECTIN RECEPTORS IN VERTRBRATES? M. Leptin and M. Wilcox, MRC Lab. of Molec. Biology, Cambridge CB2 2QH, U.K.

The Position-specific (PS) antigens were discovered in a search for molecules conveying differences in cell surface properties that might be responsible for the segregation of cells belonging to different developmental compartments. They are a family of related cell surface glycoprotein complexes showing spatially restricted patterns of distribution. Changes in these patterns correlate not only with compartmentalization but also with other morphogenetic events like the formation of folds and grooves during tissue formation and so suggest a general role for the complexes in morphogenesis. Their overall structure and their biochemical properties resemble closely those of a group of vertebrate receptors involved in cell migration and attachment (e.g. fibronectin and vitronectin receptors, VLAs and the leukocyte antigens Mac-1 and LFA1). The N termini of the α subunits of some of these molecules show homology with the N terminus of a PS antigen α subunit. We propose that the position specific antigens in Drosophila are homologous to these receptors in vertebrates. Thus, PS antigens probably act in a morphogenetic mechanism not peculiar to Drosophila, but generally required for the construction of multicellular organisms.

Wilcox et. al, Cell 25, 159 (1981).
Wilcox, M. and M. Leptin, Nature 316, 381 (1985).
Leptin, M. and M. Wilcox, Bioessays 5; 204 (1986).

K 205 MOLECULAR CHARACTERIZATION OF THE POD AND TER LOCI: TWO BLASTODERM-SPECIFIC GENES OF DROSOPHILA, Paul A. Mahoney, Paul D. Boyer, Richard M. Baldarelli & Judith A. Lengyel, Molecular Biology Institute and Dept. of Biology, UCLA, Los Angeles, CA 90024.

The polebud (pod) and terminus (ter) loci were obtained in our screen for blastoderm specific genes (Roark, et al., Devel. Biol. 109, 1985). The 7.1 kb pod locus encodes 3 overlapping transcripts of 4.5, 3.0, and 2.7 kb. The sequence of the blastoderm-specific 2.7 kb transcript encodes an 84 kd acidic protein. Database homology searches with the predicted amino acid sequence reveal a 98 amino acid domain in the pod protein with structural similarity to a domain in the fos oncogene protein, in addition to a 21 amino acid segment in the pod protein that is similar to a repeated segment in tropomyosin. Preliminary in situ hybridization experiments to embryos indicate that pod RNA first appears in the pole buds, just before the pole cells form.

The ter locus contains two 1.5 kb blastoderm-specific transcription units which encode the same predicted 49 kd protein. Within this protein sequence is a region with homology to the framework amino acids of the "DNA-binding finger" of Xenopus transcription factor IIIA. Ter RNA is distributed in a concentration gradient along the anterior-posterior axis of the embryo, and is most concentrated in the posterior. It is also distributed in a gradient along the dorsal-ventral axis of the embryo, and is most concentrated ventrally. Ter RNA concentration is maximal at early nuclear cycle 14. As nuclear cycle 14 (the cellular blastoderm stage) progresses, the concentration of ter RNA decreases sharply. During gastrulation, ter RNA continues to decrease, remaining most concentrated in the posterior midgut/proctodeal primordium.

K 206 SEGMENTATION AND SEGMENT IDENTITY FUNCTIONS OF THE DROSOPHILA HUNCHBACK GENE, Jonathan S. Margolis, Chung W. Shim, Suzanne F. Seavello, and James W. Posakony, UC San Diego, La Jolla, CA 92093.
The formation of segments and the assignment of segment identity in the insect embryo have often been interpreted as distinct processes. The existence of homeotic alleles of certain segmentation genes, however, suggests a closer connection between the two. For the gap gene hunchback (hb), two dominant homeotic alleles exist. The first (Regulator of postbithorax) exhibits a dominant postbithorax phenotype. We have isolated a second dominant hb allele which has postbithorax and bithoraxoid homeotic transformations, a dominant pair rule-like segmentation defect, and a localized depression of wild type hb function. This allele is semi-lethal in heterozygous combination with a deficiency for the pair rule segmentation gene even-skipped. A genomic region containing the wild-type hb gene has been cloned and breakpoints associated with chromosomal rearrangements affecting hb function have been mapped. Our current efforts are directed at understanding both normal hb function and the specific defects underlying the mutant phenotypes. A structural analysis of the hb gene includes DNA sequencing of genomic and cDNA clones derived from both wild-type and mutant animals. Functional interactions between hb and other genes are also being studied using antibody and nucleic acid probes to examine in situ the distribution of these other genes' products in hb mutant embryos.

K 207 THE DECAPENTAPLEGIC GENE COMPLEX OF *DROSOPHILA* ENCODES A PROTEIN HOMOLOGOUS TO THE TRANSFORMING GROWTH FACTOR-ß GENE FAMILY, Richard W. Padgett, R. Daniel St. Johnston and William M. Gelbart, Harvard University, Cambridge, MA 02138.

Recent work from our laboratory has implicated the decapentaplegic gene complex (DPP-C) in several events in pattern formation during *Drosophila* development. During embryogenesis, DPP-C participates in the establishment of dorsal-ventral specification. Later, it is required for the proper morphogenesis of the imaginal discs, which will form much of the adult epidermis. To gain insight into the role it plays in positional information, we have undertaken a molecular analysis of the DPP-C. The DPP-C appears to consist mostly of *cis*-regulatory information controlling the expression of overlapping transcripts which differ at their 5' ends, but which share the bulk of their transcribed sequences. The C-terminus of the deduced protein encoded by these RNAs exhibits strong sequence homology (25% to 38% amino acid identity) to the C-termini of a class of mammalian proteins. This class includes transforming growth factor-ß (TGF-ß), inhibin and Müllerian inhibiting substance (MIS). These proteins act on target cells to produce a variety of responses, such as stimulation or inhibition of cell division or differentiation. Considerations of protein structure and homology suggest that the DPP-C protein contributes to proper morphogenesis through its action as a secreted factor involved in the differential regulation of cell growth.

K 208 A NEW HOMEOBOX GENE IN DROSPHILA MELANOGASTER, Robert B. Saint and Bill Kalionis CSIRO Division of Entomology, GPO Box 1700, Canberra City, ACT
We have isolated a new member of the homeobox family of genes in Drosophila melanogaster using a synthetic oligonucleotide homologous to the most conserved region of the homeobox sequence. The cytological location of this gene, at 97D on the right arm of chromosome 3, does not correspond to the location of any known developmental pattern mutants. The predicted amino acid sequence of the homeobox region is equally divergent from the corresponding Antennapedia and engrailed sequences (approximately 50% homology with both). Preliminary analysis suggests that the gene is first expressed about the time of cellular blastoderm formation. We are currently carrying out a genetic analysis of this region together with a more detailed analysis of the molecular nature of the gene and its pattern of expression.

K 209 TRANSCRIPTION IN EARLY EMBRYOS OF C. ELEGANS, Irene E. Schauer and W.B. Wood, Department of MCD Biology, University of Colorado, Boulder, Colorado 80309.
Early embryos of many species appear to be transcriptionally inactive, relying on maternal messenger RNA's until their own genomes are activated. General transcriptional activation in amphibian embryos occurs at the so-called mid-blastula transition. However, recent work with Drosophila has shown that several developmentally important genes are transcribed before activation of general transcription. We are characterizing early transcription in embryos of the nematode Caenorhabditis elegans, in which the time corresponding to mid-blastula transition is unclear. General transcriptional activation in these embryos probably occurs at about the time of gastrulation.

We have established methods for obtaining large quantities of early (<30 cells) synchronized embryos. These embryos are homogenized to obtain either isolated intact nuclei or crude nuclear extracts, both of which actively incorporate ^{32}P-labeled nucleotides into run-on transcripts. Using transcripts from these preparations and from similar preparations made with post-gastrulation embryos for comparison, we have screened a genomic clone library for sequences that are only or preferentially transcribed early. So far 12 such clones have been isolated. Fragments corresponding to the early transcripts have been identified and are being used to further characterize these early genes by "Northern" analysis and in situ hybridization. We are also looking at incorporation of ^{32}P-labeled nucleotides into permeabilized embryos in order to quantitate general transcriptional activity at various stages in vivo.

K 210 DEVELOPMENTAL REGULATION OF AN INSULIN/EGF BINDING PROTEIN IN DROSPHILA, M. Patrizia Stoppelli[1], Stuart J. Decker[2] and Marsha R. Rosner[1,2] Massachusetts Institute of Technology[1], P. Cambridge, MA and Rockefeller University[2], New York, NY. A Drosophila protein (dp100) that binds both insulin and EGF related growth factors has been described (Thompson et al. (1985 Proc. Natl. Acad. Sci 82, 8443). This protein was originally identified in Kc and Schneider Drosophila cell lines using an anti human EGF receptor antibody and affinity labelling with insulin. To further characterize the biological role of dp100, we quantitated the level of the protein in response to differentiation stimuli. Initially, we determined whether dp100 synthesis is regulated by ecdysone, a hormone that induce differentiation of both Kc and Schneider L3 cell lines. The results indicate that the level of dp100 increases in response to ecdysone with a kinetics similar to that of the overall protein. In vivo studies show that dp100 is also present in the adult flies and in the different stages of development; specifically, in the early and late embryos, in the first, second and third instar larvae, in the early and late pupae, and in the bodies and heads from adult flies. A dramatic increase in the level of dp100 from the embryonal stage to the adult was observed, although the highest level of dp100 appeared to be in the Kc and Schneider cell lines. These results indicate that dp100 is developmentally regulated in the intact organism.

K 211 FOUR COMPARTMENTS MAKE A SEGMENT IN Oncopeltus, David A. Wright Edinburgh University & Albert Einstein College of Medicine, Bronx NY 10461

Growth restrictions during development in the hemipteran insect Oncopeltus fasciatus were revealed after epidermal transplantation. Drosophila clonal analysis techniques were mimicked by grafting mutant unpigmented skin cells to similar positions in pigmented wild type larvae. Thin strips of donor tissue were used to mark putative compartment boundaries in the dorsal abdominal epidermis, then followed over three moults. Constraints on the intermingling of graft and host tissue during proliferation were used to define compartments. The criterion for a compartment boundary was an unusually straight graft-host interface at a constant anatomical position respected even by overgrowing grafts.

Unlike adult Drosophila, Oncopeltus has four compartments per body segment. At the midline of segment A3 the boundaries were at 85, 62, 20 and 100/0% segment length. Surgical ablation of discrete compartments generated abnormal segment patterns. Removal of 1, 2, 3 or 4 compartments from Oncopeltus phenocopies the range of patterns in embryonic segmentation mutants of Drosophila. Since deletion of a single compartment in Oncopeltus causes loss of pattern elements without pattern reversal or intercalation (presumably because abnormal neighbours are not recognized), mutants (e.g. lin) which only delete pattern elements may define single compartments in Drosophila. I propose that the typical insect segment forms as 4 different compartments, the Drosophila embryo included.

Neurobiology, Imaginal Discs, Metamorphosis

K 300 FACTORS CONFERRING RESISTANCE TO ENDOSULFAN IN DEVELOPMENTAL BIOLOGY OF **Musca domestica**, Iqbal Ahmad and P.K. Ray, Industrial Toxicology Research Centre, Post Box No. 80, Mahatma Gandhi Marg, Lucknow 226001, India.

Insect pests and vectors resistance to insecticides has been the subject of great concern in recent years. Studies on the mechanism of resistance has been of great help in devising the appropriate combat measures against resistance. Alterations on target sites and detoxification systems have been attracting a great deal of attention to understand the mechanism of resistance. Studies on these lines were largely confined to adult stages, thereby, informations on these vital aspects during developmental biology of pests and vectors and specially with respect to endosulfan resistance remained scanty. An attempt was, therefore, made to elucidate the alterations on receptors and detoxification systems. ATPases (Mg^{++} (OS), Mg^{++}(OIS)-, Ca^{++}-, Na^{+}/K^{+}- and HCO_3^- -stimulated) were acknowledged as the possible receptors to endosulfan. The ATPases in resistant fly showed reduced sensitivity to endosulfan which was generally more in larval stage followed by adult and pupal. Sensitivity of these receptors also varied with the developmental stages. Cytochrome (P-450 and b5), cytochrome c reductase (-NADPH and -NADH) and glutathione-s-transferase, were acknowledged as key representative enzymes of detoxification system in the present study. These enzymes behaved differently in larval, pupal and adult stages. Results obtained on these lines will be presented and discussed.

K 301 CONTROL OF YELLOW GENE EXPRESSION IN DROSOPHILA DEVELOPMENT, Harald Biessmann and James M. Mason, Developmental Biology Center, University of California, Irvine, CA 92717 and NIEHS, Research Triangle Park, NC 27709.

The yellow (y) gene is responsible for the specific pattern of melanin pigmentation in the cuticle of the adult fly and of the larval mouth parts in Drosophila. It differs from other genes that affect body coloration by the fact that several alleles of this gene are known (y^2-type) that specifically alter the pattern of coloration rather than the overall intensity. Y gene expression during late pupal development, when the adult cuticle is formed, must be coordinated with the tyrosine metabolic pathway that is under control of ecdysteroid hormones and generates metabolites for cuticle sclerotization as well as precursors for black melanin. We have isolated the y gene by molecular cloning, determined its expression in all developmental stages, sequenced its cDNA and mapped DNA rearrangemnts of several y^1-type (null) and y^2-type alleles. These y^2-type alleles are very likely due to mutations that affect regulation of y gene expression, resulting in allele-specific mosaic pattern of cuticle coloration due to wild type (black) expression in some parts and mutant (yellow) in other parts of the cuticle. Mosaic expression of the y gene in the adult cuticle can either be caused by insertion of a "gypsy" element about 650 bp upstream from the start of transcription, or by terminal deficiencies that break in the 5' regulatory region of the y gene. The effect on y gene expression is presumably caused by juxtaposition of repeated telomeric DNA sequences. The y^2-type phenotype of these terminal deficiencies shows variegation in bristle pigmentation, and is unstable over a period of two years, gradually changing to the y^1-type pigmentation state.

K 302 **SENSORY NEURON TRANSFORMATION IN cut MUTATIONS**, **Rolf Bodmer, Susan Shepherd, Lily Y. Jan and Yuh Nung Jan, Howard Hughes Medical Institute, University of California, San Francisco CA 94143.**

The identity of sensory neurons in Drosophila is under genetic control. In the wild-type embryo there are three types of sensory neurons: neurons sending single dendrites to innervate external sensory structures (es neurons), neurons with single dendrites innervating internal chordotonal organs (ch neurons), and neurons with multiple dendrites (md). Mutations null for the cut gene function transforms es neurons into chordotonal neurons, while leaving md neurons and endogenous ch neurons unaffected. External sensory structures are essentially absent. Besides null mutations, mutations of the cut lethal I group cause similar but less complete transformation. Analysis of adult mosaic flies gives a similar transformation of es neurons into ch neuron, but the sensory bristles are still present in most parts of the body. Studies of mosaic larval and adult flies indicate that the effect of the cut locus is primarily cell autonomous, and that the cut gene function is required for acquiring the identity of most larval and adult es neurons.

K 303 MOLECULAR ANALYSIS OF A CHROMOSOMAL INTERVAL INCLUDING DELTA, A NEUROGENIC GENE IN DROSOPHILA MELANOGASTER. Kim Fechtel, Althea K. Alton, Casey C. Kopczynski, Scott B. Shepard, and Marc A.T. Muskavitch, Indiana University, Bloomington, IN 47405.
The neurogenic genes of Drosophila encode functions that regulate the segregation of embryonic ectoderm into neural and epidermal lineages and affect the development of imaginal, ectodermally derived structures. In an effort to analyze one locus that affects this process, we have initiated a chromosomal walk in region 92A on the right arm of chromosome three, which includes the Delta locus. We have made use of a collection of 60 Delta mutations, induced on known genetic backgrounds, to map changes in restriction fragment length along this walk that correlate with Delta mutations. These changes, when compiled and superimposed on the transcript map of the chromosomal walk, reveal the extent of the chromosomal interval in 92A1 that is essential for Delta function.

K 304 A PROTEIN MARKER FOR DIFFERENTIATION IN AN INSECT ACCESSORY GLAND. George M. Happ, Hiroshi Shinbo, and Marnix Peferoen, Department of Zoology, University of Vermont, Burlington, VT 05405.
Growth and differentiation of the bean-shaped accessory glands of mealworm beetles are dependent upon a mid-pupal ecdysteroid peak. The glands secrete adult-specific proteins, termed spermatophorins, that form the sac which encloses sperm during its transfer to the female. We have isolated and characterized a secretory protein with apparent molecular weight of 23-kDa on SDS-polyacrylamide gels. This secretory protein is precursor to a spermatophorin of the same size. Total gland homogenate was fractionated by gel filtration and the 23-kDa antigen was isolated by its affinity for a monoclonal antibody immobilized on a column of Affigel-10. On native polyacrylamide gels, the antigen has an apparent molecular weight of 370-kDa. The secretory antigen is distinguished by being high in glutamic acid/glutamine (15.4%) and in proline (25.2%). To infer the sequence of amino acids in this protein, we attempted to determine the base sequence of the corresponding messenger RNA. To establish that sequence, we prepared cDNA, copied from the total poly(A)$^+$ RNA of the adult glands and cloned the cDNAs in the expression vector λgt11. The library was screened with monoclonal and polyclonal antibodies to the 23-kDa antigen. Eleven clones producing proteins with reactive epitopes were purified to homogeniety. Inserts from selected clones were sequenced. The sequence will be discussed and the composition of this protein will be compared to that of other insect structural proteins.

K 305 MALE-SPECIFIC TRANSCRIPTS IN THE ACCESSORY GLAND OF DROSOPHILA MELANOGASTER. Scott A. Monsma and Mariana F. Wolfner, Cornell University, Ithaca NY 14853.
The accessory gland is an internal genital disc derivative found in the adult male. This tissue contributes a number of substances to the ejaculatory fluid; components of its secretion are known to affect the female's behavior following copulation, decreasing her receptivity to further copulation and increasing oviposition (1).

We are studying two polyA+accessory gland transcripts derived from the 26A chromosomal region. The transcripts, of 0.9 and 0.55 kb in length, are produced only in the adult male accessory gland. The genomic DNA fragment homologous to the transcripts has been sequenced and the transcription units have been localized within this region. The two RNAs are transcribed from the same strand of DNA and overlap.

The expression of both accessory gland transcripts has been examined in mutants affecting various aspects of sexual development. Both RNAs are made in normal males, germlineless males, XO males, and in somatically transformed females (X/X; tra/tra). Temperature shift experiments were performed using the tra2^{ts2} mutation (2) to determine the temperature-sensitive period for expression of these two transcripts. The genital disc cells become committed during early pupal life to produce both of the accessory gland transcripts in the adult. Adult expression of both transcripts is insensitive to temperature shifts of X/X; tra2^{ts2}/ tra2^{ts2} animals after the commitment is made.

1. Chen, P.S. (1984) Ann. Revs. Entomol. 29 233-255
2. Belote, J.M. and Baker, B.S. (1982) PNAS 79 1568-1572

K 306 ENTACTIN, A SULFATED GLYCOPROTEIN OF DROSOPHILA BASEMENT MEMBRANES, Pamela F. Olson, Rachel Sterne, Liselotte I. Fessler, and John H. Fessler, Department of Biology and Molecular Biology Institute, University of California, Los Angeles, CA 90024

Entactin is a sulfated glycoprotein of vertebrate basement membranes (1). A closely similar protein has been isolated from Drosophila. Entactin was purified from the culture media of Drosophila Kc cells and murine PF-HR9 cells. Drosophila and mouse entactin comigrate in Sepharose CL-6B gel filtration and SDS-PAGE; the mobility is not changed by reduction and corresponds to one 155 kd peptide. Drosophila and mouse entactins are highly glycosylated, as indicated by a strong PAS reaction. They are sulfated on tyrosine residues as determined after $Ba(OH)_2$ hydrolysis by electrophoresis of tyrosine sulfate. Antisera raised against Drosophila entactin gave immunofluorescent staining of Drosophila embryo sections that co-localized with anti-laminin staining of basement membranes.

A Kc cell cDNA library constructed in the lambda gt11 expression vector has been screened with the antiserum against Drosophila entactin, and 6 unique cDNA clones have been isolated. These clones hybridize to a 4.0 kB message by Northern blotting. The size of this message is appropriate to code for the entactin core polypeptide. Further characterization is proceeding.

1. Bender, B.L., Jaffe, R., Carlin, B., and Chung, A.E. (1981) Am. J. Pathol. 103:419-426.

K 307 CELL RECOGNITION DURING NEURONAL DEVELOPMENT IN DROSOPHILA: EXPRESSION AND MOLECULAR GENETICS OF THE FASCICLIN III GLYCOPROTEIN. Nipam H. Patel, Peter M. Snow, and Corey S. Goodman, Department of Biological Sciences, Stanford University, Stanford CA 94305.

During neuronal development, growth cones display a selective affinity for specific neuronal cell bodies and axons. The 7G10 monoclonal antibody (MAb) recognizes a surface antigen expressed on a subset of neuronal cell bodies and axon fascicles in the Drosophila embryo, and immunoprecipitates 4 highly related surface glycoproteins called fasciclin III. We isolated the fasciclin III gene by cDNA expression cloning using antisera raised against each of these related proteins; the gene maps on the 2nd chromosome to 36E1. Df(2L)H20/Df(2L)VA18 trans-heterozygote embryos are deficient for the fasciclin III gene and only a few other genes. Such embryos have a quite normal central nervous system at 12 hr, with the exception of subtle yet consistent abnormalities in the axon pathways of a small number of neurons. At the level of interactions between the growth cones, axons, and cell bodies of identified embryonic neurons which normally express fasciclin III, the phenotype of this small deficiency is consistent with the notion that fasciclin III is a neuronal recognition molecule which either modulates or mediates cell adhesion in the Drosophila embryo.

K 308 AN ANTIBODY THAT RECOGNIZES A 20-HYDROXYECDYSONE MODULATED CELL SURFACE/SECRETED ANTIGEN DURING MORPHOGENESIS IN DROSOPHILA, Wayne L. Rickoll and Samuel Galewsky, Texas A&M University, College Station, TX 77843.

20-Hydroxyecdysone (20-HOE) affects cell-cell interactions and causes extensive changes in cell surface proteins in Drosophila imaginal discs and in 20-HOE-responsive Drosophila cell lines. Some of these proteins may function in specific cell rearrangements of imaginal disc evagination and in the aggregation response in cell lines. Using the S3 cell line, we have identified, via Lens culinaris affinity chromatography, a group of cell surface antigens showing a 20-HOE-dependent increase in iodination. Several of these antigens increase in metabolic labeling in response to hormone, and are secreted into the medium as soluble glycoproteins. Experiments using the ionophore monensin indicate that this is a Golgi-mediated secretory event. We have produced an antibody against one of these hormone-dependent cell surface/secreted glycoproteins with a relative mobility of 116,000(M_r). This antibody immunoprecipitates the hormone-dependent cell surface antigen of 116,000 M_r and specifically recognizes the 116,000 M_r antigen on protein blots of either cell extracts or secreted proteins. Protein blots also show that this antigen is present in apparently increased amounts in evaginated discs compared with unevaginated. In immunofluorescent staining experiments on whole discs, we observe that this antibody stains the surface of evaginated discs much more intensely then that of unevaginated discs. Therefore hormonal stimulation of S3 cells increases the metabolic labeling of both cell surface and secreted glycoproteins, and at least one of these proteins appears to be hormonally regulated during imaginal disc evagination.

THE RECESSIVE TUMOR GENE l(2)gl of Drosophila. O. Schmidt, R. Lützel-
K 309 schwab and C. Klämbt
Institut für Biologie III, Schänzlestr.1, 78 Freiburg, FRG.
Recessive mutations in the Drosophila tumor gene lethal(2)giant larvae affect the growth and tissue specifity of determined cells in imaginal discs and presumptive optic centers of the brain. To analyse the function of the l(2)gl gene during development, we have isolated cDNA fragments and identified a protein product from a deduced amino acid sequence and with antibodies against a bacterial fusion protein.
The protein is detected in increasing amounts up to mid-embryonic stages. Antibody binding to embryo sections and indirect immunofluorescence labeling indicate that the protein is localized at the cellular membranes or in the intercellular matrix of embryonic cells. The primordia of most larval tissues are labeled in the embryo. Much less labeleing is found in the neural primordia of the central nervous system, except that within the supraoesophageal ganglion regions of the presumptive optic centers are distinctly labeled. After embryogenesis the l(2)gl protein is found at low levels until the end of the 3rd larval instar, when it is preferentially seen in the brain and imaginal discs. The protein distribution in embryonic and larval tissues correlates with already known patterns of cell division, which could indicate that the l(2)gl protein is involved in proliferation arrest of cells.

MOLECULAR MECHANISMS FOR CUTICULAR SCLEROTIZATION IN DROSOPHILA
K 310 MELANOGASTER,* Manickam Sugumaran, Univ. of Mass., Boston,MA 02125.
The molecular mechanisms responsible for sclerotization of Drosophila melanogaster exoskeleton were examined using chemical, enzymological and radioactive techniques. The chitin bound, cuticular phenoloxidase responsible for the above reactions resisted solubilization. It exhibited a pH optimum of 6.5 and oxidized a variety of catecholamine derivatives. Typical phenoloxidase inhibitors such as sodium azide, potassium cyanide and sodium fluoride inhibited this enzyme activity drastically. However, phenylthiourea showed marginal inhibition only. Studies on the mode of tanning of puparial cases and adult cuticle indicated the operation of quinone methide sclerotization as the major mechanism for stabilization of these cuticles. Accordingly, acid hydrolysis of cuticle released large amounts of ketocatechols. HPLC analysis of this mixture revealed the presence of arterenone as the major catecholamine derivative. Radioactive experiments further supported the participation of quinone methides in the sclerotization reaction.
*This work was supported in part by USPHS Grant RO1-A1-14753 from the NIH.

Gene Regulation

K 400 Stable Transformation of Mosquito Cells by a Bacterial Gene, Barry Beaty, Thomas Monroe, Vickie McGrane, and Jonathan Carlson, Department of Microbiology, Colorado State University, Fort Collins, CO 80523.

Mosquitoes are vectors for a large number of important pathogens. In order to facilitate the study and possibly the disruption of the pathogen-vector interaction we are attempting to develop methods to introduce genetic information into the mosquito genome. As a first step we have introduced the neo gene from the bacterial transposon Tn5 into cultured Aedes albopictus cells (line C6/36). The plasmid pNHP (Rio and Rubin (1985) Mol. Cell. Biol. 5, 1833-1838) contains the neo gene cloned in front of the LTR from the Drosophila transposon copia. This plasmid was introduced into C6/36 cells by electroporation and transformed cells were selected by growth in medium containing G418. Several G418 resistant lines were tested for the presence of neomycin phosphotransferase activity. Crude extracts from these lines were capable of ATP dependent phosphorylation of kanamycin. Experiments to characterize the integration events and the transcription of the neo gene are in progress. Techniques have also been developed to inoculate developing mosquito embryos with the molecular constructs.

DIFFERENTIAL EXPRESSION OF ß TUBULIN GENES IN DROSOPHILA MELANOGASTER

K 401 D. Buttgereit, A. Gasch, U. Hinz, F. Michiels, R. Renkawitz-Pohl, MPI f. Biochemie, Genzentrum, D-8033 Martinsried/FRG

We have analyzed three of the four ß tubulin genes of Drosophila melanogaster in greater detail. Comparison of the amino acid sequence revealed strong homology between Drosophila tubulins as well as to other eukaryotic tubulins. In contrast, the promoter regions of the three investigated genes are highly diverged, except of two blocks of homolgy between the developementally regulated ß3 tubulin and the testis-specific ß2 tubulins of Drosophila melanogaster and hydei. Analysis of the transcription start site of the ß3 gene revealed no differences between mid embryos or pupae, and the same splice-sites are used in both stages. Interestingly, no TATA or CAAT box-elements are found in the promoters of the ß2 and ß3 genes, while a TATA box is located at the correct position in the constitutively expressed ß1 tubulin. Experiments to characterize the ß3 tubulin promoter by transient and P-element transformation of embryos and in vitro transcription are in progress.

K 402 THE YYRR BOX: A SEQUENCE FOUND AMONG DEVELOPMENTALLY REGULATED GENES IN DROSOPHILA, Douglas Cavener, Philip Krasney, and Brian Foster, Vanderbilt University, Nashville, TN 37235

We have discovered a 72 bp tandem repeat of a GACT tetranucleotide motif in a large 5' intron of the Drosophila Gld gene. Given its dipyrimidine/dipurine nature, we have named it the YYRR box(pronounced "wire box"). Approximately 40-50 putative YYRR boxes in the Drosophila genome have been detected. Genomic Southern analysis also indicates that the locations of YYRR boxes are highly conserved within and between Drosophila species. We have identified seven YYRR boxes in 900 kb of genomic DNA clones from three chromosome walks. YYRR boxes occur in the 5' regions of the Gld, ted, Cd, and Scr genes. Like Gld, the Cd gene YYRR box is found within a large 5' intron. Three putative YYRR boxes have been found in the Bithorax Complex interspersed among the iab genes. One apparent common feature of the Gld, ted, Cd, Scr, and iab genes is that they are expressed and/or function in the epidermis during metamorphosis. Three YYRR boxes have been sequenced. They have the same length (i.e 68-72 bp) and have the same 5' to 3' orientation relative to their genes. The dipyrimidine/dipurine nature of the YYRR box has interesting structural ramifications due to its high density and unique spacing of cross chain purine-purine clash. Theoretical and empirical studies indicate that dipurine/dipyrimidine repeats lead to the largest possible variance of the helix twist angle. Such alterations in helix conformation may form recognition patterns for certain types of regulatory proteins.

K 403 REGULATION IN *TRANS* AT THE 68C GLUE GENE CLUSTER OF *DROSOPHILA*, Peter H. Mathers and Elliot M. Meyerowitz, California Institute of Technology, Pasadena, CA 91125. Three of the genes which code for the polypeptide components of the *Drosophila melanogaster* salivary gland glue, *Sgs-3*, *Sgs-7* and *Sgs-8*, are found at the 68C chromosomal locus. These genes are coordinately expressed at abundant levels and their activity is restricted to the salivary glands of late third instar larvae. Thus, they constitute a system for the study of developmental gene regulation. We have analyzed two mutations, *l(1)npr-1* and *l(1)su(f)*^{ts67g}, both of which act in *trans* to prevent the normal accumulation of mRNAs synthesized from the 68C genes. By introducing altered fragments of the 68C region into flies via P-element-mediated transformation, we have been able to delimit the regions necessary for interaction of the glue genes with the wild-type products of the genes defined by these mutations. A product of the 2B5 region, which is disrupted in the *l(1)npr-1* mutation, has been shown to act within *Sgs-3* or a region 130 base pairs 5' of the gene. The *l(1)su(f)*^{ts67g} mutation, which prevents accumulation of normal levels of the steroid hormone ecdysterone, has its effects within *Sgs-3* or in the 5' region up to 2.3 kilobases away. Experiments to further localize the sequences where these trans-acting factors exert their influence are currently in progress.

K 404 Characterization of follicle-specific protein binding sites in the 5` flanking regions of Bombyx mori chorion genes. H.T. Nguyen,N. Spoerel & F.C. Kafatos. Harvard University, Cambridge, MA. The chorion A and B multigene families in B. mori are arranged as A/B gene pairs, with the oppositely-oriented "TATA"boxes being separated by a common 276 (+1)bp 5` flanking region. DNA and RNA hybridization studies have identified two classes of genes with distinctly different expression profiles within the "middle" period of choriogenesis. Gel electrophoresis-DNA binding assays are being used to identify tissue- and temporal-specific trans-acting factors that bind to the 5` flanking region and that may be involved in the regulation of two typical gene pairs, A/B.L11 and A/B.L12. At least two follicle-specific binding sites, that do not involve "TATA" binding proteins, have been identified in each 5` flanking region. The binding sites are being mapped more precisely by using the DNase I technique. In addition, by using "linker-scanning" mutants of the 5` flanking region of the A/B.L12 gene pair, differential binding (decreased or increased) has been observed, thereby implicating the involvment of the element "TTGNGAAA" in one of the binding sites. This element is found in multiple non-identical copies in B. mori and D. melanogaster chorion genes and P element transformation assays of its functional significance are underway (N. Spoerel & S.A. Mitsialis).

K 405 MOLECULAR AND ULTRASTRUCTURAL EFFECTS OF MYOSIN HEAVY CHAIN MUTATIONS ON DROSOPHILA MUSCLE DEVELOPMENT, Patrick T. O'Donnell and Sanford I.Bernstein, Biology Dept. and Molecular Biology Institute, San Diego State University, San Diego, CA 92182. Drosophila has a single muscle myosin heavy chain (MHC) gene. Alternative splicing at the 3' ends of MHC transcripts results in RNAs that encode at least two MHC isoforms with different C-termini. One of these isoforms appears to be specific to the indirect flight muscle (IFM). We have identified two classes of dominant flightless mutations which affect the MHC gene. Mutations of the first class, such as the allele Mhc1, are recessive lethals at the embryonic or early larval stages (Mogami et al., 1986, PNAS 83: 1393). Heterozygotes (Mhc1/+) are flightless but other muscles function normally. DNA sequence analysis indicates that the Mhc1 mutation is a small deletion in the coding region for the ATP binding site, probably resulting in premature translation termination. We have used electron microscopy to examine IFM and larval intersegmental muscles of Mhc1/+ heterozygotes. Both muscle types show a decreased thick:thin filament ratio. Apparently the function of the highly organized filament array in the IFM is exceedingly sensitive to disruption. Less organized tubular muscles, such as larval intersegmental muscle, show less sensitivity to a reduction in the thick:thin filament ratio. MHC alleles in the second class of dominant flightless mutations, such as Ifm(2)2, are not recessive lethals. These mutations result in a reduction in MHC accumulation in the adult IFM, but not in other muscle types. Ultrastructural analysis of the IFM shows complete absence of thick filaments, while the ultrastructure of other muscles appears normal. The Ifm(2)2 mutation may lie in a coding region, or within an RNA splicing signal, that is only utilized by the IFM-specific MHC isoform. We have molecularly cloned the Ifm(2)2 MHC gene and are currently sequencing the IFM-specific exon and splice signals in order to define the molecular lesion responsible for the mutant phenotype.

K 406 ANALYSIS OF A GERM-LINE TRANSFORMATION EVENT IN THE MALARIA VECTOR ANOPHELES GAMBIAE, Patricia Romans, Richard K. Sakai, Hayden Coon, and Louis H. Miller, National Institutes of Health, Bethesda MD 20892.

The parasite/mosquito relationship is one of the most vulnerable facets of the epidemiology of malaria. We wish to study genes that could alter the capacity of the mosquito vector to transmit the parasite. The first step is the ability to transform mosquitoes. Pre-cellular blastoderm embryos of Anopheles gambiae were microinjected with DNA using techniques extensively modified from Drosophila transformation protocols. The DNAs injected were a Drosophila P element helper plasmid, pUChsπΔ2-3wc (gift of F. Laski and G. Rubin), and a vector plasmid, pUChsneo, which contains P element ends and adjacent sequence from the Drosophila white locus flanking the neo gene from Tn5 (Steller and Pirrotta, 1986). In the G1 generation derived from mass mating about 320 survivors of injection, 13 adults carrying sequence hybridizing pUChsneo DNA survived G418 selection as larvae. Restriction enzyme analysis indicated that all apparently derived from a single integration of vector sequence into mosquito DNA. These adults were mated and gave rise to a line of mosquitoes containing the vector sequences stably integrated into the genome. Genomic mapping of the region of the insertion indicates that one seemingly complete copy of pUChsneo with white sequence on either side but no other complete pUChsneo was integrated. Thus the germ-line integration was not a precise P-mediated event. The insertion may be within 1 kb of a telomere. We are presently studying this integration event further by plasmid rescue of the inserted vector and adjacent mosquito DNA, and DNA sequencing. PR was supported by a grant from the Rockefeller Foundation.

K 407 MOLECULAR AND DEVELOPMENTAL ASPECTS OF HISTONE GENE REITERATION IN DROSOPHILA, Linda D. Strausbaugh, David H.A. Fitch, and Carll E. Ladd, Department of Molecular and Cell Biology, The University of Connecticut, Storrs, CT 06268.

It has been widely accepted that histone gene reiteration is related in a simple way to the developmental demands on an organism, especially in early embryogenesis. Utilizing genetic perturbations to histone gene copy number and naturally occurring variants, we have gained some insight into this postulated relationship and suggest that chromosomal location also plays an important role in reiteration levels. — D. melanogaster contains 100-110 tandem copies of the histone repeat per haploid genome, located near the quasi-heterochromatic base of chromosome 2L. Two pieces of information from experiments utilizing deficiency heterozygotes suggest the possibility that these levels of histone DNA may be important to the organism. First, in heterozygotes with a deletion for the histone region on one chromosome, there is a disproportionate replication of the histone DNA on the non-deficient homolog to increase the amount of histone DNA (compensation). Second, although this increase occurs during the ontogeny of the fly, it is insufficient to correct developmental phenotypes that we associate with histone gene deficiencies in early stages. These results lead us to speculate that high histone gene copy numbers may, in fact, be required in D. melanogaster — D. hydei, a rather close relative provides a naturally occurring counterpoint. We estimate that this species contains only 5-10 copies of tandemly repeated histone genes. Surprisingly, these genes are located in a completely euchromatic interval of the polytene chromosome. These observations lead us to propose that chromosomal position either indirectly influences copy number by affecting histone gene activity or directly influences copy number by association with highly repeated sequences.

K 408 P-ELEMENT TRANSFORMATION AND RESCUE OF A DROSOPHILA TROPOMYOSIN FLIGHTLESS MUSCLE MUTANT, Terese Tansey, Margaret Mikus, Michele Dumoulin and Robert V. Storti, University of Illinois College of Medicine, Chicago, Il. 60612.

The Drosophila tropomyosin I (TmI) gene encodes two TmI isoforms by using alternate splicing pathways. A transposable element inserted into the TmI gene of the semi-dominant flightless mutant Ifm(3)3 prevents expression of normal levels of Ifm-Tm, the isoform found in indirect flight and some leg muscle, but has no effect on Scm-Tm, the isoform present in other muscles. Homozygous Ifm(3)3 flies neither jump nor fly. Using P-element-mediated transformation with a wild-type TmI gene containing 2500 bp of 5' and 1800 bp of 3' flanking sequence, we have rescued the Ifm(3)3 phenotype in 5 different transformed lines. In all lines normal flight requires two autosomal copies of the wild-type gene; a single copy restores the ability to jump in 2 of the 3 lines examined. Two-dimensional gel electrophoresis of leg and thoracic proteins shows that Ifm-Tm is expressed at approximately normal levels. Ifm-Tm-specific transcripts missing in Ifm(3)3 are expressed with the proper tissue-specificity in the transformants although different lines show some variation in levels of Ifm-Tm mRNA. Homozygous transformants have approximately the same amount of Scm-Tm mRNA as flies with only 2 copies of the TmI gene, suggesting that autosomal dosage compensation prevents excess Tm mRNA accumulation.

K 409 A STUDY OF ACTIN GENES IN ASCARIS SUUM. Michael A. Winrow, Pamela E. Martin, and Ann Sodja, Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

To gain a better understanding about the evolution of multigene families as well as the mechanisms which regulate the expression of their members, we have initiated a molecular study of actin genes in a parasitic nematode, Ascaris suum. Southern blot hybridizations, using heterologous actin specific probes from either Drosophila melanogaster or Caenorhabditis elegans, indicate that in A. suum, actin is encoded by a multigene family in which the number of members exceeds the four found in C. elegans. Analysis of pre- and post-diminutive DNAs indicates that no actin genes are lost during chromosomal diminution which is known to occur in A. suum during cleavage stages. A lambda recombinant A. suum genomic library was constructed in order to investigate these genes in greater detail. Five non-overlapping actin gene clones were isolated. None of the clones mapped so far contain more than one actin gene within the approximately 16 Kb long DNA inserts. This is in contrast to the organization found in C. elegans in which 3 of the 4 actin genes are clustered. Gene specific 3' transcribed-untranslated regions derived from C. elegans were hybridized to A. suum DNA and actin clones in search of sequence homology. At the level of Southern blot hybridizations, no conserved sequences within these regions were detected. Further studies on these regions as well as information about the structural and transcriptional features of A. suum actin genes will be presented. (Supported by NIH Grant No. RR-08167-08)

K 410 SUPPRESSOR-OF-WHITE-APRICOT IS A POSTTRANSCRIPTIONALLY AUTOREGULATED GENE. Zuzana Zachar, Tze-Bin Chou and Paul M. Bingham. State University of New, Stony Brook, NY, 11794.

Suppressor-of-white-apricot [su(w^a)] is a member of the class of allele-specific suppressor loci in Drosophila and yeast that interact with specific retrotransposon insertion mutations and are presumptive transcriptional regulatory loci. We have analyzed the developmental and tissue-specific patterns of expression of the complex array of transcripts produced by various mutant and wild type alleles of su(w^a). Our results demonstrate that the levels of mature, functional transcripts of the gene is autoregulated at the level of RNA processing (splicing). Various other results suggest that su(w^a) codes for an RNA binding protein that postranscriptionally regulates the expression of other genes. It is possible to propose a coherent, detailed model for the interaction between the autoregulatory machinery of su(w^a) and exogenous developmental cues to allow this gene to regulate the expression of other genes in a developmentally complex way.

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