Instituto Juan March
de Estudios e Investigaciones

26 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Co-sponsored by
EUROPEAN MOLECULAR BIOLOGY ORGANIZATION

Workshop on

Cellular Interactions in the Early Development of the Nervous System of Drosophila

Organized by

J. Modolell and P. Simpson

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S. B. Carroll
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INDEX

PROGRAMME

CELLULAR INTERACTIONS IN THE EARLY DEVELOPMENT OF THE NERVOUS SYSTEM OF DROSOPHILA

MONDAY, April 11th

Registration.

Organizational Remarks.

I. Proneural and Neurogenic Functions
Chairman: A. Ghysen

J.A. Campos-Ortega - Early Decisions in Insect Neurogenesis.

E. Knust - bHLH Proteins Encoded by the *Enhancer of Split* Complex of *Drosophila* Negatively Interfere with Transcriptional Activation of Proneural Genes.

S.B. Carroll - Regulation of Proneural Gene Expression in the Embryonic CNS.

D. Hartley - The Role of the *Enhancer of Split* Complex in Sensillum Development.

P. Simpson - *U-shaped* and *Pannier*: Two Transregulatory Genes of *Achaete-Scute*.

J. Modolell - The Helix-Loop-Helix Extramacrochaetae Protein Participates in Multiple Events During *Drosophila* Embryonic Development.

II. Mechanisms of Cell Interactions
Chairman: S.B. Carroll

S. Artavanis-Tsakonas - The Notch Signaling Pathway.

A. Martínez-Arias - A Function for *Notch* in the Prepatterning of Cellular Fields As a Receptor for the Product of the *Wingless* Gene and Its Relationship to the Patterning of the Nervous System.

TUESDAY, April 12th

M.A.T. Muskavitch - Regulation and Function of Delta During Early Development.

R.G. Fehon - Cell Junctions and Polarity in Cellular Interactions During *Drosophila* Development.

C. Klämbt - Molecular and Genetic Dissection of Glial-Neuronal Interaction in the Embryonic *Drosophila* CNS.

B.-Z. Shilo - The Role of the *Drosophila* EGF Receptor Homolog (DER) and Its Ligand, Spitz, in Establishment of Embryonic Dorsal-Ventral Polarity.

III. Neuroblast and Sensory Organ Specification
Chairman: J.A. Campos-Ortega

G.M. Technau - On Lineages and Specification of Neuroblasts.

C.Q. Doe - Cell Lineage and Cell Fate Determination in the Embryonic Central Nervous System.

C. Dambly-Chaudière - Genes that Control the Differentiation of Sense Organs in *Drosophila*.

A. Ghysen - The Leg of *Drosophila* As a Model System for the Analysis of Neuronal Diversity.

Short Oral Presentations:

M. Hoch - The Tip Cells of the Malpighian Tubules Are Specified and Selected By the Activities of Proneural and Neurogenic Genes.

Z. Paroush - The Groucho Protein Interacts with the Hairy and *E (spl)* bHLH Proteins and is Necessary for Segmentation.

M.A. González-Gaitán - The *Drosophila* Stomatogastric Nervous System: a Model System for Neurogenesis.

F. Schweisguth - *Suppressor of Hairless* Controls Alternative Cell Fate Decisions in the Adult Epidermis.

WEDNESDAY, April 13th

V. Hartenstein - **Genetic Control of Delamination and Proliferation in the Neurectoderm.**

D. van Vactor - **Molecular Analysis of Neuromuscular Specificity in *Drosophila*.**

IV. Eye Development
Chairman: S. Artavanis-Tsakonas

M.Freeman - **Over-Expression of the *Argos* Gene, a Regulator of Inductive Interactions in the Developing Eye.**

M. Mlodzik - ***Drosophila* Jun is Required for Ras-Dependent Photoreceptor Determination.**

Y.N. Jan - ***Atonal*, the Proneural Gene for Photoreceptors and Chordotonal Organs.**

C. Zuker - **Molecular Genetics of Sensory Signal Transduction.**

INTRODUCTION

J. Modolell

The central and peripheral nervous systems and the compound eye of *Drosophila* are excellent model systems to study the generation of the most complicated biological structure, the nervous system of the higher eukaryotes. In *Drosophila*, the conjunction of genetic, molecular and cellular biological approaches are providing many important insights into the development of the nervous system of this insect. For instance, it has become clear that cell to cell interactions play major roles throughout the development of its nervous system. During the early stages, the main focus of this workshop, cellular interactions are most important to decide the number and position of cells of the neuroectoderm or imaginal discs that become neural precursors; to implement, in these precursors, the neural differentiation program; and to specify the identity of each precursor, and consequently the specific neurones it will give rise to. The genetic and molecular identification of many of the genes that mediate such interactions are providing important clues on how cells communicate and develop according to the signals they emit and receive from their neighbours.

This workshop has brought together twenty two invited speakers and twenty participants from Europe, the U.S. and Israel who have discussed their recent discoveries in these areas. Presentations and discussions have been organized around four topics. 1) Proneural and neurogenic functions, which are paramount to decide the number and position of cells that will become neuroblasts in the central nervous system and sensory organ mother cells in the peripheral nervous system by governing the choice of epidermal versus neural fate. 2) Mechanisms of cell interactions, which focused on the molecules, mechanisms and subcellular structures that allow cell-cell communication. 3) Neuroblast and sensory organ specification, which examined different aspects

of the genes and cellular interactions that provide identity to neuroblasts and sensory organ mother cells and consequently define the specific set of neurones and type of sensory organ originated by each of these precursors, respectively, and is ultimately responsible for the cell diversity in the nervous system. 4) Eye development, which focused on the action of several gene products that control the determination of and inductive interactions between photoreceptor cells and regulate the strict sequences of cell recruitment and differentiation that yield that most complex structure, the *Drosophila* ommatidium. It is hoped that this workshop will help its participants and the readers of this booklet to develop new strategies, coordinate efforts, establish collaborations and avoid duplications by groups working in similar problems. The increasing realization that many of the genes that regulate the development of the nervous system of *Drosophila* have counterparts in mammals will certainly help understand the development of the nervous system of these higher eukaryotes.

I. Proneural and Neurogenic Functions

EARLY DECISIONS IN INSECT NEUROGENESIS

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The neurogenic ectoderm of insects contains an initially indifferent population of cells which during later development will give rise to the progenitor cells of the neural and epidermal lineages. A large body of experimental evidence indicates that cellular interactions determine which cells will adopt each one of these fates. The cell interactions are assumed to be mediated by the products of several genes forming a complex, not yet well understood, network of interrelationships. Elements of this network are the proteins encoded by the genes *Delta* and *Notch*, which appear to form a complex required to convey the regulatory signals between the cells, acting as signal source and receptor; the proteins encoded by the *achaete-scute* gene complex (AS-C), which regulate the neural development; and the proteins encoded by the *Enhancer of split* gene complex (E(SPL)-C), which regulate epidermal development.

In my talk, I will present results of experiments on overexpression and ectopic expression of genes of the AS-C and E(SPL)-C, as well as of the analysis of the promoters of some of the genes of the regulatory network. The available data indicate that the proteins encoded by the AS-C play a prominent role in the regulation of the cellular decision.

bHLH proteins encoded by the *Enhancer of split* complex of *Drosophila* negatively interfere with transcriptional activation of proneural genes

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Institut für Entwicklungsbiologie der Universität Köln, 50923 Köln, Germany

The *Enhancer of split* complex [E(SPL)-C] of *Drosophila* participates in the control of cell fate choice by uncommitted neuroectodermal cells in the embryo. It encodes seven proteins that belong to the basic helix-loop-helix (bHLH) family, which exhibit a certain degree of redundancy as shown by germ line transformation experiments. Two proteins of the complex, HLH-M5 and ENHANCER OF SPLIT, are capable of binding as homo- and heterodimers to a sequence in the promoters of the *Enhancer of split* and *achaete* genes, called the N-box, which slightly differs from the consensus binding site (the E-box) of other bHLH proteins. In transient expression assays in cell culture, both proteins were found to attenuate the transcriptional activation mediated by the proneural bHLH proteins LETHAL OF SCUTE and DAUGHTERLESS at the *Enhancer of split* promoter, showing that they act as negative regulators of the proneural gene function.

"Regulation of Proneural Gene Expression in the Embryonic CNS"

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The development of the *Drosophila* nervous system provides an excellent model for analysis of pattern formation. The development of neural precursors in the central (neuroblasts; NBs) and peripheral (sensory organ precursors; SOPs) nervous systems involve a sequence of three common steps including: 1) proneural cluster formation, where the common neural potential of adjacent ectodermal cells is conferred by the expression of proneural genes of the *achaete-scute* complex (AS-C)[1,2]; 2) the singling out of one cell within the cluster that maintains proneural protein expression and assumes a neural fate; and 3) inhibition of proneural gene expression and loss of neural potential from the surrounding cells of the cluster, mediated by a signal emanating from the neural precursor[3]. While there is considerable understanding of the genes and molecules involved in the cellular events of proneural cluster formation, neural precursor segregation, and lateral inhibition, the molecular mechanisms connecting these events are not understood. Since the deployment of the AS-C genes directly reflects these processes, we have focused on dissecting the regulation of AS-C gene expression to analyze mechanisms of neurogenesis.

We have found that in the developing embryonic CNS, the number, position, and size of proneural clusters expressing *achaete* or *scute* are dependent upon the early embryonic axis-patterning genes that act upon an array of cis-acting elements located between the two genes. Using a series of reporter genes and transposons bearing wild-type or mutated copies of the *ac* gene, we have determined that 3' elements to the transcription unit control gene activation in proneural clusters while 5' elements mediate gene expression in neuroblasts and the response to lateral inhibition. In addition, we find no requirement for proneural protein function or upstream *ac* protein binding sites in the activation of *ac* gene expression in the early embryo. Because *ac* is activated by different mechansims in the early embryo and imaginal discs, it is possible that the mechanisms for silencing *ac* expression during lateral inhibition also differ at these two stages. The presumed transcriptional effectors of lateral inhibition, the *E(spl)* proteins[6] could operate by sequestering activators of proneural genes, by binding directly to proneural gene control sequences or some combination of the two mechanisms. We are presently trying to distinguish between these alternatives using a combination of approaches.

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The role of the *Enhancer of split* complex in sensillum development

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The "neurogenic" genes have been proposed to restrict pluripotential development by cellular interaction. We have been attempting to integrate the function of the *Enhancer of split* complex of genes into a scheme whereby neural potential is provided by expression of "proneural" genes like *Achaete-scute*, and that potential is inhibited by neurogenic gene function provided by *Notch*, *Delta* and the *Enhancer of split* complex amongst others. This is complicated by the fact that the complex consists of at least eight genes, only one of which (*groucho*) mutates to give a lethal phenotype. Our experiments have been to determine the effect of overexpressing *Enhancer of split* genes on sensillum determination. We find, as expected, that ectopic expression of *Enhancer of split* leads to absence of both the internal and external features of adult sensilla, suggesting that neural determination has been blocked. Has this effect been achieved by elimination of *achaete* expression? Here the situation is less clear but we will present data which suggests that it is. What is the functional connection between *Notch* (at the cell surface) and *Enhancer of split* (in the nucleus)? In order to address this, we have been looking at the requirement of sensory precursors for *Notch* and *Enhancer of split* function. Reduction of *Notch* function (using $Notch^{ts1}$), leads to extra sensilla at the expense of cuticle. We find that directed expression of one of the *Enhancer of split* genes, m8, can suppress the formation of these ectopic sensilla. Therefore, we can bypass the requirement of epidermal precursor cells for *Notch* by expressing *Enhancer of split* m8. This suggests to us that the neurogenic gene pathway leads to the activation of expression of *Enhancer of split* complex members, rather than post-transcriptional modification. Since *groucho* is the only gene of the complex for which we have isolated point mutations, we have looked for interaction between these mutations and other genes in the genome. We find a dramatic interaction between lethal *groucho* mutations and mutations in the *Drosophila ras-1* gene. However, other members of the *ras* signalling pathway like *D-raf* and *sos* do not have such a dramatic effect. These differences in interaction between members of the *ras* pathway suggests that either the observed interaction between *groucho* and *ras* is not specific, or that there are differences between PTKR / *ras* pathway and the *ras* pathway in other circumstances.

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u-shaped and *pannier*: two transregulatory genes of *achaete-scute*.

The pattern of macrochaetae on the notum arises, in part, as the consequence of a precise spatial expression of the *ac* and *sc* genes in the wing disc. The transregulatory gene *emc* has been shown to act as a general posttranscriptional repressor of *ac*. In an attempt to identify genes involved in this spatial regulation we have performed several mutagenesis experiments and have identified two new candidates. Mutant alleles of the gene *pannier (pnr)* can cause either a dominant or a recessive gain of bristles at different sites. These phenotypes are the result of a gain or loss of function of *ac/sc*. *pannier* encodes a putative transcription factor with a domain showing homology to the vertebrate transcription factor GATA-1. Experiments are in progress to test whether *pnr* is a direct regulator of *ac/sc*. *pannier* also contains a domain of possible negative regulation that bears two α helices Allele-specific interactions are observed between *pnr* and *emc*. These only concern mutant alleles of *pnr* that carry an intact helical domain, raising the possibility of direct interactions between the *pnr* and *emc* proteins.

Mutant alleles of *u-shaped (ush)* are recessive and the flies display extra macrochaetae. Loss of function of *ush* enhances the extra bristles caused by *pnr* mutants whereas a duplication suppresses them. u-shaped encodes a putative transcription factor containing several zinc fingers and an area of homology to leucine zippers.

The helix-loop-helix extramacrochaetae protein participates in multiple events during *Drosophila* embryonic development.

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The function of the *Drosophila extramacrochaetae* (*emc*) gene has been characterized almost exclusively in relation to the patterning of the fly's sensory organs (chaetae and other types of sensilla). It is well established that the Emc protein antagonizes the function of the Achaete and Scute proteins (Botas et al., 1982; Moscoso del Prado and García-Bellido, 1984). Achaete and Scute, as well as additional related proteins encoded in the *achaete-scute* complex, confer to cells the ability to become neuroblasts or sensory organ mother cells (proneural function; see Campuzano and Modolell (1992) for a review). The proneural proteins and Emc contain the *h*elix-*l*oop-*h*elix (HLH) dimerizing domain (Murre et al., 1989; Garrell and Campuzano, 1991, review) characteristic of a large family of transcriptional regulators that perform their functions as homo- or hetero-dimers. Most proteins of this family, including the proneural ones, have, adjacent to the HLH domain, a basic region necessary for DNA binding (reviewed in Murre and Baltimore, 1992). Emc lacks this region (Ellis et al., 1990; Garrell and Modolell, 1990). Consequently, it has been proposed that Emc antagonizes proneural function, and thereby sensory organ generation, by sequestering the proneural bHLH proteins in complexes uncapable of effective interaction with DNA (Ellis *et al.*, 1990; Garrell and Modolell, 1990).

Some, albeit scarce, data suggest that during embryonic and imaginal development *emc* plays roles other than to antagonize the proneural function (García-Alonso and García-Bellido, 1988). To study the role of *emc* during embryonic development, we have analyzed the embryonic expression of *emc* by hybridization in situ to wild type and mutant embryos and we have determined the embryonic phenotype of *emc* strong, lack-of-function alleles. We find that *emc* is expressed in complex patterns that comprise derivatives of the three embryonic layers. Expression of *emc* often precedes and accompanies morphogenetic movements. It also occurs at regions of specialized cell-cell contact and/or cell recognition, like the epithelial part of the muscle attachment sites and the differentiating CNS. The insufficiency of *emc* affects all tissues where it is expressed. The defects suggest impairment of processes as diverse as cell proliferation and commitment and cell-cell adhesion and recognition. If Emc affects these processes by dimerizing with bHLH proteins, the variety of defects and tissues affected by the insufficiency of *emc* suggests that HLH proteins are involved in many more embryonic developmental processes than hitherto described.

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II. Mechanisms of Cell Interactions

Spyros Artavanis-Tsakonas
Martin Baron
Robert Diederich
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Hui Hing
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Xin Sun

The Notch signaling pathway

Extensive genetic and molecular analyses in Drosophila and other organisms, indicate that Notch acts as the receptor of an evolutionary conserved, pleiotropic, cell interaction mechanism which controls the ability of a very broad spectrum of uncommitted cells to respond to specific developmental signals. Studies involving the in vivo expression of truncated forms of Notch, have demonstrated that an activated Notch receptor is capable of blocking the differentiation of an uncommitted cell to a more differentiated state. What appears to be the most compelling working hypothesis regarding the developmental function of Notch , is that Notch signaling renders uncommitted cells competent to receive and/or interpret more specific developmental signals. Consistent with this, we will present recent results which demonstrate that Notch can modulate specific signals transmitted by the ras or the wingless pathway.

We will also present molecular and genetic evidence which further dissects the involvement of Serrate, Delta and deltex in Notch signaling and defines precisely the active part of the intracellular domain of the Notch protein. Finally, we will discuss molecular and genetic data identifying Supressor of Hairless as a key component of the Notch pathway.

A function for *Notch* in the prepatterning of cellular fields as a receptor for the product of the *wingless* gene and its relationship to the patterning of the nervous system. A. Martinez Arias and J.P. Couso. Dpt. Zoology. University of Cambridge. Cambridge CB2 3EJ. UK.

The *Notch* (*N*) gene of *Drosophila* encodes a protein with a single transmembrane domain which genetic analysis has shown to act as a receptor in a signalling event during the patterning of the nervous system . However, mutations in the *Notch* (*N*) gene affect many processes during the development of *Drosophila*. Some of them e.g. the generation of precursors for the muscles or for the lineages of the nervous systems, can be related to a function during the singling out of precursors from pools of equivalent cells. The product of the *Delta* (*Dl*) locus is widely accepted as the ligand in this process. Others, like the patterning of the wing margin or the development of epidermis from ectoderm have not been investigated in detail but are thought to involve other ligands. Because of the variety of these defects and because some of them are correlated with particular alleles of *Notch*, it has been suggested that the Notch protein might have more than one functional domain.

We have found that mutations in *N* behave as dominant enhancers of the segment polarity gene *wingless* (*wg*). This observation has led us to explore the pleitropy of *N* in some detail and has uncovered a series of embryonic and adult *N* mutant phenotypes that are similar to those produced by loss of function of *wg* suggesting the possibility that Wingless is a ligand for Notch. This suggestion is reinforced by the finding of allele and protein specific interactions between the two loci. In particular, it is interesting that some alleles of *N* mimick *wg* mutant conditions. At the very least, our results argue that, in addition to its well characterized role in lateral inhibition, *N* has an important function as a receptor in processes other than lateral inhibition.

These observations have led us to propose a general model for *N* function during development in which the extracellular domain of the Notch protein is a substrate for competition of multiple ligands. The outcome of this competition is the integration of the spatial information that exists in the patterns of expression of various signals. We suggest that, at the mechanistic level, this is implemented by N being a subunit of various receptor complexes. We have some evidence for this suggestion from the activity of single aminoacid changes in both Wingless and Notch and also from inferences from the interactions between different ligands in the patterning of the wing margin and the positional specification of neural precursors.

Regulation and function of Delta during early development

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Delta is a cell surface-associated protein (1) that appears to act as a signal (2), in concert with the Notch cell surface receptor (3), to mediate cellular interactions central to the correct specification of cell fates in numerous venues during embryonic and postembryonic development. We have been investigating the regulation and function of Delta using a number of approaches ranging from the analysis of cellular development (4,5) to the cell biology (1,3) of the Delta protein. Recent results from a number of these approaches are summarized below.

Cellular development in *Delta* (*Dl*) mutants: A broad analysis of requirements for *Dl* function during postembryonic development has revealed that Delta is required for numerous cell fate specification events during metamorphosis. The larval and pupal phenocritical periods we have defined for *Dl* function in different tissues correspond closely with those for Notch (N) function, consistent with the hypothesis that Delta and Notch act coordinately in these processes. In a more detailed cellular analysis of bristle development, we have found that Delta is required for specification of sensory organ precursor cells, as well as for the correct specification of the four derivatives of the precursor cell. In the developing retina, Delta function is required for the correct specification of all identified cell types in the retina, including photoreceptors, cone cells, pigment cells, and bristle organs.

Regulation of Delta expression during development: Analysis of *Dl* expression during the embryogenesis has revealed that *Dl* is expressed in all cells within equivalence groups, within which alternative fates are being determined, preceding and during the period during which cell fates are being specified. Delta is expressed in all cells within embryonic neurogenic regions, and is rapidly down-regulated in neuroblasts as they delaminate and are specified during embryogenesis. Delta expression is then reactivated during later stages of embryonic neurogenesis. Investigation of Delta expression in the developing retina has shown that Delta is expressed in photoreceptor and cone cells as they are being specified, and is subsequently down-regulated in these cells once they have been specified, with some curious exceptions. These data, and similar data for other

cell types, suggest that Delta may function in a permissive fashion during development, i.e., to permit the exchange of instructive signals, encoded by other genes, that immediately direct the specification of particular cell fates.

Structure-function analysis of the Delta protein: Analysis of Delta functions in Drosophila cultured cells has led to the definition of a number of activities of the Delta protein. Delta participates in heterotypic interactions with Notch and homotypic interactions with itself, as assessed in cell aggregation assays conducted with programmed Drosophila S2 cells. We have also found that Delta appears capable of participating in homotypic interactions within the same cell, based on the cis-internalization of Delta protein that is provoked by treatment of Delta-expressing cells with a monoclonal antibodies against the Delta extracellular domain. We have also found that Delta is taken up by S2 cells that express Notch, and that cytoplasmic as well as extracellular portions of the Delta protein are taken up by Notch cells. These data imply that Delta can function as a transmembrane ligand, in the manner first described for the bride-of-sevenless protein. Construction of Delta chimeras has revealed that the Delta amino-terminus is necessary and sufficient for binding of Delta to Notch, implying that the Delta amino-terminus includes an EGF-motif binding domain. Site-directed mutagenesis and functional analysis of the Delta amino-terminus have also demonstrated that structural requirements for Delta-Notch binding that are distinguishable from those for Delta-Delta binding.

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Title: Cell junctions and polarity in cellular interactions during Drosophila development

We are interested in the mechanisms by which epithelial cells establish and maintain apical/basal polarity, and in the role that this polarity plays in the functions of transmembrane signaling proteins necessary for cellular interactions. To initiate these studies in Drosophila, we have been studying the *Drosophila* homologue of protein 4.1, a junctional protein that is thought to mediate interactions between the cytoskeleton and the cell membrane. Starting from a reverse genetic approach, we have now cloned and sequenced this gene, determined the tissue and subcellular localizations of the protein it encodes, and isolated mutations that disrupt its functions. Sequence analysis reveals extensive conservation with the human gene in the N-terminal 350 aa. This same domain is conserved to a lesser extent in the entire 4.1 family of proteins, which consists of 4.1, merlin (the Neurofibromatosis-2 tumor suppressor), ezrin (found in microvilli), moesin (found in cleavage furrows and junctional complexes), talin (a component of focal adhesions that may interact with the cytoplasmic domain of integrins), the Drosophila tumor-suppressor gene *Expanded*, and several other recently discovered genes. Using immunofluorescence and immunoEM we have found that D4.1 is expressed in close association with the cell membrane and in particular that it is associated with the septate junction. While little is currently known about the function of these junctions, they are present in all epithelial cells, are thought to function in the maintenance of cellular polarity, and have recently been implicated in the regulation of cell proliferation.

To understand more about the cellular and developmental functions of D4.1, we have isolated mutations in this gene and are beginning to examine their phenotypes in detail. For example, mutations in the D4.1 gene (*coracle*) cause embryonic lethality due to a failure in dorsal closure, an important morphogenetic process. Using hypomorphic and null mutations, we are examining the effects of D4.1 mutations on septate junction structure and function to determine the role of this protein in their formation and maintenance. Interestingly, *coracle* mutations also dominantly suppress the *Ellipse* mutation, an activated form of the Drosophila EGF-receptor homologue. We are currently testing the hypothesis that this suppression may be due to disruption of cellular polarity in *coracle* mutants. In addition, we have begun to use in vitro mutagenesis together with germ-line transformation to determine the functional domains of D4.1 and in particular, to map the region of the protein necessary for proper localization of D4.1 to the septate junction. These studies, in combination with genetic and biochemical interaction screens will allow us to identify other gene products with which D4.1 interacts.

We are also interested in the functions of other members of this gene family. We have so far identified and cloned two new members of the 4.1 gene family in *Drosophila*. One of these genes is moesin-like, while the other is most similar to human merlin, the Neurofibromatosis-2 tumor-suppressor gene. Preliminary studies of their expression and subcellular localizations are currently in progress. We plan to study the cellular and developmental functions of these well conserved proteins using the combination of genetic, molecular and cellular tools that are available in Drosophila.

In the longer term, the genes that we have so far identified and characterized should serve as "probes" for the analysis of junctional formation and structure in *Drosophila*. Genetic interaction screens, using mutations in genes we have already isolated, should now allow us to identify new genes that are associated with cell junctions and signaling mechanisms. Thus, starting from these promising candidates, we can extend our analysis of cell junctions and cellular polarity in Drosophila epithelia in order to broaden our understanding of the mechanisms of cellular interactions during development.

MOLECULAR AND GENETIC DISSECTION OF GLIAL-NEURONAL INTERACTION IN THE EMBRYONIC *DROSOPHILA* CNS

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Glial-neuronal cell interactions are an important prerequisite for the correct formation of the axonal network. To understand these cell-cell interactions we study the development of commissures across the ventral midline of the embryonic CNS of *Drosophila*. The elaboration of the commissural pattern occurs in a series of discrete steps, each of which requires different cell-cell interactions. First, the midline appears to attract commissural growth cones and thus organizes the commissural pattern. In a final step the commissures acquire their well known ladder like appearance, brought about by a migration of two midline glial cells along cell processes of the VUM midline neurons. In order to understand the glial-neuronal interaction which precedes this migration, we study genes which are expressed in the midline glial cells and are required for their function, such as the spitz group gene *pointed* (*pnt*) and the *argos (aos)* mutation.

pnt is required for the differentiation of a number of tissues during development, including the embryonic CNS glial cells. To understand the role of *pnt* for glial development, we have conducted a detailed genetic and molecular analysis. Molecular cloning showed that *pnt* encodes two putative transcription factors, P1 and P2, which share an ETS domain. Remarkably, 96 of the 100 aa long ETS domain are identical compared to the human *ets1* protein. In the embryonic CNS both *pointed* proteins are expressed in different, non overlapping sets of glial cells, which fail to properly differentiate in mutant *pointed* embryos.

In the embryonic CNS two MP2 interneurons are present in each hemisegment. Their growth cones, which closely interact with the longitudinal glial cells, express the 22C10 antigen. The level of 22C10 expression is strongly reduced in *pointed* embryos. Since *pointed* is not expressed in these neurons, it is suggestive that the neuronal expression of this antigen is controlled by the glial cells. To further our understanding of the *pointed* gene, we have overexpressed both *pnt* transcript forms during embryogenesis. The results can be summarized as follows: 1) *pointed* P1 is able to ectopically induce glial cell differentiation. 2) Flanking the ectopically formed glial cells we observe ectopic expression of the neuronal antigen 22C10. This again indicates that *pointed* controls glial-neuronal interaction, which subsequently controls expression of the 22C10 cell surface molecule on the MP2 neurons. The presence of this antigen on the MP2 neurons thus provides us with a

beautiful marker to genetically dissect neuron-glia interaction in the *Drosophila* embryo.

In a second project we study the gene *argos*, which encodes a secreted protein containing an EGF like motif, is involved in the control of photoreceptor cell fate in the developing eye. During embryogenesis *argos* expression in CNS is specifically found in the midline glial cells. However, despite the precisely regulated transcription pattern, *argos* function seems dispensable for embryonic CNS development as loss of function *argos* mutants do not lead to an abnormal CNS-phenotype. This could indicate that *argos* function is indeed not required during early CNS development or that redundant *argos* like gene functions guarantee normal development in the *argos* mutants. Our genetic analysis of *argos* supports a model where interacting loci reside in the vicinity of *argos* (see figure). When *argos* alleles (Y) are placed over deficiencies that remove *argos* as well as one of the interacting genes X or Z, surviving adult flies eclose indicating that the gene functions X and Z counteract *argos* function. When X and Y (or Y and Z) are removed on both chromosomes, a characteristic embryonic CNS phenotype emerges, suggesting that the presence of all three gene functions X, Y, Z is required for normal CNS development.

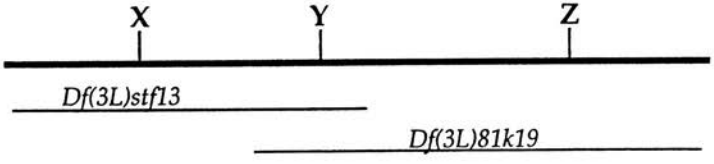

Figure1 Loss of function *argos* alleles (Y) lead to embryonic lethality. However, when *argos* is trans to deficiencies Df(3L)stf13 or Df(3L)81k19 we observe 1-5% surviving adults. Thus, a dose reduction of interacting loci X or Z suppresses the lethality associated with *argos*.

Using a dosage titration approach we have isolated genes which phenotypically interact with *argos*. Among the third-chromosomal complementation groups we had recovered in our screen, three are indeed placed in the close vicinity of the *argos* gene (0.1 to 0.2 cM) and therefore fulfill our criteria of interacting genes near the *argos* locus.

THE ROLE OF THE *DROSOPHILA* EGF RECEPTOR HOMOLOG (DER) AND ITS LIGAND, SPITZ, IN ESTABLISHMENT OF EMBRYONIC DORSAL-VENTRAL POLARITY.

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The embryonic phenotype resulting from mutations in the *Drosophila* EGF receptor homolog (DER) is complex, since the receptor is required multiple times during embryogenesis. Utilizing a temperature-sensitive allele the phenotype could be dissected and the earliest roles identified. We found that at 3.5 hours of embryonic development, DER is essential for establishing the identity of cells within the ventral ectoderm. In the absence of DER activity at this phase, alterations in cell fate are observed: Ventral cells acquire more dorsal fates, and the central nervous system, formed by neuroblasts delaminating from the ventral ectoderm, is severely disrupted. DER is thus a crucial element in establishing or maintaining the signals generating a spectrum of ventral cell fates in the embryonic ectoderm of *Drosophila*.

Some aspects of the mutant ectodermal and CNS phenotypes of the DER locus (*faint little ball, flb*) resemble the phenotype of mutations from the *spitz* group (including *spitz, Star, rhomboid* and *pointed*). Synergistic interactions between *flb* and *spitz* or *Star* mutations have indeed been demonstrated, suggesting that these genes participate in a common signaling pathway. The deduced structure of the spitz protein displays homology to TGFα, one of the ligands of the vertebrate EGF receptor, and to lin-3, the putative ligand of the *C. elegans* EGF receptor homolog. We have shown that the secreted form of the spitz protein triggers within 1 minute the tyrosine autophosphorylation activity of DER expressed in *Drosophila* Schneider cells, as well as the association of DER with the Drk protein containing SH2 and SH3 domains. Elucidating the molecular basis for the participation of the *spitz* group proteins in DER signaling, is crucial for understanding the regulation of the pathway. The possibility that other members of the *spitz* group which encode membrane proteins (most notably rhomboid and Star) may facilitate or enhance the activation of DER by spitz is being examined.

III. Neuroblast and Sensory Organ Specification

ON LINEAGES AND SPECIFICATION OF NEUROBLASTS

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Towards approaching the mechanisms leading to cell diversity in the CNS the clarification of the various neural cell types, their origin and lineage-relationships is an important issue. We developed a new method which allows to mark individual cells in their original positions with a lipophilic fluorescent tracer (DiI). Embryonic development of a labelled progenitor can be followed in vivo and subsequently the composition of the fully differentiated clone derived from that progenitor can be analyzed in detail (Bossing and Technau, 1994). This method should allow to uncover all lineages in the embryonic CNS and to assign them to the identified neuroblasts in the map (Doe, 1992). Once knowing the lineages, investigating mutant phenotypes or the consequences of experimental manipulations like transplantation or ectopic gene expression will provide insight into the mechanisms of cell fate specification.

We recently described the entire embryonic lineage of Drosophila neuroblast NB1-1 and showed that the composition of this lineage differs between the thoracic and abdominal neuromeres (Udolph et al., 1993). We address the mechanism involved in the regulation of segmental specificity of this lineage. Heterotopic transplantations of single neuroectodermal progenitor cells (nPC) from the thoracic into the abdominal ventral neurogenic region (vNR) and vice versa show that at the early gastrula stage (stage 7) nPC are already firmly committed with respect to segmental identity. Activity of the homeotic genes *Ubx* and *abd-A* is required for the expression of the abdominal variant of the lineage. However, heat induction of *Ubx* and *abd-A* expression can still override thoracic determination several hours after gastruation (stage 10/11) and antibody stainings reveal both proteins to be normally present in NB1-1 not before stage 11. The data are compatible with the assumption that early segment-specific commitment of NB1-1 is established by mechanisms acting upstream of *Ubx* and *abd-A* (Prokop and Technau, 1994).

To address intrasegmental specification of neuroblast identity we performed heterotopic transplantations along the ventrodorsal axis of the vNR (stage 7). Single nPC transplanted from dorsal (40-50% VD) to ventral sites (0-15% VD) develop according to their new position, as they give rise to clones which normally originate from ventral sites (e.g. NB1-1, MP2, midline clones). Furthermore, when transplanted into the ventral midline they express the midline specific gene *sim*. On the other hand, midline progenitors or nPC transplanted from ventral to dorsal sites of the vNR develop according to their origin, giving rise to clones typical for midline progenitors or ventral neuroblasts respectively. Thus, at the early gastrula stage midline progenitors and ventral nPC are firmly committed

with respect to further (ventrodorsal) fate, whereas dorsal nPC are not. The data suggest the existence of inductive signals leading to early commitment of CNS progenitors at ventral sites.

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CELL LINEAGE AND CELL FATE DETERMINATION IN THE EMBRYONIC CENTRAL NERVOUS SYSTEM

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Recent advances have provided molecular markers that allow every Drosophila CNS precursor to be uniquely identified [1], as well as methods for determining the complete cell lineage of each precursor [2]. We are interested in (1) defining the complete cell lineage for individual embryonic neuroblasts [in collaboration with the Technau Lab]; (2) how initial neuroblast identity is established within the neurogenic ectoderm; (3) how neuroblast and GMC cell types are specified; and (4) what are the mechanisms controlling the changes in neuroblast and GMC identity during the course of a neuroblast cell lineage.

To define the cell lineage of individual neuroblasts, we are using the newly developed DiI lineage tracing technique [2]. The lipidophilic tracer DiI dissolved in oil and applied to a single cell in the neuroepithelium; this cell can be followed in living embryos as it delaminates to form a neuroblast, divides to produce neuronal progeny, and these neurons extend axons to their synaptic targets. All cells during the lineage can be imaged by confocal microscopy in living embryos, and the DiI labeling is sufficiently bright to easily reveal synaptic terminals of neurons in mature embryos or larvae. We are using this method to document transformation of neuroblast or GMC fates in embryos lacking putative cell fate control genes.

The existence of molecular markers for individual neuroblasts [1] allows us to identify and characterize genes specifying neuroblast fate. A simple model is that an orthogonal grid of "segmentation" and "dorsoventral" genes provide positional cues that are used to specify the initial identity of each neuroblast. We have initially focused on the segment polarity gene *wingless* (*w g*), which encodes a secreted glycoprotein known to non-autonomously specify cell fate in the embryonic epidermis. *w g* is expressed in a stripe of neuroectodermal cells that produces the "row 5" neuroblasts in each segment [3]. Transient functional inactivation of the wg protein (using a temperature-sensitive allele) prevents the formation and/or specification of adjacent row 4 and row 6 neuroblasts, although the row 5 neuroblasts develop normally [3]. While most row 4 and 6 neuroblasts do not form in the absence of the wg signal, in 20% of the segments we observe a neuroblast at the position of neuroblast 4-2 (in row 4); molecular markers for neuroblast and GMC fates suggest that this neuroblast has been transformed to the neuroblast 3-2 fate (a row 3 neuroblast that is more distant from the wg signal). Thus, *w g* encodes a positional cue that directly regulates neuroblast specification [3].

We have identified a number of additional genes that act "downstream" of *w g* in a pathway controlling neuroblast 4-2 formation and specification (e.g. *armadillo*, *dishevelled*, *porcupine*, *shaggy*/*zeste-white* 3, and the *h k b*

gene; Q. Chu-LaGraff et al., in preparation). Current work involves determining the epistatic relationship between these genes. In particular, we are interested in the role of the armadillo gene product (β-catenin homologue) in mediating neuroblast 4-2 delamination and formation; the role of the shaggy serine-threonine kinase in regulating the activity of proneural and neurogenic gene products; and the function of the *hkb* gene in transducing a positional cue (the wg signal) into a unique neuroblast lineage (4-2).

With every cell division a neuroblast produces two different cell types: a new neuroblast and a smaller GMC. We are interested in how these distinct cell types are established. The prospero protein is initially the cytoplasm of neuroblasts; subsequently it is abundant in GMC nuclei before disappearing in mature neuronal progeny (E. Spana and CQD, in preparation) [4,5]. The *prospero* gene encodes a protein with a predicted highly divergent homeodomain [6]; the phenotype of *prospero* mutant embryos can be most clearly described as a failure of the GMCs to initiate GMC-specific patterns of gene expression (instead maintaining neuroblast-like gene expression). With several different collaborating labs, we have cloned the *prospero* gene from a wide range of organisms (mouse, P. Gruss; leech, M. Martindale; grasshopper, J. Broadus in my lab; nematode, R. Waterston). We observed that only the carboxy-terminal 160 amino acids of the predicted 1503 amino acid prospero protein are highly conserved; this region contains a divergent homeodomain and presumably has an evolutionarily conserved functional role. In addition to a role in GMC development, prospero protein is observed at high levels in the longitudinal glia (E. Spana and CQD, in preparation), postulated substrates for longitudinal connective pathfinding [7,8]. Null pros mutants show no longitudinal connectives. We are currently testing whether this axon phenotype is due to loss of pros function in neurons or glia.

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Genes that control the differentiation of sense organs in *Drosophila*.

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Embryonic development involves the accurate determination of cell fate according to cell position. The aim of our work is to understand the genetic programme that controls the different aspects of the formation of a complex, multicellular structure: the chemosensory organs of *Drosophila*. Each sense organ is formed by the progeny of a single precursor cell, the sensory mother cell (SMC)[1]. During the past decade, several groups have joined their efforts to analyse the process by which single cells at defined positions of the ectoderm become SMCs. Thus the early part of the programme which leads to the appearance of sensory organs at specific positions on the body has now been largely elucidated (for a recent review, see ref. 2). Very little is known, however, of the following steps whereby the progeny of a SMC is instructed to differentiate a particular type of sense organ.

We discovered recently that the precursor cells that will form chemosensory organs express the gene *pox-neuro* (*poxn*)[3]. Chemosensory organs differ from all others in their lineage (chemosensory organs are the only ones to comprise several neurons), in the morphology of their external structures (chemosensory bristles are the only one to be open at the tip), and in their axonal projection into the CNS (which is characteristic for each type of sense organ). We have shown that the ectopic expression of *poxn* is sufficient to confer chemosensory properties to other types of sense organs, indicating that *poxn* is responsible for the decision of a SMC to develop into chemosensory organs. The product of the *poxn* gene contains a putative DNA-binding domain, the paired-box, consistent with the role of the gene as a "selector" gene[4,5]. We are now exploring several avenues to identify the targets of the *poxn* gene[6-9].

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The leg of *Drosophila* as a model system for the analysis of neuronal diversity.

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The neurons innervating insect sense organs vary in numbers, shape, dendritic morphology, axonal projections and connectivity, providing a rich material for the genetic analysis of neuronal diversity[1]. Here we describe the leg of *Drosophila* as a potential model system for this analysis. The leg of *Drosophila* comprises a variety of sense organs arranged in a precise and reproducible pattern. The cell bodies of the sensory neurons are located near the organ they innervate, which greatly facilitates their identification and accessibility. The time of appearance and of divisions of the sense organ precursors is known in good detail[2]. The origin and mode of formation of the leg nerve (through which all sensory axons project into the central nervous system) has been described[3,4]. The central projections of some of the sensory neurons have been examined by horseradish peroxidase backfill[5] or DiI labelling[6].

In addition to this rich diversity, the pattern of sensory organs of the leg can be modified by interfering with the expression of its control genes, either by using mutants strains, or by inducing the ectopic expression of a control gene in transgenic flies, or by combining both methods. This allows one to manipulate the pattern to a large extent, and adjust it to experimental needs. For example, in the legs of $sc^{10.1}$ mutant flies where the genes *achaete* and *scute* are inactivated, the only remaining neuronal elements are the chordotonal neurons and the neuromuscular junctions. We have also modified the mesothoracic leg system by inducing the ectopic expression of the gene *poxn*[7], resulting in the transformation of the single neurons innervating mechanosensory bristles into clusters of chemosensory neurons[2]. Because this system is easy to modify genetically, and because the consequences of genetic modifications can be assessed in great detail, this system should facilitate the study of the genetic processes involved in controlling neuronal diversity.

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The tip cells of the Malpighian tubules are specified and selected by the activities of proneural and neurogenic genes

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The Malpighian tubules are involved in osmoregulation in insects. *Drosophila* has two pairs of tubules that float in the hemolymph from where they take up substances that have to be excreted. During organogenesis, a special cell at the tip of each of the four tubules, the tip cell, is required for the proper proliferation of the tubule cells (Skaer, 1989).

We have found that the tip cells differentiate into neural cells and become specified early in embryonic development by the concerted action of proneural genes (*AS-C, daughterless*) and neurogenic genes (*Notch, Delta)* in a manner analogous to the specification and the selection of sensory mother cells of the PNS.

During germband extension a tip mother cell is selected from a cluster of *AS-C* expressing cells by lateral inhibition through the neurogenic genes. Once singled out, the tip mother cell divides and gives rise to two daughter cells. One of the daughter cells becomes the tip cell and, as a difference to the PNS formation, maintains the expression of proneural genes until the end of embryogenesis. The segmentation gene *Krüppel* is activated in the tip mother cell after the singling out process has been carried out. In neurogenic mutants supernumerary tip mother cells appear and in mutants for proneural genes the tip cells are absent. Later in embryonic development the tip cells express neuronal proteins like 22C10, HRP and synaptic vescile proteins like synaptotagmin and synaptophysin. The tip cells differentiate into neural cells and make specific contacts with the PNS. In the physiology of the tubules, the tip cells might have a sensor or a neurosecretory function.

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The Groucho protein interacts with the Hairy and *E(spl)* bHLH proteins and is necessary for segmentation

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The product of the pair-rule gene *hairy*, a basic-helix-loop-helix (bHLH) protein, acts as a repressor in segmentation and bristle patterning. An embryonic expression library was screened in yeast for cDNA-encoded *Drosophila* proteins whose interactions with Hairy allow the growth of an otherwise auxotrophic strain on depleted medium.

One of these clones derives from the neurogenic gene *groucho* (*gro*), that interacts genetically with the bHLH genes of the *Enhancer of split* {*E(spl)*} complex. We have demonstrated physical interactions between Groucho protein and the Hairy, Deadpan and *E(spl)* bHLH proteins, in yeast and *in vitro*, and show that these associations rely on a functional carboxy-terminal WRPW domain found in the bHLH proteins. Groucho does not include an HLH motif; its interacting domain is currently being defined. To determine whether maternal expression of this gene is required for segmentation, we are examining eggs from *gro* germ-line clones for segmental defects. We find that *gro* plays an essential role not only in neurogenesis but also in segmentation, consistent with its acting in concert with Hairy in repressing *ftz* expression.

The *Drosophila* stomatogastric nervous system: a model system for neurogenesis.

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The stomatogastric nervous system (SNS), which innervates the larval gut, originates from a distinct region of the roof of the esophagus. Within this region, three distinct invaginations emerge during embryonic development, giving rise to SNS ganglia. As in the central (CNS) and the peripheral (PNS) nervous system, both neurogenic (like *Notch, Delta*...) and proneural genes (like *lethal of scute, achaete, scute*...) are expressed during SNS development. In the CNS and PNS, single neural precursors are sorted out by lateral inhibition from clusters of competent cells through the combined action of the neurogenic and proneural genes. In the SNS, however, a whole cell plate achieves neural fate, and the three invaginations emerge in a manner analogous to neurogenic processes during neural tube formation in vertebrates. This raises the question as to how proneural and neurogenic genes act in a developing system in which lateral inhibition is not obvious.

We describe the early determination of the three SNS invaginations within the esophagus. Each invagination then follows a distinct developmental program, contributing to a different set of SNS ganglia. A model is presented for SNS formation in which proneural genes play a dual role: determination of the neural fate and partitioning of the neurogenic region into the three invaginations giving rise to the different SNS ganglia.

Suppressor of Hairless controls alternative cell fate decisions in the adult epidermis.

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Suppressor of Hairless [*Su(H)*] activity is required for the singling out of adult sensory organ precursor cells (SOPs) from proneural cluster cells. Phenotypic analysis of *Su(H)* mutant clones indicates that *Su(H)* activity is also strictly required for determining the fates of sensory organ accessory cells. Conversely, conditional over-expression of the *Su(H)* gene product may prevent SOP determination, and lead to the transformation of the trichogen (shaft) cell into a second tormogen (socket) cell. These *Su(H)* and gain-of-function phenotypes thus appear similar to the *Hairless* (*H*) loss-of-function phenotypes.

Su(H) encodes a nuclear sequence-specific DNA-binding protein that has been highly conserved throughout evolution. The function of the murine (J_K-RBP) and human (IGKJRB/KBF2) proteins is unknown. A weak sequence homology between J_K-RBP and the integrase domain led to the hypothesis that Su(H)/J_K-RBP may act as recombinases. However, site-directed mutagenesis in the putative integrase domain of Su(H) indicates that this sequence similarity is not of functional significance. The role of the Su(H) protein in the Notch-mediated signalling pathway remains to be determined.

Genetic control of delamination and proliferation in the neurectoderm

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We have analyzed the pattern of delamination and mitosis of neuronal precursors in the pupa and embryo. Our results indicate that the pattern of delamination and mitosis are closely correlated: delamination occurs either immediately after the cell has divided (in case of sensory neuronal precursors), or shortly before the division (in case of the neuroblasts). In addition, cytoskeletal changes similar to those occuring during mitosis can be seen in delaminating neuronal precursors. Thus, during mitosis and delamination, the discrete apico-basally oriented microfilament-tubulin bundles break down. Microfilaments form a dense, diffuse cortical layer surrounding the entire cell body. Microtubules are concentrated at the apically located centrosome. The relationship between mitosis and delamination is supported by the finding that the neurogenic gene *Notch* (*N*) and segment polarity gene *wingless* (*wg*) affect both proliferation and delamination in the ventral neurectoderm. Thus, in embryos expressing the trunkated cytoplasmic domain of the neurogenic gene *Notch* under heatshock control (Struhl et al., 1993), ventral neurectodermal cells go into mitosis prematurely, followed by the absence of neuroblast delamination. In *wg* loss of function mutants, mitosis in the VN is irregular and generally postponed, accompanied by irregularities in the timing of neuroblast delamination in general and the absence of a subset of neuroblasts.

We have identified a mutation, *shotgun* (*shg*) in which neurectodermal cells surrounding the delaminating neuroblasts degenerate. As a working hypothesis, we proposed that the *shg* gene may code for or regulate the expression of an adhesion molecule required for the formation and maintenance of specialized epithelia, such as the neurectoderm, in which cells are subjected to excessive morphogenetic "stresses", (e.g., delamination). This model is supported by the finding that *shg* interacts with the neurogenic genes. Expression of activated *N* in a *shg* minus background almost entirely rescues the *shg* phenotype. We interpret this finding to mean that *shg* is only required in a situation where cells delaminate from the neurectoderm. This not being the case in embryos expressing activated *N*, loss of *shg* function has no detrimental effect on the cohesion of the neurectodermal cells.

ABSTRACT: Van Vactor et al.
TITLE: Molecular analysis of neuromuscular specificity in Drosophila.

The embryonic pattern of neuromuscular connectivity in Drosophila provides an attractive system for the study of axon pathfinding and target recognition at a molecular level. Evidence from a variety of experimental manipulations in this system suggests a high degree of specificity in the mechanisms that establish the pattern of muscle innervation. We have recently described a genetic screen for mutations on the second chromosome (~40% of the Drosophila genome) affecting the ability of embryonic motoneurons to find their correct targets [Van Vactor et al. (1993) Cell 73: 1137-1153]. We are presently conducting a similar screen for mutations on the third chromosome. Our genetic analysis points to three key steps in the generation of neuromuscular specificity. First, motoneurons are guided towards the correct region of mesoderm, often pathfinding across different substrates and navigating a sequence of specific choice points. Second, motoneuron growth cones display an affinity for a particular domain of neighboring muscles. Finally, each growth cone recognizes its specific muscle target(s) from within this domain. Not surprisingly, such a model suggests a developmental sequence of gene activities. We have identified genes that appear to control choice point navigation and specific muscle target recognition. Some genes may be used in a combinatorial fashion by many neurons in more than one target domain, whereas other genes may be unique to specific domains or subsets of growth cones or targets.

Our molecular characterization of the gene stranded, has revealed that this locus encodes Drosophila profilin. Drosophila profilin, originally identified by a viable, female-sterile phenotype, was previously cloned [Cooley et al., (1992) Cell 69: 173-184]. Biochemical studies in several systems suggest that this small cytoplasmic protein, which binds both actin and phosphoinositide 4,5-bisphosphate (PIP2), plays an important role in regulating the dynamic rearrangement of actin cytoarchitecture. It has been proposed that profilin mediates a link between specific cell signalling through the phosphoinositide pathway and the actin cycle. Our present results provide the first evidence that profilin regulates at the very least the general process of neuronal growth cone motility, and quite possibly, more specific aspects of growth cone guidance.

IV. Eye Development

Over-expression of the *argos* gene, a regulator of inductive interactions in the developing eye

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Interactions between cells play an important part in the mechanism by which cells acquire their identities. These interactions range from short range, between abutting cell membranes, to longer range signals mediated by diffusible factors. The *argos* gene (also known as *giant lens* and *strawberry*) encodes a protein that is predicted to be secreted, and mosaic analysis indicates that its product is diffusible. Null mutations in *argos* are lethal, but there is a class of loss-of-function alleles which are eye specific. These are characterised by a variety of defects with a common theme: too many cells are recruited into each of the main classes of cells. Thus the mystery cells are transformed into extra photoreceptors, and too many cone cells and pigment cells are formed.

Eye development starts in the third instar, when the morphogenetic furrow sweeps from the posterior to anterior of the eye imaginal disc; in its wake ommatidia start to differentiate. Cells join the growing ommatidia in a strict sequence, and it is widely accepted that this occurs by sequential inductive interactions — already determined cells recruiting neighbouring naive cells. The phenotype of *argos* mutations implies that it regulates these inductive signals.

One prediction of this view is that over-expression of the *argos* gene might lead to too few cells acquiring a given fate: if there is too much 'repressor', cells normally able to be recruited might be blocked. To test this prediction I have made fusions between *argos* and the *hsp70* promoter (HS-argos) and the *sevenless* enhancer/promoter (sev-argos). The secondary and tertiary pigment cells, which develop in the pupal retina, are sensitive to increased amounts of argos. As predicted, too few form in HS-argos flies which have been heatshocked. In contrast, the cells in the third instar imaginal

disc are much more resistant to extra argos. I have also found that the normal *argos* expression pattern is not crucial for its function: sev-argos can rescue *argos* mutants. Finally, by looking at the behaviour of argos when expressed in tissue culture cells, I have confirmed that it really is a secreted and diffusible protein.

These results suggest that argos acts in a mechanism to limit the response of inappropriate cells to inductive cues. Based on loss-of-function and over-expression phenotypes, the cells which respond to argos are the mystery cells, the cone cells and the pigment cells. Since most cells in the developing eye are exposed to extracellular argos, its restricted effects must be due to only a limited group of cells being able to receive or transduce the signal. If this model is correct, it implies that patterning in the eye occurs as a consequence of a balance of opposing influences: inductive cues and a repressor system, of which argos forms a part.

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Drosophila Jun is required for Ras-dependent photoreceptor determination

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The AP-1 transcription factor consists of Jun and Fos proteins that form dimers and are thought to be regulated by phosphorylation upon activation of the Ras pathway (Curran and Franza, 1988). *Drosophila melanogaster* Jun is transiently expressed in photoreceptor precursor cells in developing eye imaginal discs at the time of their neuronal determination. Expression of *Jun* is spatially and temporally very similar to genes that are required for photoreceptor determination such as *sevenless*(*sev*)and *sina* (Tomlinson and Ready, 1987; Tomlinson et al., 1988; Carthew and Rubin, 1990). Moreover, the competence of imaginal disc cells to develop as photoreceptors upon normal or ectopic activation of Ras (Dickson et al., 1992a) correlates well with the expression of *jun*.

To characterise the contribution of Jun to photoreceptor development, dominant negative Jun mutants were expressed under the control of the *sev* enhancer (Basler et al., 1990) in photoreceptor precursor cells. Dominant negative forms of Jun and Fos that form leucine zipper mediated dimers but do not activate transcription when binding to their DNA target sites have been shown to suppress Ras mediated transformation in tissue culture experiments. Flies expressing such mutant proteins in photoreceptor precursors, but not wild type control proteins, show a dose-dependent loss of photoreceptors, demonstrating a requirement of Jun for neuronal development in the Drosophila eye. Furthermore, the transformation of non-neuronal cone cells into R7 neurons elicited by constitutively active forms of Sevenless, Ras1, Raf and the *Sem* allele of rolled/MAPK (Basler et al., 1991; Fortini et al., 1991, Dickson et al., 1992b; Brunner et al., 1994) is suppressed in the presence of the Jun mutants. This suppression effect by the negative mutant form of Jun can be relieved when wild type Jun is overexpressed in the same cells demonstrating the specificity of the interaction. Moreover, in a weakly activated form of Sevenless, *sE-tor^{Y9}-sev*, which has reduced transformation efficiency (Dickson et al., 1992a), the cone cell transformation frequency is increased by overexpression of wild-type Jun.

These results position Jun (and AP-1) downstream of the *sevenless/ras* signalling pathway during photoreceptor development and suggest that Jun is one of the nuclear factors activated by this pathway in R7 determination. In addition, they demonstrate that the adverse effect of the oncogenic form of *Ras* can be inhibited by designed repressor molecules in an intact organism.

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atonal, the proneural gene for photoreceptors and chordotonal organs

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The Drosophila peripheral nervous system (PNS) comprises four major classes of sensory elements: external sense (es) organs (such as bristles), chordotonal (ch) organs, multiple dendritic (md) neurons and photoreceptors. Genes of the achaete-scute complex (AS-C) are responsible for the formation of es organs and the majority of md neurons. However, they are not responsible for the development of chordotonal organs or photoreceptors (Dambly-Chandiere and Ghysen, (1987) Genes & Develop., 1: 297-306; Jimenez, F. and Campos-Ortega (1987) J. Neurogenet., 4: 179-200).

We recently identified a candidate proneural gene for ch organs (Jarman et al.,(1993) Cell 73: 1307-1321). This gene atonal (ato) encodes a basic-helix-loop-helix (bHLH) protein that is similar to, but distinct from, the AS-C proteins. ato expression prefigures where ch precursors arise and ectopic expression results in ectopic ch organs.

More recently, we obtained loss-of-function mutants of ato. We found that ato is not only the proneural gene for chordotonal organs but also photoreceptors. In ato$^-$ mutants, both chordotonal organs and photoreceptors fail to form. Thus, ato and the AS-C can account for the origin of almost the entire PNS.

In the eye disc, ato expression occurs in a stripe spanning the disc on the anterior of morphogenic furrow (MF), thereby defining it as a proneural cluster for photoreceptors. Often, stronger expression is seen in smaller clusters of cells within this band. As the general expression disappears posteriorly, this stronger expression becomes confined to isolated regularly spaced cells. Double labeling experiments showed that those are R8. Thus, the expression pattern suggests ato is only directly required for R8 formation. Mosaic analysis confirms this hypothesis. Only R8 absolutely requires ato for formation. R8 must then recruit R1-7 in a process that does not require proneural gene function. The absence of R1-7 and cone cells in ato$^-$ eye is a consequence of ato requirement to form R8.

There are interesting qualitative differences between ac/sc and ato. In the ac/sc proneural cluster, the cells compete among themselves via neurogenic genes until a single cell emerges as the neural precursor. In the ato proneural cluster, the initial singling out process appears to be similar to that of ac/sc system, however, there is a crucial difference. In ato proneural clusters, following the initial singling out of "founding neural precursors", there is a second phase of recruitment of additional neural precursors by induction resulting in the formation of clusters of neural precursors (vs. the usually solitary neural precursors in the ac/sc system). We are trying to identify the domains in ato and ac/sc which may be responsible for this difference by doing domain swapping experiments.

Molecular genetics of sensory signal transduction

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Sensory signaling cascades are utilized by all metazoan organisms to detect, record and respond to environmental cues. Vision, taste, olfaction and mechanotransduction represent different modes of signal transduction processes that are highly developed and specialized in *Drosophila*.

We have used a variety of molecular and genetic screens to identify components required for the functioning and regulation of the phototransduction cascade in the adult visual system. Recently, we have shown that calcium is a key regulator of photoreceptor cell activation, deactivation and adaptation. We have now developed a novel approach to carry out a physiological dissection of the role of calcium in the modulation of the phototransduction cascade *in vivo*. These results will be presented.

We have also carried out a genetic dissection of mechanosensory transduction in *Drosophila*. Recent findings regarding the identification of mutants defective in mechanosensory transduction will be discussed.

POSTERS

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Characterization of lethal P-insertions that interact genetically with *seven-up* during *Drosophila* eye development

Members of the steroid hormone receptor superfamily have been cloned from several species. Though the target sequences of most of these receptors have been determined, few genes are known that act downstream or are regulated by these receptors during development. In addition, for many of these proteins, the so called orphan receptors, the ligands have not been identified yet.

One of these receptors, *seven-up* (*svp*), is required for embryonic nervous system development and later for the determination of a subset of outer photoreceptors.

Using the *sevenless* enhancer, we have ectopically expressed *svp* in R7 and the cone cells, resulting in a transformation of the non-neural cone cell precursors into R7 or outer photoreceptor cells. The presence of the supernumerary photoreceptors leads to a dosage dependent rough eye phenotype and therefore provided a most suitable system to genetically identify genes that interact with or act downstream of *svp*.

In a screen of 2300 lethal P-insertions on the 2nd chromosome we have isolated 11 suppressors and 1 enhancer of *svp*, as well as some already known genes that are involved in photoreceptor specification in general, suggesting that R1-6 and R7 share part of the same pathway.

Here we present an overview over the P-alleles isolated: most of them act as enhancer detectors and show β-Gal expression in the developing eye-imaginal disc. All of the lines interact specifically with *svp*, as non of them is capable to modify the phenotypes of dominant alleles of *sevenless*, *ras1* or *raf*. We have generated somatic clones in the adult eye for most of the lines, and so far one of the lines shows a phenotype expected of a gene that would be necessary for *svp*-function: homozygous clones in the eye contain a fraction of mutant ommatidia with a phenotype identical to the loss-of-function phenotype of *svp*(transformation of outer photoreceptors R1/6 and R3/4 towards R7 cells). Moreover, β-Gal is expressed in discrete clusters of photoreceptors behind the morphogenetic furrow.

We are currently generating deletions around the point of the P-insertion in order to obtain null-alleles and have isolated cDNAs that map to the corresponding genomic region.

SYNAPTOGENESIS IN *DROSOPHILA*. Kendal Broadie and Michael Bate, Department of Zoology, Downing Street, Cambridge, United Kingdom.

The construction of synaptic connections is of central importance in the development of an integrated nervous system. We would like to promote the *Drosophila* embryonic neuromuscular junction (NMJ) as a new system in which to study synaptogenesis at a cellular, molecular and genetic level. The embryonic NMJ of Drosophila is a large and accessible developing synapse which can be easily manipulated both genetically and molecularly and, thus, promises to allow rapid progress into the molecular and genetic pathways directing synaptogenesis for the first time. Over the last few years, we and others have defined the elements of the embryonic NMJ and generated a detailed map of the cellular events underlying synaptogenesis. Most importantly, we have refined electrophysiological techniques with which to record and drive synaptic communication at any stage of synaptogenesis and so can couple physiological and morphological studies in the *Drosophila* embryo for the first time. With these techniques, we are using known mutations as tools to dissect the mechanisms of synaptogenesis. For example, using a mutation (*prospero*) which eliminates the presynaptic motor innervation, we have divided synaptogenesis into innervation-dependent and independent events. We find that definition of the synaptic domain (localized expression of putative guidance molecules) is independent of innervation, whereas the constuction of the muscle's postsynaptic receptor field (expression and clustering of transmitter receptors) is innervation-dependent. Likewise, using mutations (e.g. *paralytic, Shaker*) and neural toxins (tetrodotoxin (TTX) and argiotoxin) to modulate neural and synaptic electrical activity, we have further divided synaptogenesis into activity-dependent and independent events. We find that the neural induction of the muscle's receptor field (receptor expression and clustering) is dependent on electrical activity, whereas synaptic morphogenesis is largely activity-independent.

Using these and similar techniques we have begun to define the cellular mechanisms which underlie synaptogenesis in the *Drosophila* embryo. We are just beginning to exploit the considerable genetic and molecular advantages of *Drosophila* to dissect synaptogenesis and, in particular, to ask questions about the role of individual gene products in the construction and function of a synapse. At present, we are concentrating on a dissection of the presynaptic sigalling mechanisms which have recently become accessible due to the isolation of several proteins hypothesized to play a role in vesicle trafficing and exocytosis in other sytems. For example, we have been characterizing the mutant phenotype of Synaptotagmin, a protein believed to couple calcium influx and synaptic vesicle release. The role of this and similar proteins in presynaptic development and signalling function will allow us to define presynaptic as well as postsynaptic development at the embryonic NMJ.

We now have a well-defined system in which to examine synaptogenesis. Our long-term goal is to systematically mutate those genes that are involved in the construction of a synapse and define their roles in *Drosophila* and other organisms.

ACHAETE-SCUTE COMPLEX GENES AND THE SPECIFICATION OF THE WING MARGIN SENSORY ORGANS OF *DROSOPHILA*.

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The wing margin of *Drosophila* is a very suitable model system to analyze the mechanisms involved in the specification and differentiation of several types of sensory organs at reproducible positions. The wing margin shows a stereotyped array of sensory organs (SO) which belong to three classes: chemosensory recurved bristles and mechanosensory, stout or slender, bristles[1]. Development of these bristles show distinct requirements for specific proneural genes of the *achaete-scute* complex (AS-C). Thus, emergence of the precursors (the SMCs) of the chemosensory bristles depends on both *scute* and *asense* whereas the complete deficiency of the complex only removes a fraction of the mechanosensory bristles[2].

We have recently shown that the proneural AS-C genes are also required for the correct differentiation of the wing margin bristles[2]. A reduction in the doses of AS-C genes which does not prevent SMC emergence causes malformation of the bristles. Another important aspect of SO differentiation, the specification of the type of SO, may also be affected by the specific combination or amounts of AS-C transcription factors accumulated in the SMC. The position in the imaginal epithelium at which SMCs are born and/or the moment of their emergence[3] may also be relevant for SO specification.

To test the first possibility we have carried out a detailed phenotypic analysis of wing margins of flies mutant for the different AS-C genes and the functionally related *hairy* gene. To address the second possibility we have induced the appearance of ectopic SMCs by temporally controlled overexpression of *asense* or *scute* under the control of a heat shock promoter before, during and after the period when the corresponding precursors are being singled out. Our results indicate that the AS-C genes provide information for the specification of the type of wing SOs.

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The proneural gene *lethal of scute* performs analogous functions in mesoderm and neuroectoderm.

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Previous studies on the expression and mutant phenotype of the gene *lethal of scute* of *Drosophila* have indicated the important role of this gene in early neurogenesis. We have recently analysed the expression of *l'sc* in the embryonic mesoderm. The study has revealed a very dynamic pattern of *l'sc* expression, that displays close analogies with its expression in the neuroectoderm. Thus, *l'sc* accumulates in clusters of mesodermal cells that appear at precise positions and in a sequencial order during late stage 10 and stage 11. In most cases, the expression becomes restricted to a single cell, which has accumulated higher amounts of protein than the remaining cells of the cluster. Such restriction does not occur in neurogenic mutants.

The homeo box gene *S59* is expressed in at least three muscle fibers and in their corresponding founder cells. Double staining for *l'sc* and *S59* shows coexpression in these founder cells. Furthermore, the founder cells are those which accumulate higher levels of *l'sc*.

Late *l'sc* mutant embryos, double stained with an *S59* antibody and mAb 3E2 (that stains all muscles) reveals a muscle phenotype. One of the *S59*-expressing muscles (m25/VT1) is frequently lost or shows some type of abnormality (i.e., abnormal morphology or loss of *S59* expression) in many segments. The defects in *S59* expression can be already detected at the founder cell stage. Discrete defects in other muscles are also found.

We have driven specific expression of *l'sc* in the mesoderm, using the GAL-4 system, in a *l'sc* mutant background. This results in a substancial rescue of the muscle phenotype, indicating that the muscle defects are largely due to the specific lack of *l'sc* expression in the mesoderm.

The above results support a function of *l'sc* in early myogenesis analogous to its role in early neurogenesis. *l'sc* would lead groups of cells, within a field of undifferentiated mesodermal cells, to enter the muscle founder pathway. This fate would be subsequently restricted to a single cell within each group by the action of the neurogenic genes, whereas the remaining cells of the cluster would become fusion-competent cells.

GENETIC ANALYSIS OF TWO NOTCH RELATED MUTATIONS

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During the development of the nervous system of Drosophila a decision between epidermal and neural fate is taken under the control of the neurogenic genes. They are involved in a signaling process between neighbour cells. Recently two other genes have been implicated in these process, *deltex* and *strowberry notch*. They have not been described as neurogenic genes, but their phenotype in homozygous condition as well as their genetic interactions with the neurogenic genes suggest a strong relation with the latter.

We have done a mutagenesis screen and isolated several dominant scalloping mutations. We have chosen two of them based upon their genetic interactions with the neurogenic genes. We think that they are antimorphic and are lethal in double heterozygous condition which, together with their similar meiotic location, suggest that they might be allelic. A duplication of *Notch* and the mutations *Abruptex* and *Hairless* supress the dominant phenotype of the two mutants. Otherwise, loss-of-function alleles of *Notch*, *Serrrate* and *groucho* strongly enhance the scalloping phenotype. We have done other genetic interactions in order to know these mutations in more detail.

Taken together, these interactions indicate a clear relationship between these two mutants and the neurogenic genes. We are now searching for revertants of the phenotype of these mutants. With the hope that these revertants will help us to define the loss-of-function phenotype of them. We also plan to do further genetic characterization and a clonal analysis in order to understand cellular behavior.

CIS AND TRANS CONTROL OF THE *ACHAETE-SCUTE* COMPLEX EXPRESSION.

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Two members of the *achaete-scute* complex, *achaete* (*ac*) and *scute* (*sc*), play a key role in the development of the adult pattern of sensory organs. In imaginal wing discs, both genes are coexpressed in groups of cells, the proneural clusters, that occupy highly specific positions and define the regions where sensory mother cells will emerge. *ac* and *sc* have very different cis-regulatory regions that contain enhancer like elements that would respond to site-specific combinations of factors and promote the spatially restricted expression of *ac* and *sc* genes.

By genetic and interspecific heteroduplex analyses, we have determined conserved DNA regions that may contain quite a number of these enhancers. Transformant flies harboring the *lacZ* gene driven by a *sc* promoter fragment (3.7 kb) and each of several of these DNA regions have demostrated the existence of the proposed enhancers.

The gene *iroquois* (*iro*) is one putative candidate to control the transcription of *ac/sc* genes. Flies homozygous for a viable *iro* allele lack lateral sensory organs. In *iro* mutant imaginal discs, the *ac/sc*-expressing cell clusters that will give rise to these sensory organs are missing.

We shall report the molecular cloning and the expression pattern of the *iro* gene.

U-SHAPED, A GENE REQUIRED FOR NORMAL BRISTLE PATTERNING

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The pattern of macrochatae on the notum arises, in part, as the consequence of a precise spatial expression of the *ac* and *sc* genes in the wing disc. In a attempt to identify genes involved in this spatial regulation we have performed several mutagenesis experiments and have identified several new candidates, one of which being *u-shaped* (*ush*). Loss-of-function mutant alleles of *ush* are recessive and embryonic lethal, embryos do not undergo germ band retraction . By viable *ush* mutations, the flies bear ectopic macrochaetae. Animals doubly heterozygous for a deletion of *ush* and a deletion of the *achaete-scute* genes lack certain bristles. From an enhancer trap screen we have identified an insertion in the gene *ush* . A molecular analysis of this gene has been undertaken and genomic cosmids were isolated using the plasmid rescue technique. Several mutant alleles have been mapped including the *Tegula* inversion which breaks in the coding region of the gene. Several cDNAs were isolated and sequence analysis shows that *ush* encodes a protein bearing zinc fingers without homology to known proteins. The results obtained by *in situ* hybridisation with probes for *ush* reveal a spatially regulated expression of *ush* in the wing disc. Expression is clearly limited to the posterior part of the notum including a large part of the postnotum, the whole of the scutellum and covering the middle of the scutum. Expression is also detected in the region of the wing hinge as well as in the pteropleura. It is worth noting that whereas *ush* is expressed in the scutellar proneural cluster, it is only just detectable in part of the dorso-central proneural cluster and expression does not coincide with the dorso-central precursor cells. Expression stops at a precise point very close to the precursor cells suggesting a possible role of *ush* in defining the position of appearence of these cells.

Regulation of proneural cluster formation and mediation of lateral inhibition.

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The Drosophila central and peripheral nervous systems (CNS and PNS) develop from sets of neuroblasts (NBs; CNS) and sensory mother cells (SMCs; PNS) that segregate from clusters of ectodermal cells expressing the proneural helix-loop-helix (HLH) proteins of the *achaete-scute complex* (AS-C). Unlike the developing adult PNS, activation of the *achaete* (*ac*) gene in proneural clusters of the embryonic CNS is mediated by 3' elements that respond to axis-patterning genes. Furthermore, maintenance of proneural gene expression in embryonic NBs and its down-regulation in the remaining cells of the cluster (lateral inhibition) involve 5' elements that do not require AS-C protein functions. Within sequences 5' to *ac*, we have identified and mutated an *E(spl)* binding site which may mediate lateral inhibition in both the embryo and the adult. While proneural cluster formation and neural precursor segregation are regulated by separate mechanisms that are different in the early embryo and the developing adult, the lateral inhibitory process may be mediated through a single control element.

In order to determine whether a similar process occurs in other insects, we have cloned and analyzed the expression patterns of a Tribolium AS-C homolog, Tash-1. While Tash-1 is expressed in proneural clusters and NBs, in patterns reminiscent of Drosophila AS-C gene expression, we find Tash-1 to be expressed somewhat later, in the developing ventral nerve cord as well.

Regulation of segment-specific development in the embryonic and postembryonic CNS of Drosophila

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The ventral nerve cord (vNC) of *Drosophila* shows segment specific morphological differences. We analyzed such differences during CNS development and experimentally investigated mechanisms involved in their regulation. Firstly we described the embryonic lineage of neuroblast (NB) 1-1 (Udolph et al., 1993: *Development* 118, pp.765ff.), the composition of which exhibits characteristic differences between thorax and abdomen. Secondly we investigated the segment-specificity of proliferation in the larval vNC: most NB in the thorax divide during postembryogenesis, whereas most NB in the abdomen do not (Truman and Bate, 1988: *Dev.Biol.* 125, pp.145ff.; Prokop and Technau, 1991: *Development* 111, pp.79ff.).

Neural precursor cells are committed at the early gastrula stage to a thoracic or abdominal fate, in a cell-autonomous manner. We provide evidence for this with respect to the NB1-1 lineage as well as to postembryonic proliferation behaviour: transplantation of thoracic precursor cells into the abdomen leads to ectopic thoracic development of their lineages, and *vice versa*.

The pattern of segment-specific components of the NB1-1 lineage as well as segment-specific embryonic and postembryonic proliferation patterns are altered in mutants of the bithorax complex, indicating that the activity of homeotic genes is essential for selecting the respective pathway. Accordingly, isotopic transplantations of mutant abdominal cells into wild type at the early gastrula stage, reveal that the absence of *Ubx* and *abd-A* in NB1-1 is sufficient to permit thoracic development of the NB1-1 lineage.

We show for the NB1-1 lineage that ectopic induction of *Ubx* or *abd-A* in these cells at stage 10/11 can override thoracic determination. At about the same stage both proteins can be detected in NB1-1 of abdominal segments in the wild type. These data are paralleled by analyses of NB1-1 in *Polycomb* mutants: In *Polycomb* the NB1-1 lineage expresses abdominal features in part of the thorax. The location of these defects matches the thoracic derepression patterns of especially *abd-A* in stage 11 *Polycomb* embryos.

Taken together, our data reveal an important role of homeotic genes in the regulation of segment-specificity during vNC development. They furthermore suggest that early commitment is due to genes upstream of homeotic gene activity.

Genetic and biochemical investigation of *yan* protein function in the Drosophila eye. Ilaria Rebay and Gerald M. Rubin, HHMI, U. C. Berkeley.

Loss-of function mutations in the *yan* gene of *Drosophila* result in the differentiation of supernumerary photoreceptors in the adult eye. *yan* encodes a protein containing an ETS-DNA binding domain that localizes to the nuclei of undifferentiated cells in the developing eye imaginal disc. Once cells begin to differentiate, *yan* protein expression is abruptly down-regulated. These observations have led to the suggestion that *yan* may function as a negative regulator of photoreceptor cell development by keeping the cells locked in an undifferentiated state. In particular, in the case of the R7 photoreceptor cell, *yan* may act as an antagonist to the proneural signal mediated by the *sevenless* receptor tyrosine kinase and *Ras1*.

In addition to the ETS-DNA binding homology, *yan* also contains 8 consensus sequences (Pro-X-Ser/Thr-Pro) for phosphorylation by mitogen-activated protein (MAP) kinases. Since MAP kinases are known to be involved in Ras-mediated signalling it is possible that the *yan* gene represents one of the downstream targets of the *sevenless-Ras1* signal transduction cascade. Our model is that phosphorylation of *yan* by MAP kinases may lead to rapid protein degradation which in turn would release the cells from their differentiation block and allow them to respond to appropriate signals for neural development.

To test this hypothesis several experiments are planned. First, we will check whether the *yan* protein is in fact phosphorylated. This will be done by transfecting S2 cells with a *yan* expression construct and looking at phosphorylation levels of the protein. In addition, we will determine whether this phosphorylation is dependent on MAP kinase activity and whether there are any correlations between *yan* protein stability and phosphorylation. The second set of experiments involves in vitro mutagenesis of the MAP kinase phosphorylation sites in *yan* and then examination of the effects in both cell culture assays and in vivo. For example, if all sites are mutated to a non-phosphorylatable form then we predict this construct to remain constitutively active in vivo and to effectively block photoreceptor development. Alternatively, it may be possible to mimic a constitutively phosphorylated state by mutating the Ser/Thr residues to Asp/Glu. The prediction in this case is that the protein would be highly unstable and that expression in vivo would mimic the loss-of-function *yan* phenotype of supernumerary photoreceptors. If any such phenotypes are obtained , they will be used as the starting points for genetic enhancer/suppresser screens designed to identify other components interacting with *yan* in these processes.

In conclusion, we propose that the function of the *yan* gene in the *Drosophila* eye is to hold cells in an undifferentiated state., In order for a cell to respond to proper developmental cues, this *yan*-mediated block must be removed. This may be accomplished, at least in the *sevenless-Ras* mediated pathway for R7 photoreceptor cell determination, by phosphorylation and subsequent degradation of the *yan* protein.

Expression of *knirps* in the CNS and its regulation by *ortodenticle*

Rolando Rivera-Pomar, **Susana Romani** and Herbert Jackle

The Drosophila gap gene *knirps* (*kni*) is expressed in the embryonic CNS after neuroblast segregation. This expression occurs in a discrete number of precursor cells for a transient period of time, approximately until 7hs. of development. We have isolated an 870 bp regulatory element located about 2 kbp upstream to the *kni* transcription start which drives reporter gene expression (kni^{NS}-*lacZ*) in transgenic flies. Whitin this element, deletions as small as 20 bp abolish the CNS expression. Further analysis of the deleted sequences by footprinting showed an *in vitro* interaction with the homeodomain protein encoded by *ortodenticle* (*otd*). Whole mount in situ hybridization using *kni* probe and analysis of kni^{NS}-*lacZ* on otd^{-} flies confirmed that *otd* activity is required to directly activate *kni* expression in specifis CNS cells.

The *ventral nervous system defective* gene controls proneural gene expression at two distinct steps during neuroblast formation in the *Drosophila* embryonic central nervous system

James B. Skeath*, Grace F. Panganiban and Sean B. Carroll. HHMI, Laboratory of Molecular Biology, University of Wisconsin-Madison, Madison, WI 53706
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The cellular complexity of the central nervous system of the *Drosophila* embryo arises as a result of the generation, specification and asymmetric division of neuroblasts. Neuroblasts are singled out from proneural equivalence groups within the ectoderm and then delaminate into the interior of the embryo. Within the *Drosophila* embryo the formation of many neuroblasts depends on the functions of the proneural genes of the *achaete-scute* complex (AS-C) - *achaete* (*ac*), *scute* (*sc*) and *lethal of scute* (*l'sc*) - and the gene *ventral nervous system defective* (*vnd*). We have found that *vnd* controls neuroblast formation, at least in part, through its regulation of the proneural genes of the AS-C. *vnd* is absolutely required to activate *ac*, *sc* and *l'sc* gene expression in proneural clusters in the medial column of the earliest arising neuroblasts. Further, using two different *ac-lacZ* reporter constructs we determined that *vnd* controls proneural gene expression at two distinct steps during neuroblast formation through separable regulatory regions. First, *vnd* is required to activate proneural cluster formation within the medial column of every other neuroblast row through regulatory elements located 3' to *ac* ; second, *vnd* functions to increase or maintain proneural gene expression in the cell within the proneural cluster that normally becomes the neuroblast through a regulatory region located 5' to *ac* . By following neuroblast segregation in embryos mutant for *vnd* we find that defects in NB formation mirror those observed for proneural cluster formation.

With respect to neuroblast specification results will be presented that address the role the gene *gooseberry distal* (*gsb-d*) plays in this process. The removal of *gsb-d* function affects the expression patterns of the proneural genes *ac* and *sc* and *l'sc* within the embryonic central nervous system in opposite ways: *ac/sc* expression expands posteriorly in every segment into a region where *l'sc* is normally expressed; while *l'sc* expression prematurely disappears from this region. This alteration to the pattern of proneural gene expression in *gsb-d* mutant embryos is correlated with a fate change of the first neuroblasts to segregate from this region. These results suggest that *gsb-d* does and the proneural genes may specify neuroblast identity. Experiments aimed at directly testing whether or not the proneural genes of the AS-C control neuroblast identity, as well as neuroblast formation, will be presented.

SUMMARY

P. Simpson

The workshop was a great success. Most of the participants agreed that we all learnt a lot. Much new information was discussed on many different aspects of development of the nervous system. The topics ranged from the role of proneural genes in the specification of the neural fate to the molecular genetics of sensory signal transduction. The regulation of expression of *achaete, scute* and *lethal of scute* in the development of the central nervous system was discussed by S. Carroll and we heard about a new proneural gene, *atonal* from Y.-N. Jan. This gene appears to be the provider of proneural activity for the chordotonal organs and the photoreceptors of the eye. Three new genes acting upstream to regulate *achaete-scute* expression, *pannier*, *u-shaped* and *iroquois* were described. Exciting new results were presented concerning the regulation of proneural genes during lateral inhibition. S. Artavanis-Tsakonas described how the suppresor of Hairless protein is associated with the intracellular domain of Notch and dissociates and goes to the nucleus after binding of Notch to the Delta molecules of adjacent cells. M. Muskavitch introduced evidence in favour of the idea that Delta homodimerizes and that it is this property that induces dimerization of Notch upon binding. A. Martínez-Arias discussed the interesting possibility that Notch may act as a receptor for the secreted *wingless* gene product.

Glial-neuronal interaction and lineage and cell fate determination in the central nervous system was discussed by C. Klämbt, G. Technau and C. Doe, whereas genes controlling the differentiation of sensory organs were described by C. Dambly-Chaudière. We heard about genes controlling axon guidance from D. Van Vactor and about the determination of photoreceptors in the eye from M. Freeman and M. Mlodzik.

These were only a few of the contributions. The meeting included poster sessions and there was much informal discussion during breaks. We hope that other meetings of this sort in the future will be as rewarding.

List of Invited Speakers

Workshop on

CELLULAR INTERACTIONS IN THE EARLY DEVELOPMENT OF THE NERVOUS SYSTEM OF DROSOPHILA

List of Invited Speakers

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CELLULAR INTERACTIONS IN THE EARLY DEVELOPMENT OF THE NERVOUS SYSTEM OF DROSOPHILA

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Organized by T. A. Springer and F. Sánchez-Madrid. Lectures by S. J. Burakoff, A. L. Corbi-López, C. Figdor, B. Furie, J. C. Gutiérrez-Ramos, A. Hamann, N. Hogg, L. Lasky, R. R. Lobb, J. A. López de Castro, B. Malissen, P. Moingeon, K. Okumura, J. C. Paulson, F. Sánchez-Madrid, S. Shaw, T. A. Springer, T. F. Tedder and A. F. Williams.

266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
Organized by F. X. Avilés. Lectures by T. L. Blundell, W. Bode, P. Carbonero, R. W.Carrell, C. S. Craik, T. E. Creighton, E. W. Davie, L. D. Fricker, H. Fritz, R. Huber, J. Kenny, H. Neurath, A. Puigserver, C. A. Ryan, J. J. Sánchez-Serrano, S. Shaltiel, R. L. Stevens, K. Suzuki, V. Turk, J. Vendrell and K. Wüthrich.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organized by J. M. Mato and J. Larner. Lectures by M. V. Chao, R. V. Farese, J. E. Feliú, G. N. Gaulton, H. U. Häring, C. Jacquemin, J. Larner, M. G. Low, M. Martín Lomas, J. M. Mato, E. Rodríguez-Boulan, G. Romero, G. Rougon, A. R. Saltiel, P. Strålfors and I. Varela-Nieto.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
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Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organized by R. Baker and J. M. Delgado-García. Lectures by C. Acuña, R. Baker, A. H. Bass, A. Berthoz, A. L. Bianchi, J. R. Bloedel, W. Buño, R. E. Burke, R. Caminiti, G. Cheron, J. M. Delgado-García, E. E. Fetz, R. Gallego, S. Grillner, D. Guitton, S. M. Highstein, F. Mora, F. J. Rubia Vila, Y. Shinoda, M. Steriade and P. L. Strick.

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Organized by B. Frangione and J. Ávila. Lectures by J. Ávila, K. Beyreuther, D. D. Cunningham, A. Delacourte, B. Frangione, C. Gajdusek, M. Goedert, Y. Ihara, K. Iqbal, K. S. Kosik, C. Milstein, D. L. Price, A. Probst, N. K. Robakis, A. D. Roses, S. S. Sisodia, C. J. Smith, W. G. Turnell and H. M. Wisniewski.

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16 **Workshop on Intra- and Extra-Cellular Signalling in Hematopoiesis.**
Organized by E. Donnall Thomas and A. Grañena. Lectures by M. A. Brach, D. Cantrell, L. Coulombel, E. Donnall Thomas, M. Hernández-Bronchud, T. Hirano, L. H. Hoefsloot, N. N. Iscove, P. M. Lansdorp, J. J. Nemunaitis, N. A. Nicola, R. J. O'Reilly, D. Orlic, L. S. Park, R. M. Perlmutter, P. J. Quesenberry, R. D. Schreiber, J. W. Singer and B. Torok-Storb.

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21 Workshop on DNA-Drug Interactions.
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22 Workshop on Molecular Bases of Ion Channel Function.
Organized by R. W. Aldrich and J. López-Barneo. Lectures by R. W. Aldrich, C. M. Armstrong, P. Ascher, K. G. Beam, F. Bezanilla, S. C. Cannon, A. Castellano, D. Clapham, A. Ferrús, T. Hoshi, A. Konnerth, R. Latorre, J. Lerma, J. López-Barneo, R. MacKinnon, G. Mandel, A. Marty, C. Miller, B. Sakmann, B. Soria, W. Stühmer and W. N. Zagotta.

23 Workshop on Molecular Biology and Ecology of Gene Transfer and Propagation Promoted by Plasmids.
Organized by C. M. Thomas, E. M. H. Wellington, M. Espinosa and R. Díaz Orejas. Lectures by J. C. Alonso, F. Baquero, P. A. Battaglia, P. Courvalin, F. de la Cruz, D. K. Chattoraj, E. Díaz, R. Díaz Orejas, M. Espinosa, J. C. Fry, K. Gerdes, D. R. Helinski, B. Hohn, E. Lanka, V. de Lorenzo, S. Molin, K. Nordström, R. W. Pickup, C. M. Thomas, J. D. van Elsas, E. M. H. Wellington and B. Wilkins.

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25 Workshop on Genetic Recombination and Defective Interfering Particles in RNA Viruses
Organized by J. J. Bujarski, S. Schlesinger and J. Romero. Lectures by V. Agol, P. Ahlquist, J. J. Bujarski, J. Burgyán, E. Domingo, L. Enjuanes, S. P. Goff, T. C. Hall, A. S. Huang, K. Kirkegaard, M. M. C. Lai, T. J. Morris, R. F. Ramig, D. J. Robinson, J. Romero, L. Roux, S. Schlesinger, A. E. Simon, W. J. M. Spaan, E. G. Strauss and S. Wain-Hobson.

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