GENETIC NETWORKS

Organizers: William F. Loomis, Lee Hartwell and Paul Sternberg

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Regulatory Circuits

A7-001 NITROGEN RESPONSE NETWORKS OF YEAST AND BACTERIA, Boris Magasanik, Massachusetts Institute of Technology, Cambridge, MA 02139.

For enteric bacteria and *Saccharomyces cerevisiae* growing on minimal media ammonia is the preferred source of nitrogen. Assimilation of ammonia into glutamine and glutamate provides the starting materials for all nitrogenous cell constituents. The extracellular stimulus, lack of ammonia, causes the sensor glutamine synthetase (GS) to generate the signal, intracellular deficiency of glutamine. In the case of bacteria, this signal is transduced by the enzyme UTase/UR and the small protein P_{II} to the modulators, the enzyme ATase and the protein NR_{II}. UTase causes the uridylylation of P_{II} which combines with ATase to remove inactivating adenylyl groups from the GS to render it fully active and relieves P_{II} from its association with NR_{II} allowing NR_{II} to phosphorylate the response regulator NR_I. NR_I-phosphate in turn activates the initiation of transcription at the σ^{54}-dependent promoter of the *glnALG* operon, encoding respectively GS, NR_{II} and NR_I and at other nitrogen regulated σ^{54}-dependent operons. Conversely an increase in the extracellular concentration of ammonia reverses the process: the increase in the intracellular concentration of glutamine causes UTase to remove the uridylyl groups from P_{II}-UMP resulting in P_{II} which in turn causes ATase to adenylylate GS and NR_{II} to dephosphorylate NR_I-phosphate. In *S. cerevisiae* , the transcription of *GLN1* encoding GS and of other genes encoding enzymes and permeases necessary for the degradation of nitrogen compounds requires the product of the *GLN3* gene for activation of transcription. A high intracellular level of glutamine causes the product of the *URE2* gene to disable the *GLN3* product. The *URE2* gene product is also responsible for the inactivation of GS in response to the increased level of glutamine. The similarities and differences of the regulatory networks will be the subject for discussion.

A7-002 DORSOVENTRAL SIGNALING IN DROSOPHILA OOGENESIS, Siegfried Roth, F. Shira Neuman-Silberberg, and Trudi Schüpbach, Department of Molecular Biology, Princeton University, Princeton, NJ 08544.

In Drosophila, the establishment of the dorsoventral pattern of the egg and embryo involves two signaling events. The first of these takes place in oogenesis and requires the activity of the genes *gurken* and *torpedo*/DER (Drosophila EGF receptor homolog). *gurken* was found to encode a TGF-a like molecule and may encode a germline ligand for the top/DER receptor. The *gurken* RNA and protein are localized to the dorsal anterior corner of the oocyte during midoogenesis. This localization appears to provide the spatially restricted dorsal determinant that induces dorsal development in the follicle cells. The localized *gurken* signal also serves to orient the future dorsoventral axis of the egg. It delimits the ventral region of the egg and embryo, but it does not appear to directly determine ventral and ventrolateral cell fates in the embryo. Rather, top/DER receptor activation represses an active patterning process which involves signaling from the follicle cells to the embryo through the *Toll* receptor (1,2) on the egg membrane.

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A7-003 ANALYSIS OF SIGNAL TRANSDUCING CIRCUITS, Melvin I. Simon, California Institute of Technology, Pasadena, California 91125

Signal transducing circuits in both lower and higher organisms display many of the same characteristics. They have the ability to detect large varieties of different chemical substances and to generate metabolic responses to small changes in external concentrations of hormones, neurotransmitters, nutrients and changes in the physical parameters of the environment. All of these systems generally include specific receptors and transducers that mediate the conversion of changes in concentrations of specific ligands to metabolic changes in the cell. Signal transduction results from a specific series of protein-protein interactions that generate both an excitation and an adaptation signal inside the cell. The excitation process involves specific elements that lead to amplification of the ligand binding event and to mechanisms that limit the excitation response. The adaptation signal generally modifies the receptor itself so that input to the transducer is regulated. The protein components that underlie these complex signaling circuits are being elucidated in a variety of systems. In the bacterial systems the histidine kinase-aspartyl phosphate systems have been known for a number of years. We will describe the bacterial chemotaxis system and its protein components and how it functions as a signaling circuit. We will further discuss a variety of microbial signaling circuits and the role that they play in interacting and regulating microbial behavior and function. Recent work in a number of laboratories including our own has demonstrated that these systems are not confined to prokaryotes but also exist in eukaryotic systems. Furthermore, the work suggests that in manner analogous to that found in the microbial world, histidine kinases play a role in regulating information processing through a series of parallel circuits in eukaryotic cells. We will also describe some of the complexity that arises in information processing in eukaryotic cells through a variety of pathways and demonstrate that even in complex sensory systems, the same principles that have been elucidated in simple microbial systems can be used to analyze signal transduction.

A7-004 **NETWORKS OF INTERCELLULAR SIGNALING AND SIGNAL TRANSDUCTION DURING NEMATODE DEVELOPMENT**, Paul W. Sternberg, Anna P. Newman, Robert E. Palmer, Giovanni Lesa, Thomas R. Clandinin, Linda S. Huang, Charles Yoon, Paul S. Kayne, Wendy S. Katz, Andy Golden, Kyria Tietze, Ralf J. Sommer & Marie-Anne Félix, Howard Hughes Medical Institute and Division of Biology, Caltech, Pasadena, CA 91125.

We are using the molecular genetics of *Caenorhabditis elegans* and other nematodes to confront the complexity of the cellular and genetic control of development. This complexity is manifest in several ways. First, one cell sends multiple intercellular signals. For example, the anchor cell of the *C. elegans* hermaphrodite somatic gonad becomes specified by a bilateral "lateral signaling" event, it then induces the underlying epidermis to generate vulval cells; it then induces nearby ventral uterine cells to specialize; and it then attaches to a subset of vulval cells. Second, one cell responds to multiple intercellular signals, and somehow integrates those signals. For example, tripotent vulval precursor cells (VPCs) become one of three types (1° and 2° which generate distinct subsets of vulval cells, and 3° which generates non-specialized epidermis) depending on at least three intercellular signals. An inductive signal from the anchor cell stimulates vulval differentiation; a lateral signal among the VPCs promotes 2° and inhibits 1°; a general negative signal most likely from surrounding epidermis promotes 3°. Third, one cell surface receptor, LET-23, the nematode homolog of the epidermal growth factor receptor, uses cell-specific pathways to transduce its signal. LET-23 is required for several aspects of development including viability, hermaphrodite vulval induction, hermaphrodite fertility. Its role in promoting fertility acts via a distinct pathway than its stimulation of vulval differentiation, which involves Ras, Raf, Mek and MAP kinase. Fourth, the same receptor is under the control of multiple negative regulation. LET-23 responds to at least five genetically defined pathways of negative regulation. Most of these are defined by silent mutations that confer no phenotype on their own, but in combination with another mutation result in excessive vulval differentiation. The apparent redundancy of these regulators is complex. Lastly, regulatory networks are modified during evolution concomitant in some cases with morphological evolution. In some species of the same family as *C. elegans* (Rhabditidae), vulval development and pattern formation do not depend on the anchor cell, and the VPCs are not equivalent in their potential to become 1° or 2°. In species of a distinct nematode family (Panagrolaimidae), the anchor cell specification is no longer subject to lateral signaling.

Morphogenesis

A7-005 COMBINATORIAL CONTROL AND REGULATORY LOGIC IN PROGRAMMING THE LIFE CYCLE FORMS OF BUDDING YEAST. Ira Herskowitz. University of California, San Francisco, San Francisco, CA 94143-0448

The following points emerge from studies of cell specialization in budding yeast.

(1) Cell specialization involves regulatory proteins coded by the mating type locus. In most cases, the transcriptional activities result from combinations of regulatory proteins, for example, association of two homeodomain proteins, **a**1 and α2, to form a novel repressor that is the molecular signature of the **a**/α cell type.
(2) In some cases, repressors such as **a**1-α2 **activate** transcription by inhibiting an inhibitor. This type of (-)(-) = (+) control is quite common and may occur in human sex determination (PNAS **90**:3368).
(3) Final differentiation of **a** and α cells results when the cells are in the presence of their mating partner due to action of extracellular peptide pheromones that mediate induction.
(4) Most of the components of the mating pheromone response pathway are known, though important gaps in our understanding exist. Some of the pathway components are used in more than one pathway.
(5) Cell cycle arrest results from activation of the FAR1 gene and its protein, which inhibits the cyclin-dependent kinases CDC28/CLN2. This type of inhibitory protein ("CKI", cyclin-dependent kinase inhibitory protein) is also found to play roles in multicellular eukaryotes.
(6) Yeast cells mate in a pairwise manner, which may reflect amplification of cell-cell interactions and commitment during the mating process.
(7) Yeast cells choose their plane of cell division in a manner that is cell-type specific. The choice of bud site may reflect use of pre-existing structural information, a morphogenetic template.

Protein Interactions

A7-006 PROSPECTS FOR PROTEIN LINKAGE MAPS, Paul L. Bartel, Jennifer Roecklein, Dhruba SenGupta and Stanley Fields, Department of Molecular Genetics and Microbiology, State University of New York at Stony Brook, Stony Brook, NY 11794-5222.

We define a "protein linkage map" as the description of all the protein-protein interactions in a cell. It is a linkage map because we assume that many, if not most, proteins have multiple partners. Thus the total set of linkages would define supramolecular structures such as replication and transcription complexes, the ribosome and spliceosome, and the cytoskeletal network. Other linked sets of proteins would correspond to signaling pathways, such as those involved in response to growth factors, or to metabolic pathways. Additional linkages in the map might connect various complexes, such as those involved in replication and in DNA repair. The development of the yeast two-hybrid system (1-3), which uses transcription to detect the interaction of a protein fused to a DNA-binding domain with a protein fused to a transcription activation domain, has made it possible to identify protein-protein interactions in a rapid fashion. When used on a global basis, this system should indicate that some protein A binds to B, and C binds to D. The further identification of B binding to C might then link all four proteins together in a particular structure. Continued application of this approach should expand the number of linkages to include many of the protein components of such a structure.

As a feasibility study, we sought to develop a protein linkage map for bacteriophage T7. T7 has the advantages of a completely sequenced genome of ~40 kb; the lack of introns or large intergenic regions to permit efficient cloning of genomic DNA; and the previous genetic and biochemical identification of several interactions between phage proteins. We generated libraries of randomly sheared T7 DNA in both a Gal4 DNA-binding domain vector and a Gal4 activation domain vector, and transformed these libraries into yeast **a** and α reporter strains, respectively. We then screened 5 to 10 random DNA-binding domain library transformants at a time against the total activation domain library using a mating assay (4). Positive transformants that define T7 interactions so far include homodimers of the gene 4A product (helicase/primase); gene 1.7 product; and gene 5.3 product. In addition, we have identified an interaction between the gene 0.7 product (protein kinase) and the gene 4.5 product; the gene 15 product (internal virion protein) and gene 16 product (internal virion protein); and the gene 11 product (tail fiber protein) and gene 2.5 product (ssDNA binding protein). Most unusually, the gene 18.5 product interacts with the gene 18.7 product; these proteins are encoded by overlapping but different reading frames. Such an arrangement poses fascinating questions for the evolution of both genes and proteins. Most of the interactions we have detected using this approach had not been previously characterized. It seems likely that our approach can be extended to more complicated organisms, such as *E. coli* and yeast.

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3. Fields, S. and Sternglanz, R. (1994) Trends in Genetics **10:** 286-292.
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A7-007 MORPHOGENESIS AND CELL-CELL SIGNALING IN MYXOCOCCUS, Brian Sager and Dale Kaiser, Departments of Biochemistry and Developmental Biology, Stanford University, Stanford, CA 94305

When starved, about 10^5 myxococcus cells assemble to form a fruiting body which has species-specific shape and within which individual cells differentiate into spores. Fruiting body morphogenesis begins with cells aggregating into mounds that grow as more cells enter them. In the neighborhood of these enlarging aggregates, other cells are moving in a highly organized, periodic pattern of equally spaced ridges that move as trains of traveling waves. Traveling waves outside the fruiting body may be a simplified version of the movement and cell-cell signaling that is going on inside a nascent fruiting body. The wave crests, which are heaps of cells, have a density comparable to that in the outer domain of fruiting bodies, about 10^{11} cells/cm^3. A full complement of swarming motility genes is required for traveling waves, just as they are for proper fruiting body morphogenesis. In addition, C-factor is required, because C-factor-less mutants are unable to form traveling waves. C-factor is a 20kDa protein that is cell bound. This molecule signals when cells are close together, and its transmission requires a particular alignment of the interacting cells. Cells respond to this signal by reversing their direction of movement, which helps to account for wave formation.

Within a nascent fruiting body, cells respond to C-factor by initiating spore differentiation. If cells are aligned end-to-end, corresponding to the most dense way to pack rod-shaped cells, then C-signal can be transferred. If the cell arrangement in the nascent fruiting body passes this test, then there is commitment to sporulation. Thus C-signaling serves as a morphogenetic test that the aggregative cell movements have given them an organization appropriate for differentiation of a motile rod-shaped cell into a spherical, non motile, quiescent spore cell.

A7-008 THE CADHERIN-CATENIN ADHESION SYSTEM FOR CONTROL OF COMPLEX ADHESIVE BEHAVIOR OF EMBRYONIC CELLS, Masatoshi Takeichi, Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto 606, Japan.

Cell-to-cell adhesion is highly dynamic, and precisely regulated for morphogenetic events during development. These events include epithelial-mesenchymal transition, separation of cell layers, migration of cells, and specific connections of cells, all of which are crucial for the construction of the body structures. Each process is spatiotemporally regulated, suggesting that it is under the strict control of genetic networks. As a machinery to regulate the complex adhesive behavior of cells, we have been analyzing the expression and function of the cadherin cell adhesion molecules.

While cadherins constitute a superfamily, members of its subfamily, "classic" cadherins, confer specific adhesiveness on cells via their selectivity in homophilic interactions. Each cadherin is expressed in a unique spatiotemporal pattern, leading us to propose that these molecules may regulate region-specific selective cell-cell adhesions (1). Recent analyses of the expression patterns of newly identified cadherins have shown that each embryonic tissue is a mosaic of cell groups expressing different cadherins. A good example can be seen in the brain which has numerous subdomain strucutures. The embryonic brain is subdivided into neuromeres, and further subdivided into cortical layers and nuclei. Each subdivision can be defined by an expression of particular cadherins. These observations suggest that cadherins are essential for the formation of subdivision structures in cell layers. On the other hand, the function of cadherins is regulated by proteins associated with the cytoplasmic domain, collectively called catenins. For example, α-catenin, one of the catenins, was demonstrated to be crucial for cadherin activity (2). Another catenin, β-catenin (Armadillo), is known to be involved even in cell fate determination in Drosophila. It has also been shown that catenins are tyrosine-phosphorylated in response to growth factors (3). The regulation of cadherin function via catenins, thus, seems to take part in intercellular signaling.

These observations indicate that cadherin-mediated cell adhesions are under the control of at least two lines of genetic network, one for the transcriptional regulation of the cadherin superfamily genes and the other for the functional regulation of the cadherin-catenin system. Little is known about upstream genes involved in these networks, although some candidates, such as Wnt-1, were identified (4). The cadherin-catenin system can be a target for many transcriptional factors or singaling molecules, known to be developmentally important.

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3. Shibamoto, S., Hayakawa, M., Takeuchi, K., Hori, T., Oku, N., Miyazawa, K., Kitamura, N., Takeichi, M. and Ito, F. (1994) Cell Adhesion & Commun. *1*, 295-305.
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Signal Transduction

A7-009 SENSORY TRANSDUCTION IN *DICTYOSTELIUM*, Peter N. Devreotes, Pamela Lilly, Dale Hereld, Michael Caterina, Ji-Yun Kim, Mei-Yu Chen, Jacqueline Milne, Robert Insall, Carole Parent, Brenda Blacklock, and Zhan Xiao, Johns Hopkins Sch. Med., Baltimore, MD.

The G-protein coupled signal transduction pathways initiated by activation of the chemoattractant receptors cAR1-cAR4 are essential for chemotaxis, cell-cell signaling and gene expression during the developmental program of *Dictyostelium*. We have exploited this property to carry out random mutagenesis of cAR1, the α- and β-subunits of G2, and the adenylyl cyclase ACA. To map the high affinity cAMP binding domain of cAR1, we generated a series of chimeras between cAR1 and cAR2, which has a much lower affinity for cAMP. The cAMP binding properties and the EC_{50} for agonist-induced phosphorylation of these chimeras suggests that the major determinant of cAMP affinity is due to 5 amino acid differences within the second extracellular loop. To explore the domains in cAR1 required for its activation, we randomly mutagenized the third intracellular loop (24 amino acids) as well as the entire coding sequence and selected mutants by phenotypic rescue. We found a series of activation mutants that displayed wild-type affinity, but with impaired ability to induce G protein-dependent and -independent pathways, as well as mutants resembling cAR2, which has low affinity but can be fully activated at high agonist concentrations. To investigate agonist-induced phosphorylation of cAR1 we used a collection of cAR1 mutants that lack various combinations of 18 series within its C-terminal cytoplasmic domain. Cluster 1 (comprised of serines 299, 302, 303, 304, and 308), and, to a lesser extent, cluster 2 (series 324, 325, and 331) are the principal sites of phosphorylation. Mutants lacking all phosphorylation sites were severely impaired in cAMP-induced loss of ligand binding, but, to our surprise, the cAMP-induced cAMP secretory response of cells expressing stops normally. Thus, it appears that a mechanism other than receptor phosphorylation leads to desensitization.

In addition, we have used insertional mutagenesis to isolate novel genes in G-protein linked signaling pathways. Adenylyl cyclase in *Dictyostelium*, as in higher eukaryotes, is activated through a receptor/G protein pathway. Insertional mutagenesis into a gene designated *dagA* resulted in cells that cannot activate adenylyl cyclase, but have otherwise normal responses to exogenous cAMP. Neither cAMP treatment of intact cells nor GTPγS treatment of lysates stimulates adenylyl cyclase activity in *dagA* mutants. We show that *dagA* is the structural gene for CRAC, a cytosolic protein that activates adenylyl cyclase, and contains a pleckstrin homology domain. We hypothesize that CRAC acts to connect free G protein βγ subunits to adenylyl cyclase activation. If so, it may be the first member of an important class of coupling proteins.

A7-010 YEAST CELL MATING: FROM GENES TO BEHAVIOR, Leland H. Hartwell, Department of Genetics, University of Washington, Seattle, WA 98195

Genetic Analysis has identified many of the genes involved in signal transduction during yeast mating. Physiological studies are defining the behavioral repetoire elicited through the signal transduction pathway. Can our knowledge of the former account for the latter?

Gene Interactions

A7-011 SEX AND DEATH IN *C. ELEGANS,* Pao-Tien Chuang, Chantal Akerib, Nick Rhind, Jenny Kopczynski and Barbara J. Meyer, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

In *C. elegans,* the genetic analysis of sex determination and dosage compensation has identified many components essential for these processes. In recent years, it has been possible to assemble these components into a genetic regulatory hierarchy that details how both sex determination and dosage compensation are triggered in response to the primary sex determining signal, the *X/A* ratio. Like Drosophila, both processes share common early regulatory steps, although these processes are ultimately implemented by more specialized genes that control either sex determination or dosage compensation. Unlike Drosophila, the relationship between these two processes is complex: not only are they coordinately controlled, but a feedback mechanism operates such that a disruption in dosage compensation affects sexual fate. Four genes are known to act in the coordinate control of these two regulatory processes. In *XO* animals, this coordinate control requires the activity of a single gene, *xol-1*(XO lethal) that acts as an essential early genetic switch to specify the male modes of both sex determination and dosage compensation. The choice of the hermaphrodite fate and the hermaphrodite mode of dosage compensation requires the activities of three *sdc* (sex determination and dosage compensation) genes whose activities are negatively regulated by *xol-1*. The *C. elegans* dosage compensation process is implemented by at least five genes, *dpy-21, dpy-26, dpy-27, dpy-28,* and *dpy-30,* that are required for proper *X* chromosome expression in *XX* animals and are dispensable in *XO* animals. Dosage compensation appears to be achieved by a mechanism that reduces expression of each *X* chromosome in *XX* animals by half, allowing these animals to have a level of *X*-linked gene expression equivalent to that found in *XO* animals.

Having worked out many of the gene interactions involved in the sex determination decision, we are defining the molecular mechanisms underlying both this decision and the process of dosage compensation. We have shown that the *sdc-1* gene encodes a protein containing seven zinc finger motifs. This protein motif in combination with other genetic and molecular information suggests that *sdc-1* functions as an embryonic transcription factor regulating downstream genes involved specifically in the sex determination and dosage compensation pathways, or instead regulating genes involved in the coordinate control of both processes. The *sdc-2* gene encodes a protein of 2962 amino acids bearing no similarities to proteins in current data bases. The *sdc-3* gene, while similar in some respects to *sdc-1* and *sdc-2*, is unique in that its sex determination and dosage compensation functions act independently and are separately mutable. The *sdc-3* protein uses separate domains to achieve proper sex determination and dosage compensation. *xol-1* is the earliest-acting gene in the known hierarchy, and it functions as a developmental switch: high levels of *xol-1* activity promote male development, while low levels promote hermaphrodite development. With this molecular information we have begun to define the primary sex-determination signal, *X/A* ratio. We have identified specific regions of the *X* chromosome that behave as dose-sensitive counting elements and act upstream of *xol-1*. These are excellent candidates for the *X* chromosome elements of the X/A ratio. Additionally, we have molecularly characterized two dosage compensation genes and have demonstrated that the product of one (*dpy-27*) binds specifically to the X chromosomes of hermaphrodites not males, a result consistent with our model for dosage compensation. DPY-27's localization to *X* is dependent on the activities of the other dosage compensation genes and coordinate control genes in the pathway. The protein is similar to a yeast protein SMC1 (Strunnikov and Koshland) involved in mitotic chromosome segregation and to two Xenopus proteins involved in chromatin assembly of mitotic chromosomes (Hirano and Mitchison). We are pursuing the biochemistry to discern the molecular mechanism of this global dosage compensation process.

A7-012 GENES AND GENE INTERACTIONS THAT ESTABLISH PATTERNS IN FLOWER DEVELOPMENT, Elliot M. Meyerowitz, Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Flowers originate as homogeneous collections of cells (floral meristems). Somehow, the cells learn their positions, and then divide and differentiate to form appropriate patterns of organs of appropriate types. In the *Arabidopsis thaliana* flower there are normally 16 organs: four sepals, four petals, six stamens, and an ovary consisting of two carpels. Each of these develops in a stereotyped position, with sepals forming a ring on the periphery of the flower, then a more central ring of petals, then stamens, and finally central carpels. The radial (outside to inside) pattern of organ types is controlled by a genetic hierarchy with three types of genes. The earliest-acting genes are meristem identity genes, which respond to environmental and endogenous signals to initiate the cascade of events that leads a meristem to adopt a floral fate. These genes are uniformly expressed in the floral primordium early in its development. The meristem identity genes activate organ identity genes. These come to be expressed in concentric rings, with one set (exemplified by *APETALA1*) expressed in the periphery of developing flowers, in whorls 1 and 2, where sepals and petals will later form; one set (including *APETALA3* and *PISTILLATA*) active in whorls 2 and 3, where petals and stamens will form, and a final type of gene (*AGAMOUS*) active in the center of the flower, in whorls 3 and 4. These genes combinatorially specify organ identity, as demonstrated both by loss of function mutant phenotypes and by ectopic expression phenotypes. The radial pattern of organ identity thus depends on the pattern of expression of organ identity genes. How is this pattern established? Two different types of gene networks are involved. One is the network of positive and negative cross-regulatory activities of the organ identity genes, the other the action of a set of negative regulators of the organ identity genes called cadastral genes. The meristem identity genes have the potential to activate organ identity genes in all cells of the developing flower. The cadastral genes prevent organ identity gene activation in certain domains, so that their initial expression is in some cases in limited domains. These domains are further limited by both positive and negative interactions between organ identity genes.

A different set of genes and gene interactions is involved in producing the substrate for the action of the organ identity genes, the floral meristem. These act to regulate cell number and cellular position in the early floral meristem. Yet another type of gene is responsible for organ position and appropriate organ number within a whorl.

Multigenic Processes

A7-013 GENETIC CONTROL OF CELL CYCLE PROGRESSION AND STEM CELL BEHAVIOR IN THE MALE GERM LINE, Margaret T. Fuller, Ting-Yi Lin, Sridhar Viswanathan, Cricket Wood, Mignon Fogarty, and Gary Hime, Departments of Developmental Biology and Genetics, Stanford University School of Medicine, Stanford, CA 94305.

Basic cellular functions like cell cycle regulation must be modified to meet the demands of specific developmental programs. We have identified a global control point during spermatogenesis that appears to coordinate meiotic cell cycle progression and sperm differentiation. Four *Drosophila* genes, *always early, cannonball, spermatocyte arrest,* and *meiosis I arrest,* are required for progression of male meiosis I through the G2/M transition and for the onset of spermatid differentiation. All four genes have a similar, male specific, mutant phenotype: primary spermatocytes with partially condensed meiotic chromosomes accumulate and post-meiotic spermatids are absent. The meiotic cell cycle arrests prior to both nucleolar breakdown and cyclin A degradation. The products of the spermatocyte arrest genes may act together to control progression of spermatogenesis. Alternatively, wild type function of these genes may be required either together or separately to pass through a critical cell cycle and developmental checkpoint. The global control point may be conserved to mammals, as a similar phenotype characterizes meiosis I maturation arrest in human male infertility.

During spermatogenesis, terminally differentiating germ cells are continuously replaced from a dedicated stem cell population. The germinal proliferation center in each *Drosophila* testis has two stem cell types: germ line stem cells arranged in a rosette around an apical hub and somatic cyst progenitor stem cells, which flank the germ line stem cells. We propose that intracellular signaling plays an important role in maintenance of both somatic and germ line stem cell identity in the testis. For both stem cell types, contact with the apical hub may influence the choice between maintenance of stem cell identity and differentiation: daughter cells that retain contact with the hub remain stem cells, while those displaced from the hub initiate differentiation (1). The segment polarity signaling system may function in intercellular communication in the germinal proliferation center. The signaling molecule *hedgehog,* is expressed in the apical hub, while *wingless* is expressed in the cyst progenitor cells and *decapentaplegic* in the germ line stem cells. Mutations in two novel genes, *shut off* and *one shot,* differently affect maintenance of stem cell populations in the male germinal proliferation center. Characterization of these genes and isolation of suppressor mutations should reveal underlying molecular mechanisms that maintain stem cell identity.

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Cell Interactions

A7-014 CONVERSATIONS BETWEEN PRESPORE AND PRESTALK CELLS OF DICTYOSTELIUM William F. Loomis and Gad Shaulsky, Department of Biology, University of California, San Diego, La Jolla , CA 92093

Temporal regulation of the pattern of gene expression during development of *Dictyostelium* results in the divergence of specific cell-types from a pure population of cells. Results from microsurgical and cell-type specific ablation studies using ricinA have indicated that the proportions of prespore and prestalk cells are established by a process of lateral inhibition that is mediated by previously differentiated prespore cells. Cells inhibited from becoming prespore cells differentiate into prestalk cells. This population further subdivides to generate cells with specialized functions during both the slug stage and during fruiting body formation. The proportions of the various cell types as well as the timing of terminal differentiations are integrated by several distinct mechanisms of cell-cell communication. This network of tissue interactions gates and orders the functioning of relatively autonomous genetic networks functional at different stages of development in the different cell-types.

Phenotypic analysis of several strains carrying mutations in newly characterized genes developing as pure populations as well as in chimeric mixtures with wild-type and other mutant cells has revealed that the initial prestalk cells (Pst1) differentiate into stalk cell precursors (PstA) in a process dependent on both protein kinase A (PKA) and *tagB*, a serine protease/MDR homolog. PstA cells then signal remaining Pst1 cells to differentiate into stalk cup precursor cells (PstO). During culmination PstA cells signal prespore cells to undergo terminal differentiation and encapsulate. This process requires the function of PKA in the signalling prestalk cells as well as in the responding prespore cells. A member of the two-component system plays an essential role in transducing the signal in prespore cells.

A7-015 INTERACTING SIGNALLING PATHWAYS REGULATING DICTYOSTELIUM MORPHOGENESIS. Jeffrey Williams, Tomoaki Abe, Margaret Nelson, Neil Hopper. University College London. The Dictyostelium culminant is built by a combination of cellular differentiation and morphogenetic cell movement. Differentiation down the prestalk and stalk cell pathway is induced by Differentiation Inducing Factor (DIF), a chlorinated hexaphenone. Extracellular cyclic AMP signals play multiple roles in multicellular development. They act to induce prespore cell differentiation, to repress stalk cell differentiation and as a chemoattractant to draw prestalk cells to the apex of the aggregate. One of the responses to receipt of an extracellular cAMP signal is the activation of adenylate cyclase and elevation of the intracellular cAMP concentration has multiple developmental effects. The interrelationships between these signalling pathways and the consequences for morphogenesis will be discussed.

Genetic Patterns

A7-016 PROTEIN INTERACTIONS IN DELIVERY OF HYDROLASES TO THE YEAST VACUOLE. Elizabeth W. Jones[1], Deborah Murdock[1], Rajesh Naik[1], Kathleen Becherer[1], Vicki Nebes[1], Kimberly Baldwin[1], Anand Bachhawat[1], Gene Webb[1], Stephanie Rieder[2], and Scott Emr[2], Carnegie Mellon University, Pittsburgh, PA 15213, Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA 92093.

Production of active vacuolar hydrolases in Saccharomyces depends upon protein interactions throughout transit. The initial translocation events into the lumen of the endoplasmic reticulum may require factors specific or quasi-specific to the protein being translocated, as well as general factors. Intramolecular interactions are commonly required to effect conformational changes needed to allow proper processing of hydrolase precursors and may also serve to regulate processing in time and space. It is not yet clear whether intermolecular interactions are required to effect conformational changes and/or retard passage through the early portion of the secretory and exert temporal and spatial control of processing and passage. The early evidence suggests that there may be more than one receptor required to sort vacuolar hydrolases from soluble bulk flow.[1] Similarly, the evidence suggests that some post-sorting steps may be at least quasi-specific for individual proteins.[2] However, at least one of the late steps, that requiring Pep12p, may be common to all proteins destined for the lumen and membrane of the vacuole. And, of course, the proteolytic processing events, which occur early, in the endoplasmic reticulum, and late, in the trans-Golgi network and/or the vacuole, require intramolecular(autocatalytic)[3,4] and intermolecular processing events.[4,5]

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A7-017 THE G1 TO S TRIGGER: DEVELOPMENTAL CONTROLS TURN THE REGULATORY NETWORK ON ITS HEAD, Patrick, H. O'Farrell, and Robert, J. Duronio Dept. of Biochem., UCSF, San Francisco, CA 94143-0448.

What a gene can do might be very different from what it does do, and in development the gene performing a given task might be different in different cells. We came across an example of this in our investigations of cell cycle control during early Drosophila development when we addressed the roles of the G1 cyclin, cyclin E, and the S phase transcription factor, E2F. Mutations provide conclusive evidence that these functions are required for S phase, but the detailed phenotypes suggest that the network governing the decision to enter S phase is plastic. Some early embryonic cells proliferate rapidly with no evident G1 phase. In these cycles all of the requirements for S phase seem to be continuously satisfied, and S phase appears to be governed by mechanisms that prevent a second round of S phase prior to mitosis. In these cells cyclin E, whose expression is continuous and independent of E2F, is required for E2F dependent transcription of other replication functions (poylmerase a, PCNA, and ribonucleotide reductase). Thus, in these cells cyclin E functions upstream of S phase transcription and acts either upstream of, or in parallel with E2F. In cells that have a G1, expression of cyclin E and other replication functions is confined to a short pulse that precedes and overlaps S phase. Induction of cyclin E to high levels can induce transcription of the other replication functions and premature S phase. However, transcription of the other replication functions initiates normally in a cyclin E mutant, and cyclin E expression in these cells is dependent on E2F. Thus, while it has a capacity ot act upstream in the G1 cycles, cyclin E appears to act downstream of E2F mediated transcription. Striking parallels can be drawn between the cyclin E/E2F relationship in flies and the CLN1&2/SWI4:SWI6 relationship in Saccharomyces cerevisiae. In both cases the role played by the G1 cyclins differs from the role it adopts when ectopically produced. Because of the prevalence and diversity of gene families among cell cycle regulators, we expect that the distinction between what regulators can do and what they do do is especially important. Finally, our example suggests that development takes advantage of the regulatory flexibility inherent in a system where there are a number of different genes that can be called on to perform a given task.

A7-018 TEMPORAL AND SPATIAL CONTROL OF CELL DIFFERENTIATION DURING A BACTERIAL CELL CYCLE, Lucy Shapiro, Kim Quon, Greg Marczynski and Craig Stephens, Department of Developmental Biology, Stanford University, Stanford, CA 94305

Genetic cascades for several cell cycle events, including the initiation of the flagellar transcriptional hierarchy and the initiation of chromosome replication are activated at specific times in the *Caulobacter crescentus* cell cycle. In addition to their temporal control, these events are spatially restricted within the predivisional cell: flagellar biogenesis occurs at the incipient swarmer pole and DNA replication initiates only from the chromosome in the stalked pole of the predivisional cell. To identify components which link the flagellar hierarchy to the cell cycle, we have isolated conditional lethal mutants which are defective in both processes. One such mutant alters the transcriptional regulation of the flagellar hierarchy as well as chromosome replication and cell division. This strain carries an allele of a response regulator gene, which we have named *ctrA* (for cell cycle transcriptional regulator). We have found that *ctrA* is also essential for the transcriptional regulation of a critical DNA methyltransferase and a promoter within the origin of DNA replication, both of which contribute to the regulation of DNA replication and cell division. As we have shown that phosphorylation of CtrA is essential, we propose a model in which both temporal control of flagellar gene transcription and transitions in the bacterial cell cycle are regulated by cycles of phosphorylation/dephosphorylation involving one or more two-component regulatory systems.

A7-019 SIGNAL TRANSDUCTION IN *C. elegans*, James H. Thomas, Elizabeth A. Malone, Deborah A. Birnby, Jennifer J. Vowels, University of Washington, Seattle, WA 98195.

Environmental conditions, primarily a secreted pheromone, determine whether *C. elegans* will develop into an arrested larval form called the dauer larva. The dauer formation response is controlled by the nervous system, including certain identified sensory neurons. A network of genes that regulate dauer formation has been worked out, largely by studying mutant phenotypes and genetic interactions among mutations in a large set of genes that mediate the process. Parallel pathways, redundancy, and integration pervade this complex network. Despite this complexity, genetic approaches have been quite effective in identifying genes involved and discerning their functional relationships. Most has been learned about sensory neuron events. In one pathway, a variety of evidence suggests that sensory neurons inhibit dauer formation by releasing a TGF-ß related protein that acts through two serine-threonine transmembrane kinases of the TGF-ß receptor family (Estevez *et al.*, 1993). In a parallel pathway, which may act in distinct sensory cells, the initial sensory transduction requires an apparent guanylyl cyclase, suggesting that the process is related to visual transduction in vertebrate photoreceptors. The talk will concentrate on features of complex networks that make possible their effective genetic analysis.

Estevez, M., L. Attisano, J. Wrana, P. Albert, J. Massague, and D. Riddle. 1994. Nature **365**, 644-649.

Multigenic Processes

A7-020 **IMMUNE NETWORKS AND T CELL RECEPTOR GENES, Leroy Hood, University of Washington, Department of Molecular Biotechnology, FJ-20, Seattle, Washington 98195**

The vertebrate immune response is composed of complex networks of cells, B cells, T helper cells, T killer cells, antigen presenting cells, etc., that interact directly by cell contact and indirectly through lymphokine signals. The T helper cells play a pivotal role in the generation of the three classes of lymphocytes with antigen specific receptors--B cells with antibodies and the two types of T cells with their T cell receptors. The T cell receptors of the T helper cells constitute unique molecular addresses, a single address for each different T cell specificity. These addresses can be manipulated to modify immune responses. I will discuss how the manipulation of T cell receptors can be used to deal with experimentally induced autoimmunity in mice. Presumably, the same will hold for humans. I will also discuss how two powerful tools of genomics can be used to delineate the entire repertoire of T cell receptors (large-scale DNA sequence analysis of the T cell receptor gene families in humans and mice) and the networks of genes that predispose to human and murine autoimmunity (genome-wide large-scale genetic mapping). Each general category of autoimmune disease (e.g. multiple sclerosis) will be substratified into distinct diseases--each characterized by a distinct network of predisposing genes and each employing a distinct set of T cell addresses to initiate pathology.

A7-021 GENETIC AND CELLULAR REGULATIONS OF THERMOTAXIS IN C.ELEGANS, Ikue Mori, Hidehiro Honda, Hidetoshi Komatsu, and Yasumi Ohshima, Kyushu University, Fukuoka, Japan

C. elegans can sense and adapt to thermal stimuli in the environment. When cultivated at temperatures ranging from 15 to 25℃ in well-fed condition for several hours, the animals migrate toward the cultivation temperature and then move isothermally around that temperature on a thermal gradient(1). Thus, thermotaxis provides one of the ideal behaviors in which behavioral plasticity can be directly studied. With the knowledge of an ultrastructurally analyzed nervous system consisting of 302 neurons and a genetically well-defined genome, we have been studying thermotaxis of *C. elegans* in order to investigate mechanisms of thermosensation, and storage and assessment of thermal information at molecular and cellular levels. We have characterized genetically and behaviorally thermotaxis-defective(*ttx*) mutants isolated by us and others(1,2). In population assays, *ttx* mutants showed one of the three phenotypes, cryophilic, thermophilic and athermotactic phenotypes. In single animal assays, the mutants were further classified according to the defects in isothermal movement. We found that *ttx* mutants are often defective in other sensory behaviors such as chemotaxis, indicating that different sensory processes use the same molecules or neurons. Using laser microsurgery, we have identified neurons mediating thermotactic responses. Our results suggest that AFD neuron in the amphid sensillum is a major thermosensory neuron. Further, we found that cryophilic and thermophilic phenotypes were induced when two amphid interneurons, AIY and AIZ, were killed, respectively. This result indicates that a conceptual model proposed by Hedgecock and Russell(1) based on mutant analysis also holds at the cellular level. According to this model, migration to an adaptation temperature is established by counterbalancing two opposing drives, one for upward and the other for downward. Cryophilic or thermophilic mutants may result when one of the drives is disrupted by mutation. Thus, AIY may be required for thermophilic movement and AIZ for cryophilic movement, implying that regulating the activities of AIY and AIZ neurons in opposite directions, depending on the adaptation temperature, might be the essential step for thermotaxis. Killing either AFD or AIY abolished isothermal movement, suggesting that the neural circuit including these two neurons is important for this movement. To identify molecules important for thermotaxis, we have been attempting to clone some of the *ttx* genes. We have recently identified a 15kb genomic fragment which complements a *tax-4* mutation. *tax-4* mutations affect both thermo- and chemotactic responses. This DNA region contains several putative genes, including a *C. elegans* homolog of the cyclic nucleotide-gated channel. One important aspect of thermotaxis is its regulation by starvation(1). We have begun to investigate this process behaviorally.

(1) E. Hedgecock and R. Russell, *Proc. Natl. Acad. Sci. USA* 72, 4061-4065 (1975); (2) D. Dusenbery *et. al.*, *Genetics* 80, 297-309 (1975)

Genetic Redundancy

A7-022 GENETIC INTERACTIONS INVOLVING THE YEAST CYTOSKELETON, David Botstein, Stanford University, Stanford, CA 94305

The actin and tubulin cytoskeletons are dynamic multi-protein structures of central importance to all eukaryotes. Despite the morphological and functional diversity of actin and tubulin-containing structures in the eukaryotes, it has turned out that the underlying structural interactions among the basic cytoskeletal proteins are very similar, as might have been expected on the basis of the high degree of conservation in amino acid sequence of actin and the tubulins themselves. We have been studying the actin and tubulin cytoskeletons of budding yeast (*Saccharomyces cerevisiae*) for many years by beginning with actin and the tubulins, using them to identify and studying interacting genes and their protein products. I will review our experience with a variety of genetic methods, beginning with the isolation of useful mutations in actin and the tubulins and the importance of careful characterization of their phenotypes. I will discuss screening for interacting genes and proteins by suppression, non-complementation, synthetic lethality, resistance to over-expression lethality, affinity methods (*in vivo* using the 2-hybrid system and *in vitro* using ordinary biochemistry) and, simplest of all, screening for mutations with phenotypes closely resembling the phenotypes of actin and tubulin mutants. Finally, I will try to address what kinds of inference one can or should make from observations using one or more of these methods for detecting interactions.

A7-023 ELEMENTS OF A SINGLE MAP KINASE CASCADE IN *SACCHAROMYCES CEREVISIAE* ARE REQUIRED FOR DIMORPHISM IN BOTH HAPLOIDS AND DIPLOIDS, Steve Kron, Haoping Liu, Hans-Ulrich Mösch, Rad Roberts, and Gerald R. Fink, Whitehead Institute/MIT, Cambridge, Massachusetts.

Diploid *Saccharomyces cerevisiae* strains starved for nitrogen undergo a developmental transition from a colonial form of growth to a filamentous pseudohyphal form. This dimorphism requires a polar budding pattern and elements of the MAP kinase signal transduction pathway essential for mating pheromone response in haploids. Specifically, Ste20, Stell, Ste7, and Ste12 are required for pseudohyphal growth, but the receptors and heterotrimeric G protein are not. When the cells switch to the pseudohyphal growth pattern they also switch their budding pattern from bipolar to unipolar. We have found that haploid strains exhibit an invasive growth behavior with many similarities to pseudohyphal development, including filament formation and agar penetration. Haploid filament formation growth depends on a switch from an axial to a bipolar mode of bud site selection. Filament formation is distinct from agar penetration in both haploids and diploids. We find that the same components of the MAP kinase cascade necessary for diploid pseudohyphal development (*STE20, STE11, STE7,* and *STE12*) are also required for haploid invasive growth. Thus, haploid yeast cells can enter either of two developmental pathways: mating or invasive growth, both of which depend on elements of a single MAP kinase cascade. Our results provide a novel developmental model to study the dynamics of signal transduction, with implications for larger eukaryotes.

A7-024 CASCADE REGULATION OF ADIPOCYTE DIFFERENTIATION BY A FAMILY OF LEUCINE ZIPPER PROTEINS, Steven L. McKnight, Marie Classon, and Wen-Chen Yeh, Tularik, Inc., 270 E. Grand Ave., South San Francisco, CA 94080.

Confluent 3T3-L1 fibroblasts can be stimulated to differentiate into fat laden adipocytes by a cocktail of hormonal stimulants. Two stimulants, dexamethasone and methylisobutylxanthane (a phosphdiesterase inhibitor that raises intracellular levels of cyclic AMP), are required early in the differentiation program. A third stimulant, insulin, is required throughout the program. We have observed that three members of the C/EBP family of transcription factors are expressed during adipocyte differentiation. C/EBPβ and C/EBPγ are induced early during adipogenesis. Transcription of their encoding genes is directly regulated, respectively, by methylisobutylxanthane and dexamethasone. Experimental strategies that selectively block the function of these transcription factors also block fat cell differentation. Moreover, gratuitous expression of either C/EBPβ or C/EBPγ accelerates adipogenesis. During the late, insulin-dependent stage of differentiation, C/EBPα is expressed. Conclusive evidence from a variety of laboratories has shown that C/EBPα can activate the battery of fat-specific genes required by adipocytes to synthesize and store triglycerides. By studying the temporal and hormone dependent patterns of utilization of C/EBP-like proteins, it has been possible to formulate a regulatory cascade repsonsible for controlling adipogenesis.

Genetic Networks Poster Session

A7-100 RZIP, A TRANSCRIPTION FACTOR HOMOLOG INVOLVED IN CELL DIFFERENTIATION AND PATTERNING DURING THE DEVELOPMENT OF *DICTYOSTELIUM DISCOIDEUM*.

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The gene *rzip* has been isolated from the cellular slime mould *Dictyostelium discoideum*. It is developmentally regulated, being maximally expressed from aggregation onwards through culmination. The predicted sequence of rZIP protein includes three motifs often associated with transcription factors: a **R**ING finger domain, a leucine **zip**per and a polyglutamine run. Disruption of the endogenous *rzip* gene by homologous recombination results in mutants that show poor aggregation, impaired migration of slugs and delays or arrest in development. Analysis of cell patterning in *rzip*-null organisms, using cell-type specific promoters fused to the *E. coli lacZ* gene, reveals a reduced prestalk region with a corresponding increase in the prespore zone. Prespore β-galactosidase staining is homogeneous, but there is a complete absence of stain in the most posterior region of the slug. Development of *rzip*-null and wild-type chimeras shows that mutant cells are not rescued in a wild type background and that wild type cells show abnormal patterning in a mutant background.

The rZIP protein has been purified. Initial analyses using antibodies raised against it suggest that rZIP is a nuclear protein. Further biochemical studies are in progress together with analysis of the phenotypes of organisms with altered patterns of *rzip* expression.

A7-101 INTERACTIONS INVOLVING PROTEIN KINASES IN THE PHEROMONE RESPONSE PATHWAY OF *S. cerevisiae*. Lee Bardwell, Jean G. Cook, Ernie Chang, and Jeremy Thorner, Dept. of Molecular and Cell Biology, University of California, Berkeley, CA, 94720; (510) 642-7335.

The *KSS1*, *FUS3*, and *STE7* gene products are protein kinases which are components the pheromone response signal transduction pathway of S. cerevisiae. Kss1p and Fus3p (MAP kinase family members) are phosphorylated (and hence activated) directly by Ste7p (a MEK kinase family member). MAP kinase cascades are found in signal transduction pathways in many (if not most) eukaryotic organisms.

Ste7 protein binds to Kss1 or Fus3 proteins *in vitro*. Ste7p, translated in a rabbit reticulocyte lysate, forms a stable (Kd < 50 nM) complex with *in vitro*-translated Kss1p or Fus3p. This complex forms between the proteins in their unactivated states. Several lines of evidence suggest that this complex does *not* simply represent the catalytic site of Ste7p binding to its target residues on Kss1p or Fus3p.

Kss1, Fus3 and Ste7 interact *in vivo*. Overproduction of either Kss1p or Fus3p inhibits (or promotes recovery from) pheromone imposed cell-cycle arrest. The catalytic activities of Kss1p and Fus3p are not required for their arrest-inhibiting activities. Ste7p is limiting in the cell for signal transmission, and overproduction of Ste7p suppresses the arrest-inhibiting activity of Kss1p. Although *KSS1* is required for pheromone-imposed cell-cycle arrest in a Δfus3 strain, overproduction of Kss1p in this background inhibits arrest, thereby counteracting its own function. A model which explains these genetic interactions in terms of stable protein-protein interactions is presented.

A7-102 COUPLED GENE-NETWORKS, F. A. Bignone, Istituto Nazionale Per la Ricerca sul Cancro, I.S.T., Viale Benedetto XV, 10, I-16132, Genova, Italy.

Interacting cell populations shows collective emerging behaviour in a variety of systems: from heart beating to aggregation in Slime Molds to regulation of apoptosis in the developing brain. In all these cases macroscopic behaviour arise as the sum over the population of specific microscopic local properties defined by the genetic background. These global properties are conceptually similar to those shown by theoretical models such as Cellular Automata or Neural Networks. The problem of regulation of gene-expression in cellular populations, that set at the microscopic level macroscopic properties as defined above, is of a similar conceptual nature at a different dimentional scale and with a greater complexity. A subset of the available models that seems particular promising, in order to study emerging properties of cell populations, are those developed for the study of spatially extended chemical reactions away from equilibrium. They can be useful tools in understanding general organization principles in the dynamical organization of gene expression. Specifically Coupled Map Lattices [CML] seems particularly promising to begin this kind of study. To describe cell-gene interactions in spatially organized cells, a model based on CMLs has been proposed recently -- J. Theoret. Biol., **161**, 231-249, 1993 --. The model simulate gene interactions in *trans* in genetically identical cells on a lattice through diffusible protein products. Despite its simplicity the system shows an unexpected complicated behaviour already with gene networks of two elements. The model has been developed with complexity in mind and - in principle - any possible network could be implemented and studied. Key problems in understanding biological organization and gene regulation are readely pointed out by this approach. Local properties like: cytoplasmic diffusion, binding affinities and abundance of regulatory proteins, mechanisms of molecular transport such as sliding or inter-segment-transfer, nuclear organization, become immediately apparent as important keys to understand global dynamics, and can be studied, increasing the complexity of the models, inside the same theoretical frame.

A7-103 FUNCTIONAL DISSECTION OF R, C1 AND P IN MAIZE, Ben Bowen[1], Laura Tagliani[1], Bruce Drummond[1], Grace St. Clair[1], Sheila Maddock[1], Brad Roth[1], & Eric Grotewold[2]: [1]Pioneer Hi-Bred International, Inc., PO Box 1004, Johnston, IA 50131 & [2]Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, NY 11724.

Anthocyanin biosynthetic genes in maize (including *a1* and *bz1*) require two transcriptional coactivators (R/B and C1) for expression. In contrast, activation of the first three genes leading to anthocyanin formation (including *a1*) by a single factor (P) leads to 3-deoxyflavonoid accumulation in floral organs. P and C1 each have an acidic activation domain and two Myb-related motifs that are required for binding the sequence $CC^{T}/_{A}ACC$, two copies of which are found in the *a1* promoter. *A1* transactivation by R/B and C1 requires an interaction between the Myb region of C1 and the N-terminal 245 aa of R or B, whereas transactivation by P is R-independent. Domain swaps and protein modeling have localized several key amino acids in P and C1 that are responsible for the differential dependence on R. In contrast to *a1*, *bz1* is transactivated by R/B and C1, but not by P. A minimal sequence from *bz1* that is sufficient for R/B plus C1 transactivation lacks the P/C1 binding motif, but contains a canonical E-box. So far, five functions indicative of protein-protein interactions have been delineated for R. The N-terminus of R (but not, apparently, B) not only interacts with $C1^{Myb}$, but also harbors a strong activation function that is suppressed on binding C1. C-terminal to the Myb-binding/activation region (aa 1-353) is a bHLH motif (aa 415-468) that is not required for anthocyanin accumulation *in vivo*. However, transient expression assays indicate that it may mediate synergistic transactivation from promoters with more than one copy of the P/C1-binding motif. The C-terminal region of R (aa 354-611) is a potent dominant negative inhibitor of not only R/B plus C1, but also P, suggesting that it may interact with a core component shared by both transcription complexes. A two-hybrid assay in maize suggests that this region can also form oligomers either directly or indirectly *in vivo*. Apparently, the bHLH motif is not required for oligomerization of the C-terminal region of R, and if this region does recruit other proteins *in vivo*, they do not contain an activation domain. Currently, we are delineating the various functions in R more precisely using domain swap assays and mutagenesis. In addition, we have exploited the domain structure of R and C1 to create a series of constructs that can be used as reversible genetic switches in maize.

A7-104 *CRABS CLAW*, A GENE INVOLVED IN THE SPECIFICATION OF CARPELS IN THE *ARABIDOPSIS* FLOWER, John L. Bowman, John Alvarez, and David R. Smyth, Dept. of Genetics and Developmental Biology, Monash University, Clayton, Victoria 3168, Australia

Flowers of angiosperms consist of distinct organ types in stereotyped relative positions. For example, *Arabidopsis* flowers consist of four concentric whorls of floral organs: sepals, petals, stamens, and carpels, from the outermost to the innermost whorl. It has been proposed that three classes of homeotic genes (A, B, and C) act alone and in combination to specify the identity of each of the four floral organ types (Bowman et al., Development 112: 1-20, 1991). Each class acts in two adjacent whorls with class A in the outer two whorls, class B in the middle two whorls and class C in the inner two whorls. Both genetic and molecular evidence gathered to date provide support for the simple model. However, the leaf-like floral organs of triple mutants in which the A, B, and C functions have been eliminated still retain some carpelloid character, suggesting that another class of genes is responsible for carpel identity in addition to the C class genes, of which *AGAMOUS* is a member.

Mutations in the *CRABS CLAW* (*CRC*) gene result in the failure of fusion of the carpels. When *crabs claw* mutants are combined in a quadruple mutant combination with those which result in the loss of A, B, and C functions, the floral organs produced lack most of the carpelliod features seen in the triple mutant alone, suggesting that *CRC* is involved in the specification of carpels as a member of this new class. In addition, mutations in *CRC* enhance the loss of determinacy exhibited by other mutants that cause a reduction in the determincy of the flower, such as *agamous*. *CRC* has been cloned by chromosome walking and the molecular characterization of *CRC* will be presented.

A7-105 AN INTERACTIVE NETWORK OF RETINOID, SERINE AND TYROSINE KINASE SIGNALLING PATHWAYS MODULATES BETA-CATENIN HOMEOSTASIS Stephen Byers, Mike Pishvaien, Keith Orford, Connie Sommers and Robert Lechleider. Department of Cell Biology, Georgetown University, Washington DC and the Laboratory of Chemoprevention, NCI, NIH, Bethesda MD.

Beta-catenin is an E-cadherin-associated molecule essential for the proper regulation of cell-cell adhesion. Several studies indicate a role for beta-catenin tyrosine phosphorylation in the regulation of cadherin-mediated cell-cell adhesion. In drosophila, the beta catenin homologue *armadillo* is an essential component of the *wingless* signalling pathway and is regulated by the activity of *zeste-white-3*, a member of the glycogen synthase kinase-3 serine kinase family. We now show that 1) beta-catenin protein expression is regulated by retinoids, 2) retinoic acid receptor alpha homodimers or heterodimers mediate the retinoid action, 3) retinoid regulated serine kinase activity influences the ratio of cytoplasmic to membrane-associated beta-catenin, 4) beta-catenin is associated with a serine kinase and can be phosphorylated by this kinase *in vitro*, both in complexes with and without E-cadherin, 5) beta-catenin exists as a serine phosphoprotein in cells. These data together with information from other studies lead to the following model. Firstly, cellular beta-catenin levels are regulated by retinoids and perhaps other steroid receptor ligands. Secondly, beta-catenin exists in three pools, a cytoplasmic pool and two cadherin-associated membrane pools, one Triton soluble and one Triton insoluble. Thirdly, beta-catenin interacts with different proteins in the three pools and beta-catenin stability and movement between the pools is regulated by the balanced action of serine and tyrosine kinases. In this model, alterations in the expression or function of certain retinoid receptors and/or any of several serine and tyrosine kinases would have marked effects on beta-catenin homeostasis. This could in turn influence not only cell-cell adhesion strength but could also, via the known interaction of cytoplasmic (but not cadherin-associated) beta-catenin with the tumor suppressor gene APC, regulate cell proliferation.

A7-106 ACTIN RING FORMATION AND CYTOKINESIS IN FISSION YEAST. Fred Chang, Ken Sawin, Allison Woollard, and Paul Nurse. Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London, WC2A 3PX U.K.

Cytokinesis in fission yeast involves a contractile actin ring, which forms in the middle of the cell during early mitosis and marks the site of subsequent septation and cleavage. We are interested in how this actin ring is formed, how it is regulated in the cell cycle, and how it is positioned in the middle of the cell. A large screen for temperature-sensitive mutants defective in cell division has identified 6 genes required for actin ring formation: *cdc3*$^+$, *cdc4*$^+$, *cdc8*$^+$, *cdc12*$^+$, *rng1*$^+$ and *rng2*$^+$. Mutants in these genes fail actin ring formation and cytokinesis and accumulate as large, multinucleate cells. Actin staining of these mutants revealed a number of phenotypic classes, suggesting that these genes play distinct functions in making the actin ring. We are focusing on the molecular characterization of one the actin ring genes, *cdc12*$^+$. The phenotype of *cdc12*$^-$ mutants suggests that *cdc12*$^+$ may specifically nucleate the actin ring in mitosis. *cdc12*$^+$ is a large protein with coil-coil regions and proline-rich regions, which may be SH3-binding or profilin-binding regions. Preliminary studies with a fluorescent green-cdc12 fusion protein show that cdc12 is localized to the site of the actin ring only during mitosis. In addition, we have identified a gene, *mid1*$^+$, which is involved in positioning the actin ring in the middle of the cell. A *mid1*$^-$ mutant forms an actin ring and septum at a random position and angle on the cell surface, but exhibits normal placement of the nucleus and spindle.

A7-107 CONTROL OF THE RAS-ADENYLATE CYCLASE PATHWAY IN THE YEAST *SACCHAROMYCES CEREVISIAE* BY THE *GGS1/TPS1* GENE PRODUCT. Sonia Colombo, Patrick Van Dijck, Joris Winderickx, Stefan Hohmann and Johan M. Thevelein. Laboratorium voor Moleculaire Celbiologie, Katholieke Universiteit te Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven-Heverlee, Belgium (+32)-16-321111.

In the yeast *Saccharomyces cerevisiae* two *RAS* genes, *RAS1* and *RAS2*, function as activators of adenylate cyclase. The RAS-adenylate cyclase pathway includes the *CDC25* gene product which promotes GDP/GTP exchange on the Ras proteins and the *IRA1* and *IRA2* gene products which stimulate RAS-GTPase activity.

The only known primary signals for activation of the pathway are glucose and related fermentable sugars and intracellular acidification. The mechanism sensing the glucose and responsible for activation of the Ras pathway is unclear. Transport and phosphorylation but no further metabolism of the glucose is required for the activation. Recently, it has been shown that the *GGS1/TPS1* gene product is required for triggering the glucose-induced Ras-mediated cAMP increase. However, *ggs1Δ* mutants are unable to grow on glucose because of a deficient control on glucose influx into glycolysis. Deletion of the *HXK2* gene, encoding the most active hexokinase, restores growth on glucose but also restores glucose-induced activation of cAMP synthesis. Although this appears to indicate that the *GGS1/TPS1* gene product is not required for the activation, deletion of the *HXK2* gene is known to abolish also glucose repression. Recent results show that deletion of the *GGS1/TPS1* gene strongly reduces the expression of genes that are normally downregulated by enhanced activity of the Ras-cAMP pathway. Deletion of *GGS1/TPS1* also counteracts derepression of these genes by mutations that attenuate the activity of cAMP-dependent protein kinase. These data appear to indicate that the *GGS1/TPS1* gene product might act as an inhibitor of an upstream component in the Ras-cAMP pathway.

A7-108 *enabled*, A GENETICALLY IMPLICATED SUBSTRATE OF THE DROSOPHILA ABL TYROSINE KINASE WITH SH3 BINDING PROPERTIES, Allen Comer, Frank Gertler, Shawn Ahern, F. Michael Hoffmann, McArdle Laboratory for Cancer Research, Univ. of Wisconsin, Madison, WI 53706

The Drosophila *Abl* gene encodes a non-receptor tyrosine kinase which is required for adult viability and is involved in development of the embryonic central nervous system. Heterozygous mutations in the *enabled* (*ena*) locus suppress the lethality associated with *Abl* mutations. The ena protein is bound by the c-abl SH3 domain in vitro and contains proline-rich sequences similar to those that interact with the c-abl SH3 domain. The ena protein contains phosphotyrosine and is a substrate for the Abl tyrosine kinase in vivo. The observation that ena is hypophosphorylated in *Abl* mutant animals suggests that this phosphorylation event may be important for normal development. Genetic interactions between the *ena* and *Abl* loci suggest that a balance between the levels of ena and Abl activity is required for normal development. These genetic and biochemical observations suggest a model in which a critical function of Abl is to attenuate ena function, perhaps by phosphorylation of specific sites in ena.

A7-109 MICE WITH TARGETED DISRUPTIONS IN THE PARALOGOUS HOX GENES HOXA-3 AND HOXD-3 REVEAL GENETIC INTERACTIONS

Brian G. Condie and Mario R. Capecchi, Howard Hughes Medical Institute, Department of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT 84112

The Hox genes encode transcription factors that mediate the formation of the mammalian body plan along the anteroposterior and appendicular axes. Paralogous Hox genes are very similar in sequence and expression patterns, suggesting that they may have at least partially redundant functions. Studies of mice with mutations in the paralogous Hoxa-3 or Hoxd-3 genes revealed that the single mutants share no phenotypes in common. However, double homozygotes for the Hoxa-3 and Hoxd-3 mutations reveal strong dosage-dependent genetic interactions between these genes. The structures altered in the single mutants are much more severely effected in the double homozygotes. We have interpreted these results as indicating partial genetic redundancy between these two paralogous Hox genes. Our results suggest that the Hox genes are a complex and interactive genetic network.

A7-110 **CONTROL OF THE NITROGEN CATABOLIC GENE NETWORK BY POSITIVE (GLN3) & NEGATIVE (DAL80) REGULATORY PROTEINS WHICH BIND TO IDENTICAL OR OVERLAPPING SITES IN S. CEREVISIAE**, T.G. Cooper, T.S. Cunningham, R. Rai, R.A. Dorrington & V. Svetlov, Dept. of Microbiol. and Immunol., Univ. of TN, Memphis, TN 38163

Expression of many nitrogen catabolic genes, including those of the γ-aminobutyrate (**UGA**) and allantoin (**DAL**) pathways in **S. cerevisiae**, is co-regulated by the GLN3 and DAL80 proteins. GLN3p is required for nitrogen catabolite repression-sensitive transcriptional activation. DAL80p down-regulates the inducer-independent **DAL** genes or maintains expression of the inducer-dependent **DAL** and **UGA** genes at a low level in the absence of inducer. The deduced sequences of the DAL80 and GLN3 proteins contain zinc-finger motifs homologous to those shown to bind GATA sequences in mammals. Moreover, DAL80p has been directly shown to bind to a site (URS_{GATA}) consisting of two GATA elements oriented tail-to-tail, or head-to-tail at least 15 bp apart. Two GATA-containing sites are also required for GLN3-dependent transcriptional activation (UAS_{NTR}). These observations suggested that sequences required for DAL80 binding and GLN3 function might overlap. We tested this hypothesis and found that the **DAL3** and **UGA4** GATA-containing sites required for optimal GLN3-dependent transcriptional activation and GLN3p-binding (UAS_{NTR}) also mediate DAL80p binding in *vitro* and DAL80-responsive regulation in *vivo* (URS_{GATA}). Only the 3' most GATA-containing site required for GLN3-mediated transcriptional activation of the **DAL7** gene overlaps with the URS_{GATA} site required for DAL80 binding and control. These observations suggest that DAL80p and GLN3p may regulate the nitrogen catabolic gene network by competing for the same DNA binding sites upstream of the genes contained in it. This work was supported by NIH grant GM-35642.

A7-111 STRUCTURE-FUNCTION ANALYSIS OF THE MAP KINASE ENCODED BY THE *KSS1* GENE OF *S. cerevisiae*, William Courchesne and Robert Archer, Department of Microbiology, University of Nevada School of Medicine, Reno, NV 89557-0046

We have genetically separated two distinct physiological functions for the yeast *S. cerevisiae* MAP kinase (Kss1) encoded by the *KSS1* gene. The yeast pheromone-response pathway requires the function of a kinase cascade that involves two related MAP kinases (encoded by the *KSS1* and *FUS3* genes). The function of Kss1 in the yeast pheromone response pathway has been uncertain, being proposed to function during both the initial G1-arrest response as well as for desensitization to this signal. We have begun a structure-function analysis of the Kss1 kinase by using site-directed mutagenesis to create single amino acid substitutions in Kss1 followed by analysis of their *in vivo* and *in vitro* activities. The mutant kinases fall into several classes including those retaining G1 arrest-stimulating activity but defective for desensitization, as well as those retaining desensitizing activity but lacking any G1 arrest-stimulating activity, demonstrating that these two physiological functions are genetically separable. All kinases that retained G1-arrest activity also allowed efficient mating, while none of the G1-arrest defective kinases allowed mating. Although the ability to cause G1 arrest correlated with functional *in vitro* protein kinase activity as expected, surprisingly, the desensitizing activity of Kss1 did not require functional kinase activity, suggesting that desensitization may involve the formation of a complex between Kss1 and its effector. This is the first time that such opposing activities as G1-arrest promotion and desensitization have been conclusively defined genetically for a MAP kinase.

A7-112 CONTROL OF PROLIFERATION ACTIVATION IN QUIESCENT NEUROBLASTS OF THE *DROSOPHILA* CENTRAL NERVOUS SYSTEM, Sumana Datta, Department of Biochemistry and Biophysics and Department of Biology, Texas A&M University, College Station, Texas, 77843

The *Drosophila* larval central nervous system is an excellent system to examine the regulation of neuroblast proliferation due to the stereotyped pattern of neuroblast birth and cell division. Defined populations of neuroblasts undergo cycles of quiescence and active cell division in a specific spatial and temporal pattern. I have previously identified a mutation at the *trol* locus that causes a 90% drop in the dividing cell population of the larval central nervous system (1). Studies of *trol* mutants suggest that the *trol* gene product is required for the activation of proliferation in quiescent neuroblasts, but not for the maintenance of cell division in already dividing neuroblast populations. The *anachronism* (*ana*) locus has been identified and characterized by the Zipursky laboratory (2). *ana* encodes a secreted glial glycoprotein that represses premature neuroblast proliferation in the larval brain. *ana trol* double mutants were constructed to examine developmental hierarchies in the regulation of neuroblast proliferation in the developing brain. Quantitative analysis of the thoracic neuroblast population demonstrates that the double mutant phenotype is indistinguishable from the *ana* mutant phenotype. These studies lead to model suggesting that: 1) *ana* represses premature neuroblast activation. 2) repression is released by *trol*, which functions to inactivate the *ana* repressor directly or bypasses the *ana* repressor to initiate proliferation further downstream in an *ana*-dependent pathway.

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2. A. J. Ebens, Garren, H., Cheyette, B. N. R. and Zipursky, S. L. (1993). The *Drosophila anachronism* locus: A glycoprotein secreted by glia inhibits neuroblast proliferation. Cell **74**(1): 15-28.

A7-113 **MOLECULAR GENETIC STUDIES OF MUSCLE - SPECIFIC ALTERNATIVE SPLICING IN DROSOPHILA.** Mary Beth Davis, David M. Standiford, and Charles P. Emerson, Jr. Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6058

We have designed a genetic screen to isolate mutations in genes encoding trans-acting alternative splicing factors in insect flight muscle. Proper structure and function of the indirect flight muscle (IFM) are not essential for viability in Drosophila and mutations in genes encoding IFM products generally result in flightlessness; thus we expect mutations in IFM splicing factors to exhibit a similar flightless phenotype. Our screen for these splicing factors employs the flp/FRT mosaic technique (Xu and Rubin, 1993) to generate populations of F1 mutant mosaic flies, and mosaic nonfliers are then selected from the population with a flight tester. Because the IFM are multinucleated cells it was necessary to demonstrate that flp-recombinase induced mitotic recombination in early muscle precursor cells could generate sufficient homozygous mutant nuclei in the IFM to disrupt flight, therefore we conducted a reconstruction experiment that demonstrated the feasibility of using this mosaic strategy to isolate flightless mutants. In this pilot experiment flp recombinase was used to generate mosaics in flies heterozygous for a recessive mutation in the IFM isoform of the α-actinin gene; conditions were established in which flightless mosaics were recovered. Using this method to search for muscle-specific splicing mutants, we then screened 15,000 mutagenized chromosomes and approximately 50 flightless mutants have been recovered. The characterization of these mutants in terms of the number of complementation groups and the genetic map positions is currently in progress. Mutations in many genes have a flightless phenotype; therefore we developed two splicing reporter genes as molecular assays to distinguish between mutations in IFM splicing factors and mutations in other IFM gene products. These lacZ based reporter genes contain Drosophila myosin heavy chain genomic DNA sequences which we have previously shown can direct IFM-specific alternative splicing of Mhc minigenes in transgenic flies. The patterns of expression of these Mhc reporter genes will be indicative of aberrations in splicing of the Mhc transcript. We are currently crossing the flightless mutants to flies carrying these Mhc-lacZ reporter genes to identify mutations that affect Mhc alternative splicing.

A7-114 ROLE OF YEAST HEXOKINASES IN GLUCOSE-INDUCED SIGNALLING, GLUCOSE REPRESSION AND CONTROL OF GLYCOLYSIS. Johannes H. de Winde, Joris Winderickx, Stefan Hohmann, and Johan M. Thevelein. Laboratory for Molecular Cell Biology, Katholieke Universiteit Leuven, Kardinaal Mercierlaan 92, B-3001 Leuven-Heverlee, Belgium (+32)-16-321111.

Addition of glucose or fructose to derepressed cells of *Saccharomyces cerevisiae* causes a variety of short- and long-term regulatory effects, eventually leading to an adaptation to fermentative metabolism. Hallmarks of this regulatory switch are a rapid transient induction of cAMP synthesis, inactivation of gluconeogenic enzymes and of high-affinity sugar transport, mobilization of trehalose, induction of the expression of glycolytic enzymes and glucose repression of the synthesis of respiratory, gluconeogenic and other enzymes necessary for growth on other carbon sources.

Yeast cells mutated in the *GGS1/TPS1* gene, encoding the catalytic subunit of the trehalose synthase complex, are defective in all of the glucose-induced regulatory responses and do not grow on glucose or fructose. Subsequent deletion of the *HXK2* gene, encoding the predominant yeast hexokinase, restores growth on glucose, apparently due to prevention of accumulation of sugar phosphates and depletion of phosphate. In addition; all glucose-induced regulatory responses are restored, indicating that GGS1/TPS1 may not be essential for glucose-induced signalling.

To further investigate the mechanism by which glucose-induced signalling in yeast is triggered, we have isolated several dominant *HXK2* alleles, suppressing the glucose growth defect of the *ggs1/tps1* mutation. The $HXK2^{D}$-mutants show a wide range of hexokinase activities, in combination with a wide variety of altered glucose repression patterns, including that of the second hexokinase gene *HXK1*. We discuss implications of these findings for the involvement of *GGS1/TPS1* and the yeast hexokinases in glucose-sensing and the establishment of the glucose-repressed state.

A7-115 TARGETED DISRUPTION IN DICTYOSTELIUM OF A HUMAN RAC PROTEIN KINASE HOMOLOG GENE ENCODING A PLECKSTRIN HOMOLOGY (PH) DOMAIN CAUSES HETEROCHRONIC DEVELOPMENT. Robert P. Dottin,[1] Byoung Chon Moon,[1] Bodduluri Haribabu,[1] Benjamin Ortiz,[1] Mauro Rabino,[1] Geri Reichel,[1] Stephanie Porcelli,[1] Paul Skehel, [2] Jeffrey Williams, [2] Sandrine Bouzid,[3] Michel Veron,[3] and Frantisek Puta,[4] [1]Department of Biological Sciences and Center for Study of Gene Structure and Function, Hunter College of the City University of New York, NY, [2]Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, Herts, U.K, [3]Institut Pasteur, Unite de Biochimie Cellulaire, Paris, France, [4]Department of Microbiology and Genetics, Charles University, Prague, The Czech Republic.

To study the role of reversible protein phosphorylation in signal transduction in *Dictyostelium,* we isolated and characterized a gene DdK6 encoding a serine/threonine protein kinase. DdK6, has a pleckstrin homology (PH) domain, which has recently been implicated in transient protein-protein interactions during mammalian signal transduction, and is closely related to the Rac protein kinase family. The retroviral *rac* oncogene causes T cell lymphomas in rodents. DdK6 is expressed transiently early in the development of *Dictyostelium.* Targeted disruption of the DdK6 gene created null mutant cell lines, which display heterochronic development, with delayed and asynchronous formation of mounds and fruiting bodies, and deranged expression of several stage specific genes. cAMP regulated gene expression is also similarly deranged. We synthesized a Multiple Antigen Peptide (MAP) in which eight identical C-terminal peptide molecules are linked to a polylysine core. Immuno-cytochemical studies with antiserum to this MAP detected only the DdK6 polypeptide on a western blot and localized it to the nucleus. These data suggest that DdK6 regulates the timing of development and that PH domains may function in signal transduction in *Dictyostelium.*

A7-116 DECISION POINTS AND THE GENETIC CONTROL OF MEIOTIC PROGRESSION: A YEAST MODEL

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Our previous studies have demonstrated that the cytologically defined program of meiotic differentiation is conserved between mammals and a yeast model system. In our working model, progression through the sequential stages of meiotic differentiation is mediated via a series of genetically defined decision points. We have cloned two meiosis essential genes from yeast, SPO11 and MEI5, that mediate progression through Prophase I. Based on their DNA sequence, these genes encode previously unidentified meiosis specific proteins. The SPO11 gene defines a decision point in early Prophase I and is required for proper meiotic chromosome behavior. Meiosis in a yeast strain deleted for the SPO11 gene skips late Prophase I and prematurely executes the first meiotic division, yielding grossly aneuploid and largely inviable spores. In contrast, the MEI5 gene defines a decision point in late Prophase I, just prior to the first meiotic division. Meiosis in a strain deleted for the MEI5 gene arrests at a normal stage in late Prophase I, cytologically and functionally equivalent to that observed as the arrest stage of an immature mammalian oocyte. Mei5 arrested meiocytes have initiated normal levels of molecular recombination but may not have completed or resolved these events. This result suggests a possible role for chromosome behavior in the arrest and resumption of meiosis, perhaps acting via a checkpoint control. Isolation of high copy number suppressors and genetic analysis of meiosis in multiply mutant yeast strains has allowed us to define a temporal pathway of decision points acting sequentially to control meiotic progression in yeast.

A7-117 ISOLATION OF *DICTYOSTELIUM* CELL FATE DETERMINATION GENES, R. H. Gomer, S. A. Wood, L. Y. Li, D. A. Brock, R. R. Ammann, and T. P. Spann Howard Hughes Medical Institute, Department of Biochemistry and Cell Biology, Rice University, Houston, TX 77005

We have developed a general method to identify genes in *Dictyostelium*, and have used it to isolate genes involved in the cell cycle-dependent cell-type differentiation mechanism. An autosubtracted cDNA library from vegetative and early developing cells was ligated into a *Dictyostelium* transformation vector downstream from a vegetative promoter. This set of constructs was then transformed into cells for shotgun antisense mutagenesis. Several transformants having unusual developmental morphologies were identified. The antisense DNA was isolated from the transformants, cloned, and used to re-transform *Dictyostelium*. Currently, seven cDNAs which cause an abnormal developmental morphology have been identified by their ability to regenerate the original phenotype. All seven transformants have normal growth rates and cell cycles (from assays for percentage in M, S, and the G1:G2 ratio). Sequencing of the antisense cDNAs indicates that one has some similarity to a cathepsin protease and another encodes cyclophilin.

One cDNA, Fb-, when expressed in antisense causes development to stop at the mound stage. At very low cell densities, these cells have an abnormally high percentage of prestalk cells and a normal percentage of null cells, and thus may have a defect in the mechanism which monitors the cell cycle for cell fate determination. Sequence data indicate that Fb- is not similar to any known gene, and Northern blots indicate that the single message is expressed at very low levels in vegetative and early developing cells.

Another cDNA, V28, when expressed in antisense causes fruiting bodies to have short, thick stalks and very large spore masses. At low cell densities, these cells have a higher than normal percentage of both prestalk and prespore cells and a low percentage of null cells. Sequence data indicate that V28 is not similar to any known gene, and both Northern blots and sequencing of cDNAs indicate that there are multiple, differentially spliced transcripts in vegetative and early developing cells.

For both Fb- and V28, Southern blots indicate that there is only one gene. We have isolated genomic fragments of both genes, and have made gene disruption transformants which in both cases have the original phenotype. We are using these cDNAs to examine the molecular basis of the cell-type choice mechanism.

A7-118 ***Tiam1*, AN INVASION INDUCING GENE THAT ENCODES A POTENTIAL GDP-GTP EXCHANGER FOR Rho-LIKE GTPases. Gaston Habets, Rob van der Kammen, Jord Stam, Frits Michiels and John Collard. Div. of Cell Biology, The Netherlands Cancer Institute, Amsterdam.**

Tiam1 was identified in T-lymphoma cells by combining proviral tagging with a functional in vitro selection for invasiveness (Cell V77:537). The murine gene is located on chromosome 16 and is activated in the invasive variants by truncations, due to proviral insertions, or by amplification. The human ***TIAM1*** gene is located on human chromosome 21q22 in a region frequently rearranged in human leukemias and the predicted Tiam1 protein is highly conserved between man and mouse (>94% identity). ***Tiam1*** is variably expressed in a wide variety of human tumor cell lines but not expressed or at low levels in most normal tissues, except for brain and testis. The Tiam1 gene product is located mostly in the cytoplasm and harbors a DBL- and two Pleckstrin-homologous domains, which it shares with GDP-dissociation stimulators for RHO-like proteins. In that manner Tiam1 might affect cytoskeletal processes via these GTPases. Treatment of the invasive T-lymphoma cells with C3-exoenzyme, that inactivates RhoA, abolished invasive capacity. Many of the GDS-proteins which harbor a DH-domain have been identified as transforming genes in NIH3T3 cells. Currently we are transfecting these cells with ***Tiam1*** cDNA constructs to test their oncogenic potential. The morphology of the NIH3T3 transfectants may also indicate which Rho-like GTPase is activated by ***Tiam1*** as Rac1 induces membrane ruffling whereas RhoA induces stress fiber formation. Transfection of non-invasive lymphoid cells with activated Rho-like GTPases are now ongoing to determine which GTPase can induce the invasive capacity. Our data suggest that Tiam-1 connects extracellular signals to cytoskeletal activities by modulating the activity of a Rho-like GTPase. (supported by the Dutch Cancer Society)

A7-119 ANALYSIS OF GENE NETWORKS ACTIVATED BY RECOMBINANT BONE MORPHOGENETIC PROTEIN 2 (rhBMP 2) DURING BONE CELL DIFFERENTIATION IN VITRO, Harris SE, Harris MA, Ghosh-Choudhury N, Feng JQ, Wozney J, Rosen V, and Mundy GR. Div. of Endocrinology, Dept. of Medicine, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7877 and Genetics Institute, Cambridge MA 02140

The bone morphogenetic protein family of TGFβ related ligands are involved in a diverse set of differentiation programs in different tissues from C. elegans to man. In particular recombinant BMP 2 (rhBMP 2) is capable of directing uncommitted fibroblast-like mesodermal cells to differentiate to bone and cartilage. We have developed an immortalized clonal cell line, 2T9 cells, from a transgenic mouse containing the BMP 2 promoter driving large T-antigen. 2T9 cells represent a pre-osteoblast state in which addition of rhBMP 2 activates the bone cell differentiation program. Without rhBMP 2 little differentiation occurs, while in the presence of rhBMP 2, after 16-20 days, self-organized mineralized bone structures are formed of a fractal nature, similar to bone formed in vivo. Many bone cell gene products, such as osteocalcin, are expressed in a specific temporal and spatial pattern after rhBMP 2 addition. The system exhibits a high degree of effective complexity and is suitable for analysis of gene networks. Using the 2T9 rhBMP 2 system, we are isolating and cloning rhBMP 2 modulated cDNA using differential display RT-PCR of poly(A)RNA from 2T9 cell cultures untreated and treated with rhBMP 2. One cDNA, B48/190 is activated as early as 2 hrs after rhBMP 2 treatment and is also regulated by rhBMP 2 in primary bone cell cultures. A variety of other cDNA that are modulated by rhBMP 2 in 2T9 cells are presently being characterized. With the isolation of the set of rhBMP 2 regulated genes, we can now begin to build the gene networks involved in a simple differentiation paradigm.

A7-120 POLYGENIC INTERACTIONS AND PROTEIN SYNTHESIS IN *DROSOPHILA* MORPHOGENESIS, Ralph Hillman, Sheryl Love, Cathie Lovett, and Wayne Allen, Department of Biology, Temple University, Philadelphia, PA 19122.

The mutation *Abnormal Abdomen (A)* in *Drosophila melanogaster* results in a slowdown of cell division and the loss of adult tergite and sternite formation. Evidence has been presented that the penetrance and expressivity of the phenotype is a function of a mutation in a major sex-linked gene in combination with modifiers in the residual genotype. There is also evidence that the mutant genome is responsible for the differential cellular distribution of tRNA aminoacylating enzymes and that this differential distribution has an effect on the rate of tRNA aminoacylation.

The modifier system responsible for the control of penetrance and expressivity consists of a number of genes located on both the X-chromosome and the major autosomes. Wild-type stocks showing a low penetrance of tergite loss have a greater effect on the penetrance and expressivity of *A* than do those stocks whose genomes do not result in the loss of tergite formation. Additionally, an enhancer gene located on the distal end of the left arm of chromosome 2 has no effect alone. In the double heterozygote with *A (A/+;E(A)/+)*, however, it results in 100% penetrance and high expressivity of the abnormality.

The relationship between the mutation *A*, protein synthesis and the morphological changes in the adult abdomen has been tested by combining *A* with two groups of mutations known to affect protein synthesis. The first of these is a series of *Minutes*; dominant mutations which reduce protein synthesis and slow development. The second is a multiple allelic series of *bobbed* mutations at the rDNA locus, ranging from minor to complete loss of cellular rRNA. In combination with *A* these mutations result in high penetrance and expressivity of tergite loss. In combinations with *bobbed*, the more extreme the loss of rDNA, the greater the phenotypic expression of the abdominal abnormality.

A7-121 INHIBITION OF PHEROMONE-INDUCED CELL CYCLE ARREST BY THE **a**-FACTOR RECEPTOR IS INDEPENDENT OF THE G PROTEIN MEDIATED PATHWAY, Jeanne P. Hirsch, Andrés Couve, and Jinah Kim, Department of Cell Biology and Anatomy, Mount Sinai School of Medicine, New York, NY 10029

The response of haploid yeast cells to the presence of mating factors includes arrest in the G1 phase of the cell cycle and induction of specific gene expression. We have recently shown that inappropriate expression of the **a**-factor receptor gene, *STE3*, in *MAT***a** cells produces resistance to the cell cycle arrest induced by α-factor. Expression of *STE3* in **a** cells also suppresses the constitutive signaling phenotype of null alleles of *GPA1*, the gene encoding the α subunit of the G protein involved in pheromone signaling. This effect is independent of the *STE2* gene, which encodes the α-factor receptor, so it is not the result of interference between receptors. It is also independent of the *MFA1* and *MFA2* genes, which encode **a**-factor, and is therefore not due to desensitization resulting from autocrine stimulation.

The components of the pheromone response pathway that act upstream of the MAP kinases regulate both the cell cycle arrest and transcriptional induction responses to pheromone. Inappropriate expression of *STE3* was found to specifically inhibit the cell cycle arrest response, and had little or no effect on transcriptional induction, suggesting that it acts at or downstream of the MAP kinases. The MAP kinase Fus3p is thought to play an important role in cell cycle arrest because mutations in the *FUS3* gene have a disproportionate effect on G1 arrest. *STE3* conferred α-factor resistance on an **a** strain containing a null allele of *FUS3*, demonstrating that inhibition of cell cycle arrest by the receptor does not occur by specifically inhibiting the function of Fus3p. These results indicate that the **a**-factor receptor has a fairly direct effect on proteins involved in regulating the cell cycle.

A7-122 A YEAST LEU3 PROTEIN-α-ISOPROPYLMALATE COMPLEX ACTIVATES *GDH1*, A KEY GENE IN NITROGEN METABOLISM, Yuanming Hu and Gunter B. Kohlhaw, Department of Biochemistry, Purdue University, West Lafayette, IN 47907

The Leu3 protein of *Saccharomyces cerevisiae* is a transcriptional regulator of genes encoding enzymes of the branched-chain amino acid biosynthetic pathways. Leu3p binds to upstream activating sequences (UAS_{LEU}) present in the promoters of *LEU1*, *LEU2*, *LEU4*, *ILV2*, and *ILV5*. In vivo and in vitro experiments have shown that activation by Leu3p requires α-isopropylmalate (α-IPM), an intermediate in the leucine pathway. In at least one case (*LEU2*), Leu3p actually represses basal level transcription when α-IPM is absent. Following identification of a UAS_{LEU}-homologous sequence in the promoter of *GDH1*, the gene encoding $NADP^+$-dependent glutamate dehydrogenase, we show that Leu3p specifically interacts with this UAS_{LEU} element. We then show that Leu3p is required for full expression of the *GDH1* gene. First, a *leu3* deletion mutant shows significantly lower levels of *GDH1-lacZ* expression and of $NADP^+$-dependent glutamate dehydrogenase activity, compared with wild type. Second, a decrease is also seen in the steady state level of *GDH1* mRNA. Third, the removal of UAS_{LEU} from the *GDH1* promoter also causes a strong decrease in *GDH1-lacZ* expression. We further show that the level of *GDH1-lacZ* expression correlates with the cells' ability to generate α-IPM and is lowest in cells unable to produce α-IPM. We conclude that Leu3p exerts its regulatory role beyond the branched-chain amino acid pathways. We suggest that the Leu3p-α-IPM complex may function as a more general communications link between amino acid biosynthesis and the early stages of nitrogen assimilation.--Supported by NIH Grant GM15102.

A7-123 GENETIC ANALYSIS OF EYE DEVELOPMENT IN DROSOPHILA USING FLP-FRT RECOMBINATION SYSTEM, Naoto Ito and Gerald M. Rubin, Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA 94720-3200

Drosophila eye offers an excellent system for genetic analysis of genes involved in the formation of an adult structure. Many of these genes are essential for viability or early development and mutation of these genes will result in lethality, making an extensive genetic screen difficult. The use of the site-specific recombination system of the yeast 2μm plasmid provides an efficient method of the identification of such genes. This plasmid encodes a recombinase, FLP(flipase), which acts on specific target sequences(FRT) and induces recombination between FRT sites. Xu and Rubin (Development 117, 1223-1237) have constructed a number of fly strains carrying FRT sites at the base of each major chromosome arm and have demonstrated the ability to generate mosaic clones in adult eye and other tissues.

We are currently conducting a genetic screen to identify genes essential for eye development using fly strains with FRT site at the base of chromosome arm 3L. Our approach involves an X-ray mutagenesis followed by induction of recombination using FLP-FRT system. In many cases mutation of genes involved in eye development will disrupt the regular smooth array of unit eyes(ommatidia), causing a roughening of the lattice within the clone. We have screened 150,000 mutagenized F1 flies and 26,000 produced clones in the adult eye. 93 mutant lines were established from this screen and further analyzed. Initial characterization of some of these mutants will be presented.

A7-124 IDENTIFICATION AND CHARACTERIZATION OF NEW GENES REQUIRED FOR DORSAL-VENTRAL AXIS FORMATION IN *DROSOPHILA*, Noriko W. Ito, Marcia P. Belvin, Kathryn V. Anderson. Department of Molecular and Cell Biology, LSA555, University of California at Berkeley, Berkeley, CA 94720-3200

Many genes that are required maternally for dorsal-ventral axis formation are important for viability. Because mutations in such genes are likely to cause lethality, they would not have been identified by a maternal effect on dorsal-ventral axis formation. Looking for mutations which enhance the weak phenotype of a recessive allele provides a novel and efficient method for the identification and study of genes required for dorsal-ventral axis formation, irrespective of whether such genes are necessary for viability. We are currently analyzing mutations isolated in a screen to identify mutations that enhance the phenotype of an unusual *cactus* allele, *cactus E10*. *cactus* encodes an IκB-like molecule and is presumed to retain the dorsal protein in the cytoplasm, just as IκB retains NFκB in the cytoplasm. *cactus E10* causes dorsalized embryos when in trans to loss-of-function cactus alleles. However $cact^{E10}/cact^{+}$ females produce wild type embryos. Females that are heterozygous for both *cactus E10* and another loss-of-function mutation in a dorsal group gene, such as *dorsal, tube, pelle, Toll,* and *spätzle* do lay dorsalized embryos. We isolated 33 mutants (*Enhancer of E10*) that reproducibly enhanced *cactus E10* and mapped to a single genomic region. Mapping, complementation and mosaic analysis of these mutations will be presented.

A7-125 STRUCTURE-FUNCTION ANALYSIS OF THE PAIRED PROTEIN IN DROSOPHILA, Susie Jun, Claudio Bertuccioli, Guojun Sheng, David Wilson, and Claude Desplan, Howard Hughes Medical Institute, Rockefeller University, New York, NY 10021

paired (*prd*), a pair-rule gene involved in early *Drosophila* embryogenesis, regulates the expression of known genetic targets. *prd* encodes a transcription factor that contains two independent and conserved DNA binding domains, the paired domain (PD) and the homeodomain (HD). The Paired (Prd) protein belongs to the family of Pax proteins which include vertebrate PAX3 and PAX6 that are associated with human genetic disorders. We describe a structure-function analysis of Prd, focusing on the general role of the conserved DNA binding domains on function.

Optimal binding sites for the PrdPD and the PrdHD were isolated from a library of random DNA oligos. The optimal binding site for the PrdPD is unusually long and is similar to optimal sites identified for other PDs from other Pax protein classes. We obtained a crystal structure of the PD bound to its optimal site. The PD binds as a monomer to its site, and folds as two structurally similar subdomains. Known missense mutations found in Pax proteins associated with mutant phenotypes map to specific residues that make important DNA contacts. The structure also reveals that the C-terminal subdomain does not contact DNA and may play a distinct role from that of the N-terminal subdomain. We also obtained a crystal structure of the PrdHD binding as a cooperative dimer on its preferred palindromic site.

The functional consequences of the above structural relationships were tested. Using transient transfection assays, we show that Prd activates transcription by binding either through the PD or the HD and that the two DNA binding domains can act independently. In addition, using an in vivo rescue assay, we show that the N-terminal subdomain of the PD is required for Prd function, and that the C-terminal subdomain is dispensable. The C-terminal subdomain has the potential to bind to DNA, and may play an accessory role in other Pax proteins.

A7-126 THE *PRY-1* AND *SPY-1* GENES MAINTAIN PATTERNED GENE EXPRESSION IN *C. ELEGANS* Julin Maloof and Cynthia Kenyon, Dept. of Biochemistry and Biophysics, U. C. S. F., San Francisco, CA 94143-0554

The *C. elegans Hox* genes are expressed in region-specific domains along the anterior-posterior body axis. They function within these domains to specify region-specific fates. *Hox* expression begins normally in *polyray–1(pry–1)* mutant animals, but later the *Hox* genes become expressed outside of their normal domains. This suggests that the wild-type function of *pry-1* is to keep *Hox* genes repressed where they are not originally activated. The ectopic *Hox* expression seen in *pry–1* mutants leads to dramatic homeotic transformations.

The homeotic transformations seen in *pry-1* mutants are suppressed by *spy-1 (suppressor of polyray)* mutations. Do mutations in *spy-1* suppress *pry-1* mutations by preventing ectopic *Hox* expression? Yes, a reporter construct for the *Hox* gene *mab-5*, which is ectopically expressed in the *pry-1* mutant, is expressed in a pattern similar to wild-type in the *pry-1; spy-1* double. *spy-1* is required for wild-type *Hox* expression as well. Expression of the *mab-5* reporter is initiated correctly in *spy-1* mutants, but in at least one cell, QL, the expression is not correctly maintained, suggesting that the wild-type function of *spy-1* is to maintain *Hox* gene activation.

The *pry-1* and *spy-1* mutant phenotypes suggest that the function of these genes may be similar to the *Polycomb* and *trithorax* group of *Drosophila* which function to maintain initial patterns of gene expression throughout development. However in *C. elegans* (and other organisms), the *Hox* expression patterns are dynamic, changing throughout development. We have found that in the V5 lineage *pry-1* repression of early *mab-5* expression is required for normal development. Later in the lineage, *mab-5* must be derepressed for normal development to continue. This derepression suggests the surprising possibility that the repression of *Hox* gene expression by maintenance genes is reversible.

A7-127 *STATHMIN PARTNERS* IN VIVO: *INTERACTION CLONING IN YEAST,* Alexandre Maucuer, Jacques Camonis* and André Sobel, *INSERM U153, 17 rue du Fer à Moulin, 75005 Paris, *INSERM U248, 10 Av. de Verdun, 75010 Paris, France.*

Stathmin, a ubiquitously expressed cytoplasmic and soluble protein, appears as a major phosphorylation substrate associated with numerous cell regulations. Its expression is regulated also, with a peak of stathmin level around birth in all tissues, suggesting, together with the phosphorylation studies, the implication of stathmin in the controls of cell proliferation and differentiation.

Molecular analysis indicates that it is a highly conserved 19 kDa polypeptide composed of an N-terminal signal integrating, regulatory domain, - phosphorylatable on up to four sites at least by PKA, cdks and MAP kinases - , and of a C-terminal region predicted to participate in coiled-coil interactions. We propose that stathmin acts as a relay and integrator of regulatory signals, the phosphorylation events on stathmin controlling the functions of multiple interacting proteins.

On the basis of this model of stathmin action, we used the two-hybrid system in yeast to screen a mouse embryonic cDNA library and identified several proteins whose interactions with stathmin are potentialy involved in its molecular function :

- KIS is a newly identified putative serine/threonine kinase, interacting with stathmin through the larger lobe of the catalytic domain; It might either be regulated by or phosphorylate stathmin, or both.

- CC1 and CC2 are strongly predicted to form alpha-helical coiled-coil structures and therefore most probably interact with the C-terminal part of stathmin. They are good candidates as stathmin regulated proteins.

Molecular and biochemical characterisations of these proteins are underway, in order to analyse the possible functional significance of their interactions with stathmin.

A7-128 AN *IN VIVO* METHOD FOR THE IDENTIFICATION OF GROWTH FACTOR-RESPONSIVE GENES IN DEVELOPING MICE, Barbara S. Mroczkowski and Sharon Tracy, The Agouron Institute, 505 Coast Blvd. S., La Jolla, CA 92037

Epidermal growth factor (EGF) administration to neonatal mice represents a powerful biological system to study the complex effects of growth factors on development. Administration of EGF to neonatal mice results in the rapid tyrosine phosphorylation of a number of tissue-specific substrates, most notably in liver, lung and brain. Within minutes after intraperitoneal injection of growth factor, one observes the appearance in liver nuclei of several tyrosine phosphorylated proteins. Nuclear substrates that are phosphorylated in an EGF-dependent manner include transcriptional factors that bind specifically to the *sis*-inducible element of the *c-fos* promoter (Ruff-Jamison *et al.*, *Science* **261**, 1993). We propose to extend these studies to identify genes that mediate the biological responses elicited by EGF in the developing mouse. The experimental approach we are employing to examine differentially expressed genes responsive to EGF is based on the arbitrarily primed polymerase chain reaction (AP-PCR), a fingerprinting method used to detect differences in sequence or sequence complexity of genomic DNA or cDNA samples. This methodology is similar, in goals, to subtractive hybridization, but is far more sensitive and has the advantage of being technically much simpler. Briefly, RNA from tissues from control and EGF-treated mice is reverse transcribed in the presence of arbitrarily selected primers. Subsequent PCR reactions performed at low stringency generate a complex fingerprint of each tissue. Fingerprints are being examined for differences between control and EGF-treated tissues by S1 and Northern analysis. The cloning and characterization of novel gene products represent the ultimate goal of this investigation. The speed and sensitivity of this PCR-based finger-printing technique, in conjunction with the nanogram amounts of cytoplasmic RNA used to sample different tissues, should make it technically feasible, for the first time, to rapidly and efficiently identify genes instrumental in the regulation of mammalian growth and development.

A7-129 DIFFERENTIATION OF RHIZOBIUM DURING NODULATION OF ALFALFA, Valerie Oke, Brenda Rushing, Michael Willits, and Sharon Long, Department of Biological Sciences, Stanford University, Stanford, CA 94305.

Rhizobium meliloti is a bacterium that establishes symbiosis with plants such as alfalfa by eliciting formation of nitrogen-fixing nodules on roots. In response to a signal from the plant, the bacteria produce Nod factor which stimulates plant cells to divide and organize a nodule. The bacteria enter the developing nodule through an infection thread, are released into plant host cells, and undergo metamorphosis into a distinct cell-type called a bacteroid which is capable of fixing nitrogen. Little is known about the developmental events that occur during bacteroid differentiation. These events are critical for successful symbiosis in that the entry of the bacteria must be controlled so that a plant defense response is not mounted, the bacteria must adapt to living within the specialized environment of a nodule cell, and the bacteria must become capable of fixing nitrogen. We are initiating a screen to identify genes that are involved in the differentiation of free-living bacteria to the bacteroid state.

Two lines of ongoing research relate to the topic of *R. meliloti* differentiation. First, three genes encoding RNA polymerase sigma factors have been cloned and sequenced. One sigma factor is the vegetative sigma factor, whereas the other two are alternative sigma factors. Currently we are investigating conditions under which these alternative sigma factors may direct transcription. Second, we are studying the expression of two homologous loci (*nodPQ*$_1$ and *nodPQ*$_2$) that encode enzymes which synthesize a high energy sulfate donor which is then used in the formation of active sulfurylated Nod factor. Interestingly, both loci are subject to growth-rate dependent regulation such that gene expression increases as cells enter stationary phase. Analysis of these promoters may lead to the identification of elements required for gene expression during stationary phase.

A7-130 ANALYTICAL RESULTS RELATED TO THE STATISTICS OF GENE PRODUCTS SYNTHESIS. Jean Peccoud, TIMC-IMAG, Institut Albert Bonniot, Faculté de médecine de Grenoble, 38706 La Tronche, France. Bernard Ycart, LMC-IMAG, BP 53 38041 Grenoble Cedex 9, France

A global view of inducible gene expression is usually broken up into two main steps. First a transcription complex is formed leading to an activated gene. After activation, gene products can be synthesised and accumulate in the cell. Since some of the molecules that tune gene expression such as the genes themselves, RNAs or transcription factors are present at a few copies per cell, a kinetic model of gene product accumulation in the cell has to be stochastic. In order to investigate the dependence of the gene product statistical distribution on the stability of the activated state and the rate of product synthesis, the Markov process defined by the following set of interactions has been worked out : $I \xrightarrow{\lambda} A; A \xrightarrow{\mu} I; A \xrightarrow{\nu} A + P$ We give an analytical expression of the mean value and standard deviation of the gene product distribution. Moreover the asymptotic normality of this distribution is demonstrated and its numerical approximate is computed by the mean of a power series expansion of its generating function. These theoretical results are very helpful to decipher the asymmetrical role played by the key parameters of gene expression : the transcription complex stability and the rate of gene product synthesis. Different sets of parameters can result in gene product distributions with similar means but with different standard deviations. The biological consequences of these fluctuations are discussed.

A7-131 ELUCIDATING BMP SIGNALING BY GENETIC APPROACHES IN DROSOPHILA, Andrea Penton, Yijing Chen, Karen Staehling-Hampton, Shigeru Morimura, Patricia Rohwer-Nutter P. David Jackson, and F. Michael Hoffmann, McArdle Laboratory for Cancer Research and Laboratory of Genetics Medical School, Madison, WI 53706

In Drosophila there are three known ligands which represent members of the Bone Morphogenetic Protein family, decapentaplegic (dpp), 60A and screw. dpp is necessary for dorsal/ventral patterning in the embryo, regulation of homeotic gene expression in the gut, and for proliferation and pattern formation in the imaginal disks. Like their mammalian counterparts dpp and 60A can induce endochondral bone formation in rats. Two type I receptors have been identified in Drosophila which are encoded by the genes *thickveins* (*tkv*) and *saxophone* (*sax*). These receptors have been shown to bind BMP2 which is the mammalian homologue of dpp. In addition certain alleles of *tkv* and *sax* have been shown to interact genetically with some *dpp* alleles. Unlike null *dpp* alleles which ventralize Drosophila embryos, *tkv* null alleles lead to a lack of embryonic dorsal hypoderm. We are using both genetic and molecular methods to identify genes that participate in BMP signaling in Drosophila. We have been looking for genetic interactions between *dpp* or *tkv* and other genes that have a dorsal open phenotype. This has resulted in the cloning of a gene called *shnurri*. Results utilizing these approaches will be presented.

A7-132 IDENTIFICATION OF COMPONENTS INVOLVED IN THE OSMOTIC SIGNAL TRANSDUCTION PATHWAY IN THE YEAST *SACCHAROMYCES CEREVISIAE*, Bert Pöpping and Martin D. Watson, Department of Biology, University of Durham, England, UK.

The osmotic signal transduction pathway in the yeast *Saccharomyces cerevisiae* has recently been described [1]. It shows obvious similarities in its structure to the earlier discovered cell wall integrity and pheromone response pathway as the same type of protein kinase cascade is responsible for transducing the signal from the cell surface to the nucleus. Several components of this pathway, including HOG1, PBS2, SLN1 and SSK1, were identified [1,2]. So far, neither a MAPKKK nor any further component of this pathway have been found.

We are using the two-hybrid system to examine interactions of some of the known components with other - currently still unknown - proteins involved in this pathway. The known components were cloned into the GAL-binding domain vector and screened for interactions against a genomic library cloned into a vector containing the GAL-activator domain. Several putative positive clones have been isolated, some of them showing abnormal morphologies as well as budding defects. These observations tally with the latest results of Brewster et. al [3] showing that the polarity of cell growth and division after osmotic stress requires a MAP kinase pathway. Therefore other links into the osmosensing signal transduction pathway remain to be described.

[1] Brewster J.L. et al. (1993), An osmosensing signal transduction pathway in yeast, Science Vol. 259, pp 1760-1763.

[2] Maeda T. et al., A two-component system that regulates an osmosensing MAP kinase cascade in yeast, Nature Vol. 369, pp. 242-245.

[3] Brewster J.L. and Gustin M.C. (1994), Positioning of cell growth and division after osmotic stress requires a MAP kinase pathway, Yeast Vol. 10, pp. 425-439.

A7-133 THE WAVE OF SPORULATION INDUCTION IN *Dictyostelium* IS MEDIATED BY A POSITIVE TRANSCRIPTIONAL ELEMENT, Delwood L. Richardson, William F. Loomis† and Alan R. Kimmel. Laboratory of Cellular and Developmental Biology, NIDDK, NIH, Bethesda, MD 20892 and †Dept. of Biology, UCSD, La Jolla, CA 92093.

*spi*A, a marker for sporulation in *Dictyostelium*, is expressed when the mass of prespore cells has moved partly up the newly formed stalk. Strains containing a full-length *spi*A promoter/*lac*Z fusion were stained for β-galactosidase activity at intervals during development. Expression of *spi*A initiates in prespore cells at the prestalk/prespore boundary (near the apex) and extends downward into the prespore mass as culmination continues. Treatment of early developmental structures with 8-Br-cAMP in situ to activate the intracellular cAMP-dependent protein kinase (PKA) precociously induces *spi*A expression and sporulation. The absence of an apparent gradient of staining in 8-Br-cAMP-treated structures suggests that PKA is equivalently activatable throughout the prespore region and indicates that all prespore cells are competent to express *spi*A. Thus, we postulate that the pattern of *spi*A expression reveals the progression of an inductive signal for sporulation and suggest that this signal may originate from the prestalk cells at the apex. Analysis of the *spi*A promoter by 5' and internal deletions has identified a positive element responsible for sporulation specific expression. This element works in concert with a pair of G-box elements to direct high level expression during sporulation. Gel shifts to identify this PKA-activatable transcription factor are in progress. A screen has been developed to identify mutants defective in transducing the signal which induces sporulation.

A7-134 SimKinase, A SIMULATOR USED TO EXPLORE NETWORKS OF KINASES, Frank D. Russo, Department of Genetics SK-50, University of Washington, Seattle, WA 98195, fdrusso@u.washington.edu.

Kinases are ubiquitous in biological regulation. Some systems involve a single kinase, whose role in transducing a signal into a regulatory response is fairly clear. Others involve several, whose roles may be more complex. Although usually described as linear pathways, or 'kinase cascades', it is becoming clear that these systems may actually involve a complex network of interactions among several kinases. By analogy to electronic networks or circuits, it is possible that such kinase networks are capable of a great deal of information processing. However, other than general ideas such as feedback or amplification, little is understood about the mechanisms at work in these networks, or what sorts of complex behavior they may produce.

The purpose of this work is to develop a tool that will facilitate the study of such networks. The SimKinase program, written in C for Macintosh, allows the user to create and explore a kinase network of arbitrary design. A given protein in the network can exist in a number of different phosphorylation states, each of which has distinct abilites to bind other proteins, or to catalyze kinase and phosphatase reactions. The simulator applies first-principle chemistry to calculate rates of binding and catalysis at a given point in time, then adjusts the concentration of each species as a result. As the process iterates, the user can simply observe the behavior of the network over time, or can explore the effects of changes introduced while the simulation is running. The ease of observation and degree of manipulation possible within such a simulation should allow the user to develop a feel for how different configurations of interacting kinases behave. This knowledge can then be applied to analyze and understand existing kinase networks in nature.

A7-135 MODELLING MULTIGENIC INTERACTIONS WITH BOOLEAN NETS, Bruce K. Sawhill and David C. Torney, Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, MS K-710, Los Alamos, NM 87545

The well documented effects of genes on other genes such as inhibition, switching, and catalysis are incorporated into a Boolean Net mathematical model which vastly increases the descriptive power over previous models of epistasis. Information theory is used to derive bounds on the amount of data required to isolate the multigenic basis of a disease. An efficient search strategy is presented.

A7-136 A NETWORK OF DUELLING CELL-CELL INTERACTIONS IN THE EARLY *C. ELEGANS* EMBRYO, Ralf Schnabel, Max-Planck-Institut für Biochemie, D-82152 Martinsried, FRG

During the first four cleavage rounds of the *C. elegans* embryo the stem-cell-like P cells (P0-P3) produce in unequal divisions the somatic founder cells AB, EMS, C and D which later form the tissues of the embryo. The classical criterium for a cell-autonomous specification of a tissue is the capability of primordial cells to produce this tissue in isolation from the remainder of the embryo. By this criterium the somatic founder cells EMS, C and D develop cell-autonomously. Laser ablation experiments however reveal that within the embryonic context these blastomeres form a network of cellular interactions.

During normal development the blastomere P3 inhibits muscle specification in the EMS and hypodermis specification in the C blastomere. The C blastomere itself inhibits muscle specification in the D blastomere. These inhibitory interactions are counteracted by two activating inductions. The inhibition of body wall muscle in EMS is overcome by an activating signal from the AB lineage. This inductive event between MS and the ABa descendants is reciprocal, specifying subsequent fates in both lineages. The ABa lineage specifies body wall muscle in MS and MS induces the left-right asymmetry in the AB lineage. Both induction events are blocked by mutations in the gene *glp-1*, known to encode a Notch-like transmembrane receptor protein. The fates of hypodermis and body wall muscle in C and D respectively are reactivated by an induction emanating from the EMS lineage into the posterior embryo. This induction most likely occurs after one more division of the embryo when two MS cells are born just before the onset of gastrulation.

Blastomeres which behave cell-autonomously in isolation are nevertheless subjected to cell-cell interactions in the embryonic context. Why this should be is an intriguing question. This work demonstrates that a classical criterium for the elucidation of the general mechanisms of fate determination may lead to incorrect conclusions about the general pathway by which a tissue is formed.

A7-137 **AN INITIAL CHARACTERIZATION OF THREE *KN1* LIKE HOMEOBOX GENES IN *ARABIDOPSIS THALIANA*.** **Kyle A. Serikawa, A. M. Laborda, S. Han, E. Vollbrecht, S. Hake and P. Zambryski, Department of Plant Biology, 111 Koshland Hall, University of California, Berkeley, CA 94706**

We have been examining three homeobox-containing genes from Arabidopsis that are members of the *KN1*-like class. *KN1* was originally described as a dominant leaf mutation in maize. Cloning and sequencing of the *KN1* gene revealed that it encoded a homeodomain. *KN1* is a member of a large, evolutionarily conserved gene family within plants and has been implicated in developmental processes such as determinancy and morphogenesis. We have cloned three *KN1*-like homeobox genes from Arabidopsis (KNAT3, 4 and 5) and are characterizing them on the sequence, expression and functional levels. Northern analysis of RNA extracted from different tissues has shown that each of these genes has a unique expression pattern. We have made transgenic plants expressing both overexpression and antisense constructs for these three genes and are analyzing their phenotypes. Preliminary results suggest KNAT3 may play a role in the timing of flowering and in the development of leaves.

A7-138 *WNT* HOMOLOGS IN *CAENORHABDITIS ELEGANS*. Supriya Shivakumar, Gregg Jongeward, Cynthia Kenyon and Harold Varmus, Departments of Biochemistry and Microbiology and Immunology, UCSF, San Francisco, CA 94143

The *wnt* gene family encodes cysteine rich secreted proteins which have been shown to act as important regulators of early development in the frog, fly and mouse. *wnt* genes have a crucial role in intercellular signalling in the mouse CNS development and in pattern formation in the fly. We chose to study the role of *Ce-wnt-1* gene in signalling in *C. elegans* development.

Two members of the wnt gene family have been identified in *C. elegans*, *Ce-wnt-1* and *Ce-wnt-2*. The *Ce-wnt-1* gene encodes a 372 amino acid protein and shares 22 of the 24 cysteines found among other members of the wnt family. The *Ce-wnt-2* cDNA encodes a protein of 362 amino acids with 22 of the cysteines shared among other Wnt proteins. Both *Ce-wnt-1* and *Ce-wnt-2*. are detected at high levels in embryos and at lower levels at all other stages. Neither *Ce-wnt-1* and *Ce-wnt-2* are direct homologs of any specific wnt genes in other organisms.

To elucidate the functions of the *Ce-wnt-1* gene product, we have screened for new mutations in the gene as the *Ce-wnt-1* locus maps to the left arm of chromosome II, but does not appear to correspond to any existing mutations. We made the assumption that the *Ce-wnt-1* gene is required for viability based on wnt mutations in other species.

To isolate *Ce-wnt-1* alleles we used an F2 screen for psoralen-induced lethal mutations balanced by an extrachromosomal array comprising the *Ce-wnt-1* gene. 1500 F2 animals were screened and 2 embryonic lethal alleles were isolated. Both contain deletions in the *Ce-wnt-1* coding region, which should create a nonfunctional Ce-wnt-1 protein. Ce-wnt-1 protein activity is required to rescue the early embryonic lethality. We are now studying this early phenoype in greater detail.

A7-139 Characterization of patterns of developmental gene expression using PCR-generated expression spectra

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A molecular characterization of genetic networks could be achieved by analyzing the expression of known genes, or by generating large scale, unbiased gene expression spectra. The former strategy ignores the population of potentially critical gene sequences not yet available to the researcher. The latter approach is currently being explored by arbitrarily-primed PCR and its variant, differential display. We selectively reverse transcribed mRNA with a T12VN primer, providing a 3' VN-anchor to eliminate frame shifting on the poly-A tail. An arbitrarily designed forward primer was used to prime multiple cDNA templates under reduced-selectivity conditions. This enables multiplex amplification of a template group, visualized as RTPCR product spectra on high-sensitivity autoradiography films of electrophoretically separated PCR products. However, the principle of arbitrary priming can also result in the production of artifacts during high-gain amplification. We have eliminated these artifacts by using a high-fidelity, low-cycle PCR protocol with modified primers to amplify the first strand PCR products created under low fidelity conditions. Several combinations of forward and reverse primers were used in the generation of expression spectra corresponding to different stages of embryonic spinal cord development, and pre- and post-differentiated PC12 cells. The method allowed the identification of up- and down-regulated genes, and provided statistical information about the number of genes which were transcriptionally altered during differentiation. This opens the door to the identification of developmentally critical elements and general definition of cell type in terms of a characteristic expression matrix.

A7-140 THE MOLECULAR CONTROL OF CELL POLARITY,

Lisa Stowers, Debra Yelon, Leslie J. Berg, and John Chant, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, 02138

A variety of cell types respond in a polarized manner to external stimuli, however, the molecular mechanisms which underlie the asymmetry of this process have been elusive. Our recent findings reveal that the ras-related GTPase, CDC42, and PI(3) kinase comprise a pathway which governs the establishment of cell polarity. Specifically, both constitutively active and dominant interfering alleles of CDC42 abolish the polarization of the actin cytoskeleton that occurs in a T cell as it responds to a cognate antigen presenting cell. This disruption of asymmetry extends to the tubulin cytoskeleton, as the orientation of the microtubule organizing center no longer polarizes in a predictable manner in the mutant T cells. Other signaling processes, such as that which leads to the production of interleukin-2, remain intact in these mutant T cells. We have been able to duplicate the phenotype associated with the mutant CDC42 alleles by treating wild-type cells with an inhibitor of PI(3) kinase, wart mannin, prior to their interaction with antigen presenting cells. In addition, recent work has shown direct interaction of PI(3) kinase and CDC42 by immunoprecipitation which leads to activation of PI(3) kinase[1]. Immunolocalization of CDC42 reveals that the dominant interfering CDC42 resides primarily in the golgi region, while constitutively active CDC42 is associated with the plasma membrane. This suggests that upon external signaling, CDC42 simultaneously binds GTP and moves from the golgi to the plasma membrane to direct the polarization and orientation of both the actin and tubulin cytoskeleton.

[1]Zheng, Y., Bagrodia,S., & Cerione, R.A., J. Biolol. Chem., 269:18727-18730.

A7-141 *MITF* (*microphthalmia*-associated transcription factor) regulates melanocyte-specific genes,

Masayoshi Tachibana[1], Kazunori Urabe[2], Kimberly A. Meyers[3], Kazuhisa Takeda[1], Stuart A. Aaronson[3] and Toru Miki[3], [1]LMG, NIDCD, NIH; [2]LCB, NCI, NIH; [3] LCMB, NCI, NIH

The human *MITF* gene encodes a protein, most likely a transcription factor, with a basic-helix-loop-helix (bHLH) motif. This gene is assigned to the chromosomal region 3p14.1-p12.3 where Type II Waardenburg syndrome (WS) is closely mapped. Since melanocytes are deleted in Type II WS and in mice with mutations in the *mi* gene, a mouse homologue of *MITF* gene, it is likely that *MITF* is involved in mediating the program of melanocyte differentiation. To assess this possibility, NIH/3T3 cells were transfected with a eukaryotic expression vector containing *MITF* cDNA.

Ectopic expression of *MITF* induced foci of morphologically altered cells, often with melanocyte-like dendritic processes. Electron-microscopic analyses of the transfectants revealed membrane bound vesicles resembling the premature melanosome as in albino melanocytes.

The expression of tyrosinase and tyrosinase-related protein 1, known to be expressed exclusively in melanocytes and involved in melanin biosynthesis, was consistently found in *MITF* transfectants but not in vector control transfectants, although the expression level was low compared to melanocytes or melanoma cells. In contrast, tyrosinase mRNA was expressed at the same level to that in mouse melanocytes or melanoma cell lines. Transactivation of the tyrosinase gene by *MITF* was confirmed by a reporter assay. A plasmid, which carries the promoter region of the human tyrosinase gene fused to the luciferase gene, was transiently transfected into *MITF*-transfected cells. The transfected cells showed a high level of luciferase activity. The consensus binding site for some bHLH proteins is known to be CANNTG. Since a CATGTG sequence has been identified in the tyrosinase gene promoter, the bHLH motif in the MITF protein probably binds this sequence, which is required for melanocyte-specific transcription.

Taken together, our findings indicate that *MITF* regulates melanocyte specific genes and is involved in induction of the melanocyte differentiation.

A7-142 QUALITATIVE DYNAMICAL ANALYSIS OF GENETIC REGULATORY NETWORKS.

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In the field of biological regulations, one usually deals with complex networks comprising intertwined feedback loops. Unhappily, formal analysis of such regulatory networks is often complicated by the presence of non-linearities and by the lack of quantitative knowledge.

During these last .years, our group has developed a powerful qualitative method which formalises the interactions between the elements of regulatory networks in terms of logical variables, functions and parameters. One aspect of this approach is a clear distinction between the roles of the two types of feedback loops, namely "positive" and "negative" feedback loops, which generate deeply different dynamical and biological properties. Recently, we have introduced the concept of "loop-characteristic state", which clarifies the relation between feedback loops and steady states, and greatly simplifies the analytical procedure. Now, to predict the dynamical behaviour of a network, we take advantage of the possibility to dissociate it into its constituent feedback loops and check their dynamical role, yet keeping a complete control on the ways in which these loops are interconnected.

As concrete applications, we briefly present logical models of genetic networks involved in the regulation of virus expression.

A7-143 THE cAMP-CRP/CYTR NUCLEOPROTEIN COMPLEX IN E.COLI:IS CYTR MORE THAN A LOCAL REGULATOR

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The CytR regulon in *E.coli* constitutes a combinatorial regulatory system in which the cAMP-CRP complex functions both as an independent transcriptional activator and as an "adaptor" for the CytR repressor. This regulon comprises at least eight operons/genes that codes for proteins involved in transport or catabolism of nucleosides. Detailed investigations have shown that CRP and CytR bind to promoter regions in a highly cooperative fashion. The cooperative binding is mediated by direct protein-protein interactions and the presence of CRP can *in vitro* enhance CytR binding more than 1000 fold.

An important aspect of this system is that nucleoprotein complex formation can trigger a repositioning of cAMP-CRP from high affinity targets to pseudo-targets present in promoter regions. Thus, in these complexes CytR acts as an "adaptor" for CRP. These features make this regulatory system extremely flexible and makes nucleoprotein complex formation possible in structurally diverse promoters. Moreover, CytR-CRP can act either as a repressor or as an activator, depending on the context. This implicates that CytR, once thought to be a negatively local acting gene regulator, could play a much broader role in gene regulation. In support of this view new members of the CytR regulon have recently been identified.

Cell, 75, 557-566 (1993)
EMBO J., 11, 3635-3643 (1992)
J. Mol. Biol. 227, 396-406 (1992)

A7-144 **AMINO ACID AND ADENINE CROSSPATHWAY REGULATION ACTS THROUGH THE SAME 5'-TGACTC-3' MOTIF IN THE YEAST HIS7 PROMOTER.**

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The HIS7 gene of Saccharomyces cerevisiae encodes a bifunctional glutamine amidotransferase:cyclase catalyzing the fifth and sixth step of the histidine biosynthetic pathway. We have previously shown that the HIS7 gene is activated by the GCN4 protein under environmental conditions of amino acid starvation. Here, we provide genetic evidence that GCN4 activates HIS7 transcription synergistically through two in vitro binding sites in the HIS7 promoter. The proximal GCN4-binding site is also required for activation of the HIS7 gene by the joint action of BAS1 and BAS2 under adenine limitation conditions. Under simultaneous amino acid starvation and adenine limitation conditions both GCN4 and BAS1/2 are necessary for maximal HIS7 transcription. These results suggest that GCN4 and BAS1/2 are able to simultaneously recognize the same DNA sequence in vivo and to use this site independently from each other. In addition, we show that both GCN4-dependent and GCN4-independent HIS7 transcription are significantly reduced by a deletion in a TATA-like sequence element in the promoter.

A7-145 A NOVEL CELL CYCLE INHIBITOR IN FISSION YEAST, Alison Woollard and Paul Nurse, Cell Cycle Laboratory, Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

In a genetic screen for novel cell cycle inhibitors, we have identified genes which inhibit progression through the fission yeast cell cycle when over-expressed. One of these genes , gip1, encodes a 14kD protein which appears to interact with both G1/S and G2/M functions. Analysis of fission yeast cells deleted for gip1 points to a potential role for this gene in G1 regulation. Biochemical analysis of the protein to investigate a potential role in cdk inhibitory networks is in progress.

A7-146 REGULATORY CIRCUITS GOVERNING COPPER AND IRON METABOLISM IN YEAST. Daniel S. Yuan, Yuko Iwai, Andrew Dancis, Richard D. Klausner. Cell Biology and Metabolism Branch, NICHD, NIH, Bethesda, MD 20892.

Copper and iron are indispensible cofactors in enzymatic redox reactions, and yet as simple ions they are highly toxic. We are studying the mechanisms by which eukaryotic cells harness these reactive species without injury using the genetically accessible eukaryote, Saccharomyces cerevisiae. In this organism, copper uptake requires a copper-specific transporter in the plasma membrane (Ctr1p) whose expression is homeostatically regulated at the transcriptional level by a sensor of cytosolic copper (Mac1p). Delivery of cytosolic copper to the extracytosolic space requires a second copper transporter (Ccc2p). Molecular components that sense (Ace1p) and detoxify (Cup1p) excesses of cytosolic copper are also known. High-affinity uptake of extracellular iron requires at least one of two externally-directed reductases (Fre1p, Fre2p) and an extracytosolic oxidase activity (Fet3p), all subject to transcriptional regulation by a sensor of cellular iron (Aft1p). Iron uptake also requires integrity of the Ctr1p/Ccc2p copper utilization pathway, since the oxidase activity of Fet3p requires copper, and in addition iron uptake requires integrity of vacuolar function and a period of growth in molecular oxygen. The regulatory circuits governing copper and iron metabolism are therefore not only elaborate homeostatic systems in their own right, but also linked to each other and to other major aspects of metabolism.

A7-147 DETERMINATION OF CELL IDENTITY IN *C. ELEGANS* RAY DEVELOPMENT BY *MAB-18*, A *PAX-6* HOMEODOMAIN CONTAINING GENE, Yinhua Zhang, Scott W. Emmons, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461

C. elegans males have nine peripheral sense organs called rays on each side of the tail, which are used to sense contact with hermaphrodites in mating. Although all rays are generated with the same cell lineage and have similar structure, each ray has its own shape and length in addition to its specific location. We are interested in how these rays are generated and how their identities are determined during development. Taking advantage of genetic analysis in *C. elegans*, we are using genetic approaches as well as molecular and cell biological approaches to address these questions.

Among the rays, ray 6 is different from other rays in having a fat, tapering shape. We isolated mutations that transform fat ray 6 into a thin ray. One gene we identified is *mab-18* (*m*ale *ab*normal), which is defined by two recessive mutations, *bx23* and *e1796*. No other rays are affected by these mutations. Based on this phenotype, we believe that *mab-18* is a key gene in specifying the thick ray identity. We cloned *mab-18* by genetic mapping and transformation rescue of the ray 6 defect. The putative *mab-18* protein contains a conserved paired type homeodomain (HD), which has 56 out of 60 (93%) amino acids identical to the HD of *pax-6* protein of vertebrates. However, unlike *pax-6*, *mab-18* only contains the HD. A paired domain similar to *pax-6* is present about 8kb 5' of the HD. The paired domain and the HD form the worm homolog of *pax-6* (Chisholm *et al*, personal commun.). The expression pattern of *mab-18* revealed by using a *lacZ* reporter gene suggests that *mab-18* acts cell autonomously to specify ray 6 identity.

After expression of mab-18 cDNA from a heat shock promoter, only the defective ray 6 was rescued. No transformation of any other thin ray into a thick ray was seen. This suggests other molecules are also required to generate a thick ray. This is consistant with data that *mab-18*, *mab-21* and *egl-5*, which is a HOM-C gene for specifying the posterior region, interact to specify the thick identity of ray 6 (Chow and Emmons, 1994, *Develop.* in press). Since mab-18 has a HD, we expect it to act as a transcription factor for target genes required to produce a fat ray. The target genes may include cell matrix and cell surface molecules which form the thick ray identity of ray 6.

A7-148 THE USE OF A SITE-SPECIFIC RECOMBINATION SYSTEM FOR FATE MAPPING IN MICE, Zinyk D.L.,[1] Mercer E.H.,[2] Anderson D.J.,[2] and Joyner A.L.[4] [1]Samuel Lunenfeld Research Institute, Toronto, Canada; [2]California Institute of Technology, Pasadena, CA; [3]Skirball Institute, New York, NY.

Fate mapping in mammals is complicated by the system's complexity and inaccessibility. We have designed a new approach for fate mapping in mice which takes advantage of the bacteriophage P1 Cre/*loxP* site-specific recombination system.

Gene expression has served as both a marker for developing regions and as a possible determinant of regional and cell fate. In our lab, the *En-1* and *En-2* genes have been shown to mark overlapping domains in the mouse embryonic mid/hindbrain region. *En* mutant mice exhibit mild to severe mid/hindbrain deletions. To further understand the role of the *En* genes during brain development, it is necessary to have a detailed fate map of the embryonic mid/hindbrain region.

The strategy for fate mapping the mouse embryonic mid/hindbrain region involves creating two lines of transgenic mice and breeding them to obtain double transgenics for analysis. One line (*En-2/cre*) will express Cre recombinase from an *En-2* enhancer/promoter fragment identified in our lab which drives specific expression to the mid/hindbrain region from 8.5-13.5 dpc. The second line of mice (βSTOP*lacZ*) will have a *loxP*–STOP-of-translation–*loxP*–*lacZ* cassette driven either by the chicken β-actin promoter (transgenic mice) or the endogenous β-actin promoter (targeted mice). By breeding the *En-2/cre* and βSTOP*lacZ* mice, double-transgenic progeny can be obtained. As a result of Cre expression in the mid/hindbrain region, the STOP will be excised by recombination, permitting *lacZ* expression under the control of the ubiquitous β-actin promoter. Since *lacZ* expression will be independent of Cre expression after activation, cells of the embryonic mid/hindbrain region and **all their progeny** should stain blue upon X-gal staining.

Both types of transgenic mice have already been obtained. Analysis of double-transgenic progeny resulting from crossing the *En-2/cre* mice and one of the transgenic βSTOP*lacZ* lines which does not express *lacZ* in the absence of Cre and has the potential to express *lacZ* in PNS and CNS neurons as well as muscle cells, indicates that only a subset of adult brain neurons stain for β-gal. This supports our hypothesis that the Cre/*loxP* system can be used for fate mapping in mice. Analysis of the transgenics will be presented.

Late Abstracts

DETERMINATION AND REDUNDANCY: THE STRANGE CASE OF NOTCH, LATERAL INHIBITION, AND R8 PHOTORECEPTOR DEVELOPMENT IN *Drosophila*, Nick Baker, Doreen Han, E-Chiang Lee, Rosaleen Tallon and Anne Zitron, Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461.

Much work on the role of lateral inhibition in defining neural precursor cells in insects has yet to provide a complete picture. Studies of the development of R8 photoreceptor cells has led us to some new perspectives. R8 cells are the first retinal cells to differentiate, and appear in a spaced pattern that initiates the recruitment of neighboring cells for other retinal cell types. Scabrous, a secreted protein related to fibrinogen and tenascin, provides a unique marker since its expression pattern changes **during** R8 determination. We find that *N* is required to single out R8 cells, and that large clusters of R8 cells differentiate when *N* function is reduced. Therefore, *N* is required to select one cell to be R8 out of many that are competent. However, *N* is **not** required subsequently to suppress supernumerary R8 cell determination. Furthermore, activating *N* function in R8 cell precursors can block their further differentiation and permit them to adopt other fates. Remarkably therefore, the 'determined' cell type is developmentally labile **longer** than the 'undetermined' cells are. We also find the *N* pathway alone insufficient to generate the spaced pattern of R8 cells seen in wildtype. Expression of sca is a necessary condition for a properly spaced pattern to result; *sca* mutants develop an irregularily spaced pattern. Our model is that in wildtype sca provides the bias that initiates orderly lateral inhibition, which occurs at random in *sca* mutants. *sca* mutants illustrate that incomplete penetrance need **not** imply genetic redundancy. *N* and its homologues may act to "canalize" preceding inhomogeneities in other systems too.

REGULATION OF THE PEPTIDE TRANSPORTER, *PTR2*, IN *SACCHAROMYCES CEREVISIAE*: A CONTROL CIRCUIT INVOLVING GENES ACTIVE IN NITROGEN RESPONSE AND UBIQUITIN METABOLISM. R.D.Barnes[1,2], H-Y.Steiner[1], K. Alagramam[1,2], F.Naider[3], J.M.Becker[1,2]. [1]Department of Microbiology, [2]Program in Cellular Molecular and Developmental Biology, University of Tennessee, Knoxville, TN, USA; [3]Department of Chemistry, City University of New York, Staten Island, NY, USA.

PTR2 encodes a polypeptide 601 amino acids in length, is predicted to contain 12 transmembrane spanning domains, and is thought to be a structural component of peptide transport in *S. cerevisiae*. Previously, it had been shown that peptide transport and *PTR2* expression are sensitive both to the type of nitrogen source and the presence of specific amino acid inducers in the growth medium. The sensitivity to nitrogen source suggested involvement of the proposed nitrogen responsive effector/modulator pair, *GLN3* and *URE2*, in *PTR2* regulation. Although the *PTR2* promoter region contains several copies of GATAA, the GLN3-binding UAS_{NTR} sequence, a *gln3*Δ mutant did not differ from a wild-type strain in its ability to transport dipeptides or to express *PTR2*. However, a *ure2*Δ strain displayed a 5-10 fold greater peptide transport rate, relief of nitrogen repression, and inducer-independent transport. Also, high levels of *PTR2* transcript were demonstrated in the *ure2*Δ strain when grown under repressive or inducing conditions. These results indicate that *PTR2* transcription is *URE2*-dependent and *GLN3*-independent. Other studies revealed that *PTR2* transcription was dependent upon the expression of *PTR1/UBR1*, a gene previously described as encoding the recognition component of the N-end rule pathway of the ubiquitin-dependent proteolytic system. In summary, these studies have revealed regulatory relationships among genes of apparently disparate function.

The pheromone communication system in the fission yeast *Schizosaccharomyces pombe*.

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Communication between two haploid yeast cells is controlled by the reciprocal action of diffusible pheromones, cells of each mating type releasing factors which induce mating-specific changes in cells of the opposite type. These changes include an arrest of cell growth, altered patterns of gene transcription and elongation of the cell to form a shmoo. The process begins when the pheromone binds to its receptor on the surface of the target cell and the mechanism by which this binding is transmitted to the relevant intracellular machinery is similar to signalling pathways used in higher eukaryotes. While most studies have concentrated on the budding yeast *Saccharomyces cerevisiae*, considerable advances are being made in defining the response pathway in the fission yeast *S.pombe*. Both pheromone receptors are seven-transmembrane proteins which interact with a heterotrimeric G protein such that binding of pheromone releases a Gα subunit that is believed to activate an effector system and generate a second messenger. Propagation of the intracellular signal involves a series of protein kinases that are functionally homologous to the mitogen-activated kinases involved in the control of proliferation and differentiation in higher eukaryotes. We are currently investigating several aspects of the signalling process with particular emphasis on the synthesis of the pheromones, the role of the receptors, the identity of the effector system and the pheromone-induced arrest of the cell cycle. I will present our latest results in these areas.

METABOLIC SWITCHING IN EARLY INTESTINE AND MUSCLE DEVELOPMENT IN THE NEMATODE *CAENORHABDITIS ELEGANS*

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The reaction of an abundant 106 kDa polypeptide with a specific monoclonal antibody has been localized in intestinal and muscle cells of the nematode *C. elegans*. This protein appeared to be expressed most highly in cells of embryos that were actively differentiating and was first detected in the clonal E lineage at 4-6 cell proliferation. This lineage is committed to the intestinal cell fate. Molecular cloning and sequencing showed that the largest cDNA clone contained 3274 bp and encoded a sequence of 1005 amino acids. The predicted polypeptide of 112,799 molecular weight contains separate domains for the glyoxylate cycle enzymes isocitrate lyase and malate synthase. Their enzymatic activities had been shown previously to be highest in embryos and larvae (Khan, F. R. and B. A. McFadden. 1980. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 115:312-314: Wadsworth, W. G. and D. L. Riddle. 1989. *Dev. Biol.* 132:167-173). The domain-specific sequences were shown to be contiguous in genomic DNA and are separated by an intron of 68 bp. A single polypeptide is precipitated by the antibody, and peptide fragments resulting from limited proteolytic digestion contained amino acid sequences which overlap the predicted junctional region. The location of the genetic locus within the physical map of nematode genomic DNA correlated with a small region of the left arm of Linkage Group V to which multiple embryonic lethal mutations have been mapped.