Instituto Juan March
de Estudios e Investigaciones

78 CENTRO DE REUNIONES INTERNACIONALES SOBRE BIOLOGÍA

Co-sponsored by
NATIONAL SCIENCE FOUNDATION (U. S. A.)

Workshop on

Notch/Lin-12 Signalling

Organized by

A. Martínez Arias, J. Modolell and S. Campuzano

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INTRODUCTION

A. Martínez Arias, J. Modolell and S. Campuzano

The genes Notch from *Drosophila*, and lin-12 and glp-1 from the nematode *Caenorhabditis elegans*, encode single transmembrane proteins which are prototypes of a large family of receptors whose structure and function have been conserved from nematodes to humans. These receptors are basic elements of a signal transduction system which involves ligands and intracellular proteins.

Extensive studies in *Drosophila* and *C. elegans* have laid down a paradigm for the functioning of these receptors. This paradigm is derived from two basic and general observations: first, that the mechanisms which recruit cells for a particular developmental pathway generally select more cells than those that will ultimately follow the pathway; and second that, once some cells have been selected the fate is inhibited in the others. This process is iterative and occurs over and over again in development. Notch is the central element in this decision making process and, in the absence of Notch, cells tend to adopt premature and erroneous fates. As might be expected, constitutive activation of Notch leads to the suppression of cell fates in an indiscriminate manner. The process of cell fate suppression during development is termed "lateral inhibition" and, by regulating the assignment of cell fates, is instrumental for generating pattern.

The conceptual framework derived from studies in invertebrates has been extended to vertebrates. A variety of experimental systems have shown that the notion of Notch as a central element in the process of cell fate assignment is widespread and that ligands as well as signal transducers and, in some cases, nuclear targets of the system are very conserved. A dramatic observation in support of this notion is the association of mutations in Notch with leukemias and other tumours. In these instances the activation of Notch leads to the maintenance of a specific undifferentiated state.

This meeting was convened as a response to the growing realization of the importance that Notch signalling and the processes with which it is associated play in development. It attempts to address many emerging or unanswered questions. For example, the conservation of Notch and of the networks of ligands and, in some cases of transducers, bears the question as to how the pathway is regulated in different biological process and to what extent the lessons from *Drosophila* and *C. elegans* are applicable to vertebrates. In addition, and at a more basic level, there are important questions about the molecular details of Notch signalling that are just beginning to be unraveled. And last, but not least, the association of mutations in Notch with pathological conditions as diverse as cancers and dementia, prompt questions about how general is the picture that is emerging of Notch signalling.

Session 1

Notch and its ligands

Chairpersons: Michael W. Young

François Schweisguth

Notch signaling and cell proliferation

Masahiro Go and Spyros Artavanis-Tsakonas

On the basis of genetic and developmental analyses it appears that the role of Notch signaling is very broad during development. Our working hypothesis is that Notch controls the progression of precursor cells to the next developmental state (e.g. Artavanis et al 1995, Fleming et al 1997). In general, the Notch pathway does not transmit specific developmental signals but rather modulates the ability of a precursor cell to respond to such signals. Several studies have demonstrated that Notch pathway activity affects the responce of a precursor cell to differentiation signals, thus controling cell fates. Chosing a cell fate in responce to differentiation signals is only one aspect of morphogenesis and multicellular development depends on the coordinate implementation of cellular differentiation, proliferation and apoptotic programs. Little is known about how cell fate controlling mechanisms link differentiation to these other programs and given the fundamental role Notch plays in development we are interested to explore links between Notch signaling and proliferation as well as apoptosis.

We have carried out experiments aimed to examine the cosequences of modulating Notch activity during imaginal disc development, especially during wing morphogenesis, using the *UAS-GAL4* system . We examined the relationship between Notch signaling and the wing margin patterning genes *vestigial* (*vg*) and *wingless* (*wg*), whose expression was hown by several workers to depend on Notch activation. We find that Notch activity not only controls cell differentiation, but can also influence cell proliferation.

Activation of Notch signaling induces strong mitotic activity in the wing disc in a Su(H) dependent manner. We have gathered evidence indicating that the effect of Notch signaling on cell proliferation is indirect and is not the simple consequence of either *vg* or *wg* induction. In fact, we find misexpression of Vg in the wing pouch results in small wing discs and loss of *wg* expression, a phenotype opposite to that associated with the activation of the Notch receptor. However, we demonstrated that either Vg or Wg display synergistic effects with Notch signaling, profoundly affecting cell proliferation.

Regulation of Delta signalling during *Drosophila* development
T.L. Jacobsen, T.R Parody, S.S. Huppert, and M.AT. Muskavitch

Cis-interactions in Delta-Notch signalling
Expression of wild type Delta in the neurons of developing bristles can lead to adoption of the tormogen fate by the pre-trichogen cell of the four cell organ. The expressivity of this trichogen to tormogen transformation varies among positions for different notal macrochaetae. Expressivity is also sensitive to the dosages of different neurogenic pathway components: increases in *Notch* dosage and decreases in *Hairless* dosage increase expressivity, while decreases in *Su(H)* and *E(spl)-C* dosages decrease expressivity. Curiously, increases in background *Dl* dosage decrease expressivity and decreases in background *Dl* dosage increase expressivity. These data, based on a two-cell interaction in which essentially only one cell expresses Delta, strongly support the hypothesis that the level of Delta protein expressed by a cell modulates its ability to receive a Notch-mediated signal: increased levels of Delta expression diminish the ability of the Delta-expressing cell to receive a Notch-mediated signal, decreased levels of Delta enhance the ability of the cell to receive a Notch-mediated signal. Co-expression of Delta and a membrane-tethered Notch extracellular domain (ECN) diminishes the ability of the neuron to send a signal, and elimination from this construct of the EGF-like motifs required for Delta-Notch binding (ECNΔ10-12) eliminates this inhibition of Delta signalling (ECN and ECNΔ10-12 responder lines were gifts from K. Brennan and A. Martinez-Arias). These data support the hypothesis that Delta and Notch can associate within the protein export pathway in a manner that impedes the net ability of the cell to generate a Delta-dependent signal. Finally, we find that co-expression of fringe and Delta in the neuron inhibits the ability of that cell to send a Delta-dependent signal. These findings imply that, in contrast to the deduced relationship in the wing margin, fringe can impede Delta signalling in developing bristle organs.

Delta-Notch feedback regulation that acts downstream of the Notch receptor
Ectopic expression of wild type Delta (DeltaWT) or a dominant-negative form of Delta that lacks the intracellular domain (DeltaDde) during metamorphosis induces development of supernumerary notal microchaetae, based on specification of supernumerary sense organ precursors (SOPs). Ectopic SOPs induced by DeltaWT or DeltaDde expression arise by qualitatively distinct mechanisms. Induction of DeltaWT expression during prepupal development leads to an initial increase in net neurogenic signalling, which is followed by a depression in signalling capacity during SOP specification. Induction of DeltaDde expression leads to an initial decrease in net signalling, and this decrement persists through SOP specification. When DeltaWT expression is induced, high levels of DeltaWT protein persist and Notch expression levels are not reduced detectably during SOP specification. We conclude that initial hyperactivation of neurogenic signalling feeds back to reduce net neurogenic signalling capacity during later development by repressing the expression and/or activity of one or more components of the signalling pathway, other than the signal or receptor. Therefore, neurogenic signalling can exert

feedback regulation on components of the signal transduction pathway other than the ligand and receptor, in the developing notum.

Neurogenic-ras pathway interactions

Previous work on wing vein development led to the inference that Drosophila EGF receptor (DER)-mediated signalling is required for activation of Delta expression within metamorphic veins. Reduced rhomboid activity resulting from the rho^{ve} mutation causes distal vein loss and loss of Delta expression in distal proveins (Sturtevant and Bier, 1993), and reductions in rhomboid and vein function lead to the absence of wing veins and the absence of Delta expression in pupal proveins (de Celis and Bray, 1997). We have tested this hypothesis directly by assessing Delta expression following ectopic activation of the DER pathway in metamorphic wing discs. Expression of rho, activated Dras1, activated Dras2, or activated Draf, under control of a dpp disk driver leads to ectopic Delta expression in pupal wings. Expression of a dominant-negative form of DER under dpp^{disk} control leads to reductions in native Delta expression within proveins in the pupal wing. These findings directly and strongly support the hypothesis that DER pathway activation is necessary and sufficient for activation of Delta expression within proveins in the pupal wing.

References

Parks, A.L. and M.A.T. Muskavitch. 1995. Relationships between complex Delta expression and the specification of retinal cell fates during *Drosophila* eye development. Mech. Dev. 50:201-216.

Huppert, S.S., T.L. Jacobsen and M.A.T. Muskavitch. 1997. Feedback regulation is central to Delta-Notch signalling required for *Drosophila* wing vein morphogenesis. Development. 124:3283-3291.

Parks, A.L., S.S. Huppert and M.A.T. Muskavitch. 1997. The dynamics of neurogenic signalling underlying bristle development in *Drosophila melanogaster*. Mech. Dev. 63:61-74.

Notch/LIN-12 signaling in *C. elegans*

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LIN-12/GLP-1 signaling in *C. elegans* is similar to Notch signaling in flies and vertebrates in that both are used broadly during development to mediate cell interactions that include induction and lateral signaling.[1] In addition, both rely on the same core components: DSL ligands, Notch/LIN-12 receptors, and a similar transcription factor called CBF1 in vertebrates, Su(H) in flies and LAG-1 in nematodes (CSL proteins). In nematodes, the receptor is processed to generate a mature receptor composed of an extracellular fragment (EGF and LNG repeats) plus a membrane associated intracellular fragment (TM/IC).[2] The degree of conservation of the downstream target genes and various regulators is not yet known.

LAG-2 shares the primary architectural features of Delta-like DSL ligands. Work by my lab and by the Greenwald lab has shown that the signaling part of the molecule resides at its N-terminus and includes the DSL domain plus a 100-amino acid stretch extending N-terminally.[3-5] For mutant rescue, the EGF-like and intracellular domains are not required, but membrane association is essential.[5]

The LIN-12 and GLP-1 receptors share the primary architectural features of the Notch receptors, but they bear substantially fewer EGF-like repeats and have a shorter C-terminal region in the intracellular domain. Both EGF-like and the family-specific LNG repeats are essential for receptor function.[6] We suggest that LAG-2 may dock on the EGF-like repeats, and then induce a conformational change in the region of the LNG repeats that releases the TM/IC domain of the receptor from repression. The intracellular ANK repeats are essential for signaling.[6-8] Binding occurs between the intracellular domain of receptor and the downstream transcription factor (LAG-1): the binding is weak and non-specific between the ANK repeats and LAG-1, but is strong between the RAM domain and LAG-1.[8] However, no dominant negative effect of expressing the RAM domain on its own could be detected.

The *C. elegans* LIN-12/GLP-1 pathways have been useful in the past because the genetics is so powerful and the intercellular interactions so well-defined. Unique to this organism is the precise knowledge of which cells are signaling and receiving and when the signal is transmitted. In future studies, the availability of the complete genomic sequence and the ability to assess the function of molecularly defined genes by RNA mediated interference (RNAi) will permit a powerful complementary approach to the analysis of the pathway in this small nematode.

1. Kimble, J. & Simpson, P. The LIN-12/Notch signaling pathway and its regulation. *Annual Review of Cell and Developmental Biology* **13**, 333-361 (1997).
2. Crittenden, S.L., Troemel, E.R., Evans, T.C. & Kimble, J. GLP-1 is localized to the mitotic region of the *C. elegans* germ line. *Development* **120**, 2901-2911 (1994).

3. Henderson, S.T., Gao, D., Lambie, E.J. & Kimble, J. *lag-2* may encode a signaling ligand for the GLP-1 and LIN-12 receptors of *C. elegans*. *Development* **120**, 2913-2924 (1994).
4. Fitzgerald, K. & Greenwald, I. Interchangeability of *Caenorhabditis elegans* DSL proteins and intrinsic signalling activity of their extracellular domains in vivo. *Development* **121**, 4275-4282 (1995).
5. Henderson, S.T., Gao, D., Christensen, S. & Kimble, J. Functional domains of LAG-2, a putative signaling ligand for LIN-12 and GLP-1 receptors in *Caenorhabditis elegans*. *Molecular Biology of the Cell* **8**, 1751-1762 (1997).
6. Kodoyianni, V., Maine, E.M. & Kimble, J. Molecular basis of loss-of-function mutations in the *glp-1* gene of *Caenorhabditis elegans*. *Molec. Biol. Cell* **3**, 1199-1213 (1992).
7. Roehl, H. & Kimble, J. Control of cell fate in *C. elegans* by a GLP-1 peptide consisting primarily of ankyrin repeats. *Nature* **364**, 632-635 (1993).
8. Roehl, H., Bosenberg, M., Blelloch, R. & Kimble, J. Roles of the RAM and ANK domains in signaling by the *C. elegans* GLP-1 receptor. *EMBO J.* **15**, 7002-7012 (1996).

The Notch signalling pathway in zebrafish neurogenesis and somitogenesis

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We have cloned zebrafish homologues of a number of Drosophila genes that are known to be involved in the Delta - Notch signalling pathway. The cloned genes include *Delta, Notch, Su(H), hairy-E(spl)* and *groucho* homologues. In addition, an Id gene, a homologue of *extramacrochaete*, and a E12 gene, a homologue of *daughterless* were cloned. To assess the function of these zebrafish genes during development, injections of mRNA encoding different variants were made in one of the two blastomeres resulting from the first cleavage division. Two developmental processes were assessed: the development of islet-1 positive cells and somitogenesis. The results indicate that the essential elements of the Notch pathway are conserved in the zebrafish. The signalling pathway participates in a process of selection of individual cells from equivalence groups. With respect to somitogenesis, fusion of somites and myotomes was found in the injected animals. Results of additional experiments suggest that in zebrafish the Notch signalling participates in the process of subdivision of the presomitic mesoderm into somitomeres.

The control of spatial and temporal patterning by Delta-Notch signalling in vertebrates

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Competitive lateral inhibition, based on Delta-Notch signalling with feedback regulation of Delta, can be used in several ways to control patterns of cell differentiation. We have studied three examples in vertebrates:-

1) The singling-out of primary neurons. In the neural plate of Xenopus or chick, the earliest cohort of neurons - the primary neurons - originate as isolated cells expressing *Delta1*. How do these cells become singled out? We have examined this question in the zebrafish, which has at least four *Delta* homologues. In the neural plate during primary neurogenesis, *deltaA* and *deltaD* are expressed in patches of contiguous cells, within which scattered individuals expressing *deltaB* become singled out as primary neurons. RNA injection experiments show that all three genes have the properties required for competitive lateral inhibition: they all code for products that can deliver lateral inhibition so as to block neurogenesis, and they are all themselves downregulated in cells where the lateral-inhibition pathway is activated. When Delta-Notch signalling is artificially blocked, expression of the *delta* genes rises dramatically, and singling-out fails: in place of isolated primary neurons, we find clusters of contiguous primary neurons; in place of a single Mauthner cell on each side of the hindbrain, a cluster of Mauthner cells.

The *mindbomb* (*mib*, alias *white tail*) zebrafish mutant shows similar overproduction of neurons and upregulation of the *delta* genes; other Delta-Notch dependent processes, including somitogenesis, are also disturbed. We presume that *mib* codes for a component of the Delta-Notch pathway, which we are actively seeking to identify.

2) Spacing patterns in the inner ear. The sensory patches in the vertebrate inner ear are comparable, in development and in function, with the sensory bristles of *Drosophila*. In zebrafish, as in chick, these patches express homologues of *Notch* (ubiquitous in the otic epithelium), *Delta* (in scattered cells - apparently prospective hair cells) and *Serrate* (in all cells of the sensory patch). Normally, each sensory patch develops as a regular fine-grained mosaic of cell types, with sensory hair cells isolated from one another by intervening supporting cells. In *mib*, the *delta* genes are upregulated in all cells of the prospective sensory patch, again suggesting a failure of Delta-Notch signalling, and these cells all differentiate, prematurely, as hair cells, with no supporting cells between them. Delta-Notch signalling, with feedback regulation of *delta* expression, may thus be the mechanism that normally generates the alternating pattern of cell types.

3) Temporal regulation of progenitor/stem cell function in the central nervous system. The vertebrate CNS develops over many days or weeks, during which additional neurons are continually generated from dividing progenitor cells (loosely speaking, stem cells). The nascent neurons transiently express *Delta1*, while the progenitors express Notch1. Studies in the embryonic chick retina (see abstract by D. Henrique) show that if the Delta-Notch signalling is blocked, the whole population of progenitors differentiates prematurely; conversely, if the Delta-Notch signalling pathway is activated in every cell, the progenitors remain as progenitors and no neurons are produced. Thus Delta-Notch-mediated lateral inhibition, delivered to the progenitors by progeny that are beginning to differentiate, provides a negative feedback to regulate the rate at which progenitors enter the differentiation pathway. In this way, a balanced production of progenitors and differentiating progeny is maintained, enabling neurogenesis to continue. The balance depends on the ratio of the progenitor cell cycle time to the duration of Delta1 expression in the nascent differentiating cells; this may explain why neurogenesis eventually comes to an end as the cycle time increases in the developing cerebral cortex.

References

Collier, J. R., Monk, N. A. M., Maini, P. K. and Lewis, J. H. (1996). Pattern formation by lateral inhibition with feedback: a mathematical model of Delta-Notch intercellular signalling. *J. Theor. Biol.* 183, 429-446.

Haddon, C., Smithers, L., Schneider-Maunoury, S., Coche, T., Henrique, D. and Lewis, J. (1998). Multiple *delta* genes and lateral inhibition in zebrafish primary neurogenesis. *Development* 125, 359-370.

Henrique, D., Hirsinger, E., Adam, J., Le Roux, I., Pourquié, O., Ish-Horowicz, D. and Lewis, J. (1997). Maintenance of neuroepithelial progenitor cells by Delta-Notch signalling in the embryonic chick retina. *Curr. Biol.* 7, 661-670.

Jiang, Y.-J. et al. (1996). Mutations affecting neurogenesis and brain morphology in the zebrafish, *Danio rerio*. *Development* 123, 205-216.

Maintenance of neuroepithelial progenitor cells by Delta-Notch signalling in the embryonic chick retina

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Neurons of the vertebrate central nervous system (CNS) are generated sequentially over a prolonged period from dividing neuroepithelial progenitor cells. Some cells in the progenitor cell population continue to proliferate while others stop dividing and differentiate as neurons. The mechanism that maintains the balance between these two behaviours is not known, although previous work has implicated Delta-Notch signalling in the process.

We show that in normal development, the proliferative layer of the neuroepithelium includes both nascent neurons that transiently express *Delta-1* (*Dl1*), and progenitor cells that do not. Using retrovirus-mediated gene misexpression in the embryonic chick retina, we show that where progenitor cells are exposed to Dl1 signalling, they are prevented from embarking on neuronal differentiation. A converse effect is seen in cells expressing a dominant-negative form of Dl1, $Dl1^{dn}$, which we show renders expressing cells deaf to inhibitory signals from their neighbours. In a multicellular patch of neuroepithelium expressing $Dl1^{dn}$, essentially all progenitors stop dividing and differentiate prematurely as neurons, which can be of diverse types. Thus, Delta-Notch signalling controls a cell's choice between remaining as a progenitor and differentiating as a neuron.

We conclude that nascent retinal neurons, by expressing *Dl1*, deliver lateral inhibition to neighbouring progenitors; this signal is essential to prevent progenitors from entering the neuronal differentiation pathway. Lateral inhibition serves the key function of maintaining a balanced mixture of dividing progenitors and differentiating progeny. We propose that the same mechanism operates throughout the vertebrate CNS, enabling large numbers of neurons to be produced sequentially and adopt different characters in response to a variety of signals. A similar mechanism of lateral inhibition, mediated by Delta and Notch proteins, may regulate stem-cell function in other tissues.

NOTCH SIGNALING IN MICE

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We have been studying the role of the Notch signaling pathway during embryonic development in mice by creating targeted mutations in several ligands and receptors in the Notch pathway, as well as in one of the mouse Fringe genes. We have made mutations in three Notch receptors (Notch1, 2 and 4), two Notch ligands (Jagged1 and 2), and the Lunatic Fringe gene.

Embryos homozygous for mutations in either the Notch1 gene or the Jagged1 gene die during midgestation, while animals homozygous for mutations in Notch2, Jagged2 or Lunatic Fringe complete embryogenesis but generally die the first day of birth. Double mutant analyses reveal dosage-sensitive synergistic effects in Notch1/Notch2 double mutants and in Notch1/Notch4 double mutants. I will summarize the phenotypes of these various mutants, and will describe in more detail what we have learned from these mutants about the role of Notch signaling during somitogenesis and limb development in mice.

EXPRESSION OF NOTCH GENES IN ADULT MAMMALIAN NEURONS: PROTEOLYTIC ALTERATIONS WITH DEVELOPMENT Daniel R. Foltz[1], Maria-Grazia Nunzi[2]*, Enrico Mugnaini[2] and Jeffrey S. Nye[1]. [1]Molec. Pharm., Pediatrics, & [1,2]Inst. Neurosci., Northwestern Univ. Medical School, Chicago, IL 60611

Member of the Notch/lin12/glp1 family of transmembrane proteins have essential roles in early mammalian development and cell fate decisions in neurogenesis. A role for Notch family members in neurological disorders of adulthood has recently been indicated by the observation of a genetic interaction of lin12, a Notch family member and sel12, a homologue of the human Presenilin gene family, the etiology of some cases of familial Alzheimer's disease, and by the discovery of a neurodegenerative disorder caused by mutations in Notch3. However, little is known about the roles of Notch family members in adult animals and in cells where fate decisions have already been made. Using in situ hybridization, we have localized Notch family members (Notch 1, 2, 3 and 4) and Delta1, a putative ligand to the adult brain. We find that a neuronal distribution of the Notch mRNAs with overlapping and complementary distribution throughout the brain. Using immunohistochemistry, we observed Notch1 expressed most highly on large neurons, notably the pyramidal cells of the cerebral cortex and hippocampus, as well as purkinje cells of the cerebellum. Preliminary electron microscopical studies confirm a distribution on dendritic spines and axons. Notch1 proteins in adult brain differs from embryonic brain with predominantly higher molecular weight forms and multiple cleavage products. These data show that Notch1 species are localized to adult CNS neurons and imply a role in mature neurons. Since proteolysis of Notch appears to participate in mediating the Notch signal, these data suggest a regional and temporal variation in the quantity of active signal.

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NOTCH SIGNALLING POSITIONS GERM-LAYER BOUNDARIES IN THE SEA URCHIN EMBRYO

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We are interested in the molecular basis of cell-cell interactions that lead to cell fate determination during early sea urchin development. Towards this goal, we have identified a homologue of the *Notch* receptor, *LvNotch*, in the sea urchin *Lytechinus variegatus*. Immunolocalization of LvNotch during sea urchin development has suggested a potential role for the Notch pathway in early patterning along the animal-vegetal axis (Sherwood and McClay, Dev. 124, 3363-74, 1997) To directly assess the function(s) that the Notch pathway may play in early sea urchin development, we have injected mRNA encoding either activated or dominant negative forms of the receptor into fertilized sea urchin eggs. Injections of activated LvNotch lead to a great expansion in the number of secondary mesoderm cells at the expense of cells that would have normally become endoderm. A normally patterned and proportional endoderm, however, still forms in these embryos, which are smaller in overall size. Marker and lineage analysis reveal that the endoderm is shifted animally along the animal-vegetal axis into territory that would normally be fated to become ectoderm. Injection of a dominant negative form of the receptor significantly reduces the number of secondary mesoderm cells. Interestingly, the overexpression of the dominant negative receptor does not affect the specification of an endoderm, but may cause the endoderm to shift vegetally along the sea urchin animal-vegetal axis and thus increase the total amount of ectoderm territory. Taken together, these results suggest that the Notch pathway plays a critical role in secondary mesoderm specification, and that the division of germ-layers in the sea urchin embryo is a coordinated process that involves cellular interactions between these layers.

The role of the Notch signalling pathway in murine CNS and mesoderm development

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Two of the developmental processes in which the Notch signalling pathway is involved in the vertebrate embryo, are neurogenesis and the segmentation of the paraxial mesoderm. Neurogenesis in vertebrates occurs by the regulated withdrawal from the cell cycle of a homogeneous population of progenitor cells in the neural tube. Prospective neurons individually cease division, migrate centrifugally, and differentiate. These events are reiterated throughout development, generating radially arranged layers of neurons, with the last-born neurons in the outermost layer. Expression of the ligand Delta directs cells to a neuronal fate[1] and, through the activation of the Notch receptor(-s), inhibit their neighbours from becoming neurons. Thus, in the vertebrate CNS, Notch signalling controls the timing of neuronal differentiation, rather than the decision between an epidermal and a neuronal fate[1, 2], as in Drosophila.

During segmentation, the paraxial mesoderm is subdivided into metameric units called somites, that are arranged with a cranio-caudal polarity. The Notch pathway is involved in the formation of somites[3,4,5] and maintenance of segment borders[5]. In contrast to the CNS, Notch signalling in the somites does not function at the single cell level, but affects the fate of cells in an embryonic field.

Currently, using mutant mice for different elements of the Notch pathway, we are analyzing the role of Notch signalling in the generation and/or maintenance of neural stem cells in the CNS, and the mechanism by which Notch signalling influences somite formation.

1. Chitnis, A. et al. (1995). Nature 375, 761.
2. de la Pompa, J. L. et al. (1997). Development 124, 1139.
3. Conlon , R. A. et al. (1995.) Development 121, 1533.
4. Oka, C. et al. (1995). Development 121, 3291. 5. Hrabe de Angelis, M. et al. (1997). Nature 386, 717.

Gerry Weinmaster

Notch signaling in mammalian cells

Studies with vertebrates and invertebrates suggest that Notch/LIN-12 receptors inhibit cellular differentiation when activated by members of the DSL (for Delta, Serrate, Lag2) ligand family. However, while such Notch-mediated inhibition of differentiation has been demonstrated both in vitro and in vivo, the intracellular signaling pathway activated by ligand-Notch interactions is not well understood. To investigate Notch signaling in mammalian cells we have isolated a number of different Notch genes as well as genes encoding Notch ligands. To uncover the molecular mechanisms of Notch signal transduction we have developed an in vitro assay in which activation of Notch, either in a ligand-dependent or independent manner, blocks myogenesis. A number of studies from different organisms have indicated that Notch signaling results in the activation of a DNA binding protein referred to as Su(H) in Drosophila and Xenopus, Lag-1 in C. elegans and RBP-Jk/CBF1/KBF2 in mammalian systems. The activation of Su(H)/CBF1 in turn upregulates the expression of downstream genes such as the transcription factors E(spl) in Drosophila, ESR in Xenopus, and HES-1 in mammals. Consistent with these reports, we have found that Notch cytoplasmic forms containing CBF1-interacting sequences activate CBF1, upregulate endogenous HES-1, and inhibit muscle cell differentiation. However we have also identified cytoplasmic forms of Notch, in which the major CBF1-interaction domain has been deleted, that prevent myogenesis but do not activate CBF1. These data imply that Notch signaling activates at least two pathways in the cell: one that involves CBF1 and one that does not. Moreover, they indicate that Notch activation of just the CBF1-independent pathway is sufficient for Notch signaling to suppress muscle cell differentiation.

It has been suggested that Notch signaling inhibits myogenesis by antagonizing the function of MyoD, a muscle specific bHLH transcription factor that orchestrates muscle cell differentiation. However, we have found that truncated forms of Notch lacking the major CBF1-binding domain are unable to inhibit MyoD activity and function in two different assays. Firstly, C2C12 myoblasts stably expressing constitutively active forms of Notch, deficient in CBF1 activation and HES-1 upregulation, are unable to differentiate; however, infection with a retrovirus encoding MyoD induces the expression of muscle specific regulatory and structural genes and the formation of myotubes. Secondly, in transient cotransfection assays these same activated forms of Notch are unable to suppress MyoD-dependent MCKCAT activation in 3T3 cells. Taken together these data reveal the unexpected finding that Notch signaling does not always antagonize MyoD activity and function. Furthermore, the ability of ectopic MyoD to override Notch-induced inhibition of muscle cell differentiation places the Notch specific target involved in the repression of myogenesis upstream of MyoD. Interestingly, forms of Notch that contain CBF1-interacting sequences are able to antagonize MyoD in both the C2C12 and 3T3 cell assay systems indicating that, as previously reported, cytoplasmic forms of Notch can inhibit MyoD activity and function. Thus, CBF1 activation, HES-1 upregulation, and MyoD antagonism all correlate with the presence of the major CBF-1 interacting sequences within the Notch cytoplasmic domain. However, our data argue that the functional repression of MyoD by truncated cytoplasmic forms of Notch is irrelevant and inconsequential since MyoD is never expressed by myoblasts undergoing Notch signaling.

HES-1 has also been implicated in Notch-induced repression of myogenesis. However, we have found that the same constitutively active forms of Notch that are unable to activate CBF1, or antagonize MyoD also fail to upregulate HES-1. In fact, overexpression of HES-1 in C2C12 myoblasts does not inhibit their differentiation into myotubes. Our data question the role of HES-1 both in Notch-mediated inhibition of myogenesis and myogenesis in general. Consistent with results from other systems, we have identified cytoplasmic forms of Notch that can activate CBF-1 and upregulate HES-1, indicative of

CBF1-dependent Notch signaling. If the CBF1 pathway is not necessary for suppression of myogenesis what then is the role of CBF1 activation and HES-1 upregulation in Notch signal transduction? We have found that activation of Notch signaling or expression of HES-1 in C2C12 myoblasts leads to an increase in both Notch1 and Notch2 protein expression. These data suggest that Notch signaling, through HES-1, positively regulates Notch receptor expression identifying Notch as a target gene in the CBF1-dependent pathway. Importantly, the HES-1 stimulated increase in Notch expression in myoblasts makes them responsive to the inhibitory effects of the Notch ligand Jagged1. Therefore, increases in HES-1 expression through Notch signaling may function to increase the expression of the Notch receptor and thereby potentiate the cell's capacity for signal reception. In this way, a positive feedback mechanism between Notch signaling and Notch expression would ensure that cells maintain their ability to respond to ligand and continue to be inhibited by ligand-expressing cells.

Multiple Notch receptors and ligands have been isolated from invertebrates and vertebrates. In C. elegans, both the APX-1 and LAG-2 ligands can activate both of the receptors, LIN-12 and GLP-1, which in turn are interchangeable, despite the fact that they regulate different cell fates. In Drosophila, there is a single Notch receptor for the ligands Delta and Serrate; however, these ligands through interaction with the same receptor are thought to regulate distinct functions during development. In vertebrates where there are four Notch genes and at least as many ligand encoding genes, the question of which ligand activates which receptor is not completely known. We have shown using a coculture assay that Jagged1 can activate Notch1 expressed in myoblasts to block muscle cell differentiation. Expression studies identify specific ligand–receptor pairs that may function during development, but overlap between the different Notch receptors and their potential ligands is also found. Interestingly, using the muscle coculture assay, we have found that while both Jagged1 and Delta1 can efficiently activate Notch1, Jagged1 is more effective than Delta1 at inhibiting the differentiation of Notch2-expressing myoblasts. Therefore in mammalian cells, in contrast to invertebrate systems, all the ligands do not appear to efficiently activate all the Notch receptors. Our observation of differential activation of Notch1 and Notch2 by Jagged1 and Delta1 provides a unique opportunity to characterize the functional determinants of receptor-ligand interactions. Preliminary data suggest that the Jagged1 cysteine-rich domain contributes to the differences in Notch1 and Notch2 activation by Jagged1 and Delta1.

Session 2

Signal processing

Chairperson: Spyros Artavanis-Tsakonas

The metalloprotease-disintegrin Kuzbanian participates in *Notch* activation during growth and patterning of *Drosophila* imaginal discs.

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The Notch transmembrane protein is the receptor of an evolutionary conserved pathway that mediates intercellular signaling leading to the specification of different cell types during development[1]. Many aspects of this signal transduction pathway remain poorly understood, specially the role of the proteolytic processing of Notch. We present genetic evidence indicating that the metalloprotease-disintegrin *kuzbanian* [2] is a new component of the Notch signaling pathway and is involved in Notch activation. *kuzbanian* genetic mosaics demonstrate that during neurogenesis, wing margin formation and vein width specification *kuzbanian* is autonomously required in the cell where Notch is activated. During sensory organ (SO) development, N signaling limits singling out of the sensory organ mother cells (SMCs) in the proneural clusters, and, subsequently, helps implement the correct fates to the SMC descendants[3,4]. We have shown that the alterations in the pattern of SOs found in *kuz* mutants - the development of groups of adjacent SOs at places where in the wild type only one SO is present and the appearance of patches of naked cuticle devoid of SOs - correspond to failures in Notch-mediated lateral inhibition processes leading first to the development of all or most cells of the proneural clusters as SMCs followed very often by the differentiation of all SMC descendants as neurons.

Genetic interactions between *kuzbanian* and different genes of the *Notch* pathway indicate that *kuzbanian* is required upstream of *Suppressor of Hairless*. Moreover, the requirement of *kuzbanian* for signaling by a ligand-dependent Abruptex receptor, but not by a constitutively activated form of Notch, suggests that *kuzbanian* is involved in the generation of a Notch functional receptor and/or in its activation. However, the incomplete neurogenic transformation found in *kuz* null mutants and the ability of *kuz* cells to proliferate normally suggest the existence of mechanisms other than Kuz-dependent proteolysis to generate N functional receptors.

References.

1.- Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995). *Science* 268, 225-232.

2.- Rooke, J., Pan, D., Xu, T. and Rubin, G. M. (1996). *Science* 273, 1227-1231.

3.-Shellenbarger, D. and Mohler, J. (1978). *Dev. Biol.* 62, 432-446.

4.- Hartenstein, V. and Posakony, J. (1990).*Dev. Biol.* 142, 13-30.

Mechanism of activation of mammalian Notch

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The biochemical events associated with Notch signaling have remained so far elusive. We and others have proposed a model according to which ligand binding induces processing of Notch, followed by nuclear translocation of an intracytoplasmic fragment of the receptor, that associates with the Su(H)/RBP-Jκ DNA-binding subunit to activate target genes . However the biochemical and physiological relevance of this model has remained unclear. Recent reports indicate that the Notch receptor exists at the plasma membrane as a heterodimeric molecule, as a result of constitutive processing in the extracellular region by a protease which has been suggested to be the product of the gene *kuzbanian (kuz)*. We report here that constitutive processing of murine Notch 1 is not due to KUZ, but to a protease whose identity will be discussed. Activation of KUZ results in a second processing event that takes place in the extracellular region of Notch, C-terminal to the first site. This processing in turn leads to a third proteolytic step, which results in the release of an intracellular fragment of the receptor by an activity which can be blocked by a proteasome inhibitor.

References

1. Jarriault, S., *et al. Nature* **377**, 355-358 (1995).
2. Kopan, R., Schroeter, E.H., Nye, J.S. & Weintraub, H. *Proc Natl Acad Sci USA* **93**, 1683-1688 (1996).
3. Blaumueller, C.M., Qi, H.L., Zagouras, P. & Artavanis-Tsakonas, S. *Cell* **90**, 281-291 (1997).
4. Pan, D.J. & Rubin, G.M. *Cell* **90**, 271-280 (1997).

Processing and release of the Notch intracellular domain is induced by ligand and is required for signaling.

The precise molecular mechanism of Notch signal transduction is unknown. We have investigated the role of a proteolytic cleavage event which releases the intracellular domain of Notch (NICD) from the plasma membrane. Cleavage at a site located at the cytoplasmic side of the transmembrane domain is required for signaling by truncated Notch molecules such as those found in neoplasms. The DNA binding protein CSLRBP3 interacts preferentially with cleaved, nuclear targeted NICD in tissue culture cells; these factors then act in concert to activate transcription of target promoters. Moreover, such processing need not generate large amounts of NICD since levels of nuclear Notch protein undetectable by immunostaining are sufficient to elicit a maximal response. We also show that when full length mNotch1 interacts with its ligand Jagged in tissue culture cells, a cleaved product that comigrates with NICD is generated. These results confirm that proteolytic processing at an intracellular site is an important step in Notch activation.

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Evidence for dimerisation and nuclear translocation as mechanisms of Notch signalling.

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Activation of the Notch transmembrane receptor by its transmembrane ligands leads to the transcription of various target genes, including *vestigial* and those of the *Enhancer-of-Split* Complex. However, the mechanisms of receptor activation and of signal transduction into the nucleus are currently poorly understood. We shall present evidence, based on experiments using an *in vivo* assay in *Xenopus* embryos, that receptor dimerisation may be important in activating Notch signalling. We shall also discuss evidence that translocation of the Notch intracellular domain into the nucleus plays a direct role in activating target gene transcription.

Session 3

Signal transduction and implementation

Chairperson: Juan Modolell

Lateral signalling in development: on equivalence groups and assymetric developmental potential.

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Notch-mediated lateral signalling takes place between equivalent cells that then adopt alternative fates[1]. An example is the epidermal-neural choice in the *Drosophila* neuroectoderm. All cells initially produce both the ligand and the receptor, but, with time, one cell(s) comes to dominate(s) and signals to the other(s)[2]. Resolution depends upon a feedback loop within each cell, linking production of the ligand with activation of the receptor[3,4,5]. The choice of signalling cell is in some cases, such as the thoracic microchaetes, random[3]. A random choice of cell fate could arise from stochastic fluctuations in the turnover of different components of the signalling pathway, causing small differences that can be amplified by the feedback loop. A random positioning of bristles is characteristic of many other, more primitive, insects[6,7]. In other cases, such as that of the thoracic macrochaetes of *Drosophila*, the outcome is biased and the same cell is generally chosen to become the dominant signalling cell[8,9]. In the case of the dorsocentral macrochaetes, two signals have been shown to bias the choice of cell fate by increasing the levels of *achaete/scute*, one component of the feedback loop[10,11,12]. Finally, during embryonic neurogenesis in *Drosophila*, a highly derived process, choice of the neuroblasts is almost completely predetermined. Notch-mediated signalling is still required but 80% of the precursors segregate normally in the absence of the feedback loop[13]. It is likely that the Notch pathway is extremely old. A random choice of fate generated through lateral signalling is probably a ancient process in terms of evolution, that may be unstable.

References:

1. Greenwald I, Rubin GM: Making a difference: The role of cell-cell interactions in establishing separate identities for equivalent cells. *Cell* 1992, 68: 271-281.

2. Kooh PJ, Fehon RG, Muskavitch MAT. Implications of dynamic patterns of Delta and Notch expression for cellular interactions during Drosophila development. *Development* 1993, 117:493-507.

3. Heitzler P, Simpson P: The choice of cell fate in the epidermis of Drosophila. *Cell* 1991, 64:1083-92.

4. Heitzler P, Bourouis M, Ruel L, Carteret C, Simpson P: Genes of the *Enhancer of split* and *achaete-scute* complexes are required for a regulatory loop between Notch and Delta during lateral signalling in *Drosophila. Development* 1996, 122:161-71.

5. Kunisch M, Haenlin M. and Campos-Ortega J A: Lateral inhibition mediated by the *Drosophila* neurogenic gene Delta is enhanced by proneural proteins. *P.N.A.S.* 1994. 91: 10139-10143.

6. Wigglesworth VB: Local and general factors in the development of "pattern" in *Rhodnius prolixus* (Hemiptera). *J Exp Biol* 1940, 17:180-200.

7. Lawrence PA, and Hayward P: The development of a simple pattern: spaced hairs in Oncopeltus fasciatus. *J. Cell Sci.* 1971, 8: 513-524.

8. Cubas P, de Celis J-F, Campuzano S, Modolell, J: Proneural clusters of *achaete-scute* expression and the generation of sensory organs in the *Drosophila* imaginal wing disc. *Genes and Development* 1991, 5:996-1008.

9. Skeath J, Carroll S. Regulation of *achaete-scute* gene expression and sensory organ pattern formation in the *Drosophila* wing. *Genes and Development* 1991, 5:984-95.

10. Phillips RG, Whittle R: *wingless* expression mediates determination of peripheral nervous system elements in late stages of *Drosophila* wing disc development. *Development* 1993, 118:427-438.

11. Cubadda Y, Heitzler P, Ray R, Marc Bourouis M, Ramain P, Gelbart W, Simpson P, and Haenlin M. *u-shaped* encodes a zinc finger protein that regulates the proneural genes *achaete* and *scute* during the formation of bristles in *Drosophila. Genes and Dev.*, in press.

12. Haenlin M, Cubadda Y, Blondeau F, Heitzler P, Lutz Y, Simpson P and Ramain P. Transcriptional activity of Pannier is negatively regulated by heterodimerization of the GATA DNA-binding domain with a co-factor encoded by the *u-shaped* gene of *Drosophila. Genes and Dev.*, in press.

13. Seugnet L, Simpson P, Haenlin M: Transcriptional regulation of *Notch* and *Delta:* Requirement For neuroblast segregation in *Drosophila Development* 1997, 124, 2015-2025.

Analysis of Notch-Dependent Transcription in *Drosophila*.

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Notch acts as a receptor for extracellular signals regulating cell determination. The signal of Notch activation at the membrane is relayed in receiving cells by Suppressor of Hairless [Su(H)], a DNA-binding protein (1-4).

It has been suggested that signal transduction may be mediated by the ligand-induced release of Su(H) from membrane-bound Notch (2). This predicts that Su(H) should co-localise with Notch at the membrane in unactivated cells, and be found in the nucleus of activated cells. However, immuno-localisation of Su(H) in fixed tissues indicate that nuclear localisation of Su(H) does not depend upon Notch activation (5). Moreover, when detected in the cytoplasm, as in socket cells, Su(H) did not co-localise with Notch at the plasma membrane. Yet, these data do not rule out this model, as it is conceivable that signal transduction is mediated by the nuclear translocation of a specific pool of « Notch-activated » Su(H).

Another model proposes that Su(H) activates transcription in response to Notch activation by tethering a processed form of Notch that would act as a trancriptional co-activator (6). This activated form of Notch would consist of the intracellular domain of the receptor released from the plasma membrane by an hypothetical ligand-induced proteolytic cleavage. This model was tested in vivo using a nuclear activity assay for Notch, based on the ability of the Notch intracellular domain to activate transcription. First, the intracellular domain of Notch can activate transcription when fused to the DNA-binding domain of Gal4. UAS-mediated transcriptional activation by Gal4-Nintra is independent of *Delta* gene activity, but is still partly dependent on *Su(H)* gene activity. Second, UAS-mediated transcriptional activation was also observed with a fusion protein consisting in a full-length Notch receptor in which the DNA-binding domain of Gal4 was inserted in frame within the RAM23 domain. Transcriptional activation by this fusion protein appeared to be *Delta*-dependent but *E(spl)-C* independent. This indicates that Notch can be cleaved upon ligand binding to produce a proteolytic fragment acting as a transcriptional co-activator for Su(H). The known regulatory properties of Su(H) in mammals thus suggest that the binding of Nintra to DNA-bound Su(H) triggers a transcriptional switch, from repression to activation. It is intriguing that the presence of Su(H) binding sites, althougth necessary, is not sufficient for this swith to occur.

1: Artavanis-Tsakonas et al. (1995) *Science* **268** 225-232
2: Fortini and Artavanis-Tsakonas (1994) *Cell* **79** 273-282
3: Schweisguth (1995) *Development* **121** 1875-1884

4: Lecourtois and Schweisguth (1995) *Genes Dev.* **9** 2598-2608
5: Gho et al. (1996) *Development* **122** 1673-1682
6: Jarriault et al. (1995) *Nature* **377** 355-358

Molecular mechanism of myogenic suppression by Notch signalling

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Notch is involved not only in cell fate determination of the nervous and muscular cells but also in transformation of T lymphocytes. Myogenenic cells provide useful *in vitro* models for studying the cell differentiation. When C2C12 myogenic precursor cells are cultured in media containing low serum, they differentiate into myotubes. Myogenesis is positively regulated by transcription factors such as MyoD, Myf5, Myogenin, and MRF4, but negatively by Id and Twist. Since the overexpression of the Notch intracellular region prevents myogenic precursor cells from differentiation, Notch is thought to be one of the negative regulators of myogenisis. RAMIC of Notch1 transactivates genes by interaction with a DNA binding protein RBP-J (*1*).

We have compared mouse RAMIC, and its derivatives for activities of transactivation and differentiation suppression of C2C12 cells. RAMIC is comprised of three separate domains, *i.e.* RAM and ankyrin repeat regions for RBP-J binding and C-terminal transactivation domain. Although physical interaction of IC with RBP-J was much weaker than RAM, transactivation activity of IC was shown to involve RBP-J by using an RBP-J null mutant cell line. IC showed differentiation suppression activity generally comparable to its transactivation activity. The RBP-J-VP16 fusion protein that has strong transactivation activity also suppressed myogenesis of C2C12 (*2*). The RAM domain, which has no other activities than binding to RBP-J, synergistically stimulated transactivation activity of IC to the level of RAMIC. The RAM domain was proposed to compete with a putative

co-repressor(*3*) for binding to RBP-J because the RAM domain can also stimulate the activity of RBP-J-VP16. IC was further devided into the ankyrin repeat region for RBP-J interaction and the C-terminal transactivation domain.

In addition, we generated cell lines stably expressing a Notch ligand, Delta 1 (D10 cells) and transduced signals through Notch endogenously expressed in C2C12 cells. C2C12 cultured with D10 cells in low serum did not differentiate to myotubes whereas parental X63 cells did not affect differentiation, indicating that lignd-induced Notch signal leads to myogenic suppression of differentiation. To determine if this suppression is caused by the same mechanism as the intracellular region of Notch, the transcriptional activity through RBP-J recognition sequences was measured in this system. The RBP-J dependent transcriptional activity in C2C12 cells was enhanced by interaction with D10 cells. These results taken together, indicate that differentiation suppression of myogenic precursor cells by Notch signalling is due to transactivation of genes carrying RBP-J binding motifs.

References

(1) Honjo, T.(1996). The shortest path from the surface to the nucleus: RBP-Jκ/Su(H) transcription factor *Genes to Cells* 1 1-9

(2) Kato, H., Taniguchi, Y., Kurooka, H., Minoguchi,S., Sakai, T., Nomura-Okazaki, S., Tamura, K. and Honjo, T. (1997).Involvement of RBP-J in biological functions of mouse Notch1 and its derivatives *Development* 124 4133-4141

(3) Taniguchi, Y., Furukawa, T., Tun T., Han, H. and Honjo, T. (1998). LIM protein KyoT2 negatively regulates transcription by association with the RBP-J DNA-binding protein *Mol. Cell. Biol.* 18 644-654

Members of a novel gene family indicate a role for post-transcriptional regulation in Notch pathway signaling

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The past several years have seen impressive advances in the identification of new components of the Notch signaling pathway and in the elucidation of their regulatory relationships and mechanisms. Nevertheless, it is clear that much remains to be discovered in this arena. Recently our laboratory has described molecular genetic studies that link two related genes, *Bearded* (*Brd*) and *Enhancer of split m4* [*E(spl)m4*], to the function of the Notch pathway in controlling the development of the adult PNS of *Drosophila*. *Brd* is expressed specifically in imaginal disc proneural clusters under the direct transcriptional control of the proneural activators achaete (ac) and scute (sc)[1]. Gain-of-function alleles of *Brd* confer mutant phenotypes that mimic at the cellular level those caused by loss-of-function mutations in Notch pathway genes, including a failure of lateral inhibition in proneural clusters[2]. These *Brd* dominant phenotypes are sensitive to the dosages of both *Notch* and *Hairless*. *E(spl)m4* is likewise expressed specifically in proneural clusters, under dual transcriptional control: Like *Brd*, it is activated directly by ac and sc, but it is also a direct target of activation by Suppressor of Hairless in response to Notch receptor activity[3]. Thus, *E(spl)m4* is an integral member of the Notch pathway.

The *Brd* and *E(spl)m4* genes are structurally related, and we have suggested that they constitute a small gene family[4]. First, the predicted Brd (81 aa) and E(spl)m4 (152 aa) proteins show weak sequence identity, and both contain a small domain that is strongly predicted to form a basic amphipathic α-helix. In addition, the two genes exhibit a very unusual degree of nucleotide sequence identity in their 3' UTRs, including sharing two novel motifs, the Brd box (AGCTTTA) and the GY box (GTCTTCC). Both of these motifs are specifically conserved in the *D. hydei* ortholog of *E(spl)m4*. Remarkably, these same sequence elements are also widely distributed in the 3' UTRs of the other, bHLH repressor-encoding, genes of the *Enhancer of split* Complex [E(spl)-C]. While we have yet to assign a specific function to the GY box (see below), we have recently shown that the Brd box acts *in vivo* as a negative post-transcriptional regulatory element that principally mediates translational repression[5].

The known complexity of post-transcriptional regulation of E(spl)-C gene expression has been extended recently by our finding of yet a third widely shared sequence motif. The K box (TGTGAT) occurs in one or two copies in the 3' UTRs of seven of the nine genes of the Complex, and is specifically conserved in the *D. hydei* orthologs of both *m4* and *m8*. We have shown that, like the Brd box, the K box

functions as a negative post-transcriptional regulatory element, but instead mediates principally RNA instability. Moreover, an *E(spl)m8* genomic DNA transgene lacking its two copies of this motif causes gain-of-function defects in PNS development. Interestingly, the mutation that originally defined the E(spl)-C, $E(spl)^D$, is known to involve a deletion of the 3' UTR of the *m8* gene[6]. We find that an *m8* transgene with mutant K boxes largely mimics the interaction of $E(spl)^D$ with the *split* allele of *Notch*.

Recently we have discovered two new members of the *Brd/E(spl)m4* gene family. *Brother of Bearded* (*Bob*) encodes a small (78 aa) protein that displays significant sequence identity with Brd, and is likewise predicted to include a highly basic amphipathic α-helical domain. *Twin of m4* (*Tom*) encodes a 158-aa protein with regions of strong sequence identity to E(spl)m4, including a bipartite domain with significant basic amphipathic character. *Brd*, *Bob*, and *Tom* are all located in the 71A1-2 region of the third chromosome, and despite its closer relationship to *E(spl)m4*, *Tom* lies only a few kilobases upstream of *Brd*. Remarkably, the very same 3' UTR sequence motifs as are found in the E(spl)-C genes and *Brd* are present in the 3' UTRs of both *Bob* and *Tom*. *Bob* has two GY boxes and two K boxes, while *Tom* includes a Brd box, two GY boxes, and a K box. Thus, it seems likely that, as in the case of the E(spl)-C, the genes of the "*Bearded* Complex" will prove to be subject to shared modes of post-transcriptional regulation.

Insight into the possible function of the GY box has been provided by our recognition of a novel 3' UTR motif [the proneural (PN) box] in three of the known *Drosophila* proneural genes, *ac*, *atonal* (*ato*), and *lethal of scute* (*l'sc*). All three genes have one exact copy of the PN box's 13-nucleotide sequence, AATGGAAGACAAT, while *ato* and *l'sc* each have an additional variant copy. The PN box of *ac* is fully conserved in the *D. virilis* ortholog. Intriguingly, the core seven nucleotides of the PN box (GGAAGAC) and the GY box (GTCTTCC) are exactly complementary, and are often centrally located within even more extensive regions of complementarity (up to 18 contiguous base pairs) between PN box- and GY box-containing 3' UTRs. We suggest from these findings that *Drosophila* proneural genes and their regulators may participate in a novel post-transcriptional regulatory mechanism mediated by RNA:RNA duplexes.

1. Singson, A., Leviten, M. W., Bang, A. G., Hua, X. H. & Posakony, J. W. *Genes Dev.* 8, 2058-2071 (1994).
2. Leviten, M. W. & Posakony, J. W. *Dev. Biol.* **176**, 264-283 (1996).
3. Bailey, A. M. & Posakony, J. W. *Genes & Dev.* **9**, 2609-2622 (1995).
4. Leviten, M. W., Lai, E. C. & Posakony, J. W. *Development* **124**, 4039-4051 (1997).
5. Lai, E. C. & Posakony, J. W. *Development* **124**, 4847-4856 (1997).
6. Tietze, K., Oellers, N. & Knust, E. *Proc. Natl. Acad. Sci. USA* **89**, 6152-6156 (1992).

Parameters modulating Notch activity: view from the *Enhancer of split* locus.

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The activation of Notch has wide ranging effects on cell morphology, proliferation and gene expression. However few direct targets of Notch signalling have yet been identified. The best characterised are the basic-helix-loop-helix genes encoded by the *Enhancer of split* complex in *Drosophila*, which were first linked with Notch through early genetic studies. Subsequently it has been shown that the proteins encoded by the *E(spl)* genes are expressed in response to Notch activation, and this expression is regulated at the level of transcription. The DNA-binding protein encoded by *Suppressor of Hairless* is a key intermediary. Binding sites for this transcription factor have been identified in the promoters of all of the *E(spl)* genes, and in several cases it has been demonstrated that these sites are essential for their expression. Similar Notch dependent regulation has also been described for genes related to *E(spl)*, the so-called HES genes, in vertebrates. So far *E(spl)* gene expression has always been linked to events where Notch signalling is implicated, making these genes currently the best indicators of Notch activity, and thus a means to assay factors modulating Notch activation.

In the mesothoracic imaginal disc, which gives rise to the wing and thorax of the adult *Drosophila*, *Notch* is required for many different processes. These include the development of the wing veins, the sensory organs and the organiser at the dorsal/ventral boundary. Expression of the *E(spl)* genes is associated with all these processes in a Notch dependent manner. However, the individual *E(spl)* genes are expressed in distinct but overlapping patterns, for example three of the seven genes are expressed at the dorsal/ventral boundary, and only one is associated with developing veins. These distinct patterns suggest that there is a synergy between Notch and other pathways in the activation of the *E(spl)* genes. In order to understand what makes a gene Notch responsive, and how the response to Notch is integrated with other signals, we have been dissecting the regulation of three *E(spl)* bHLH genes which are located at the proximal end of the *E(spl)* complex. Two of the genes, *E(spl)mγ* and *E(spl)mδ*, have very similar patterns of expression in the imaginal discs, whilst the third *E(spl)mβ* is quite distinct. We have identified small fragments from the individual genes which confer many aspects of normal regulation, although there is evidence both for repetition of regulatory elements and for sharing of enhancers between genes. The majority of fragments conferring expression contain binding sites for Su(H) as expected, however, the Su(H) binding sites alone do not appear to be sufficient to render a heterologous gene responsive to Notch in vivo. In addition, individual genes/fragments are only responsive to Notch in certain domains. The results indicate that a combination of position specific activators and repressors operate with Notch signalling to determine the places where the individual *E(spl)* genes are activated.

In spite of the diverse transcription patterns of the *E(spl)* genes there are two unifying features linking expression of all the genes. First, during processes where Notch acts to restrict the number of cells which adopt a particular fate, such as vein and sensory organ development, *E(spl)* expression is excluded from the cells which become the specified precursors. Thus during vein development, the vein precursors form as stripes of cells which lack *E(spl)*mβ expression. Second, expression of *E(spl)* is not restricted to the cells immediately adjacent to the precursors, but rather is detected in a broad field of neighbouring cells. These factors lead us to favour a hypothesis whereby a key step in the selection of the precursor cells is that Notch, and thus *E(spl)* expression, cannot be activated in these cells and we have been investigating how this could be regulated. One mechanism that is important in restricting Notch activation in the wing vein precursors and in cells flanking the dorsal/ventral boundary is the levels of the ligands Delta and Serrate. High concentrations of either ligand appear to make cells unable to respond to Notch, and we propose that they have a dominant negative effect on the Notch protein. The most abundant Delta expression is detected within the vein precursor cells, supporting this model. However, no such clear variations in Delta or Serrate expression are associated with the precursors of the sensory organs, suggesting that there are additional mechanisms that restrict Notch activation. A possible candidate is the Wingless signalling pathway, which is necessary for the development of some sensory organs and which has been reported to antagonise Notch through the action of Dishevelled. We therefore tested whether miss-expression of Dishevelled was able to perturb the activity of Notch as detected by *E(spl)* expression. No inhibition of *E(spl)* was detected in these assays. Furthermore, similar adult phenotypes are produced by miss-expression of an activated Armadillo molecule, which acts after Dishevelled in Wingless signalling and thus bypasses any effects of Dishevelled on Notch. Therefore the inactivity of Notch in sensory organ precursors cannot be explained by interactions between Dishevelled and Notch, suggesting that there are other mechanisms involved.

Post-Translational Regulation of bHLH Activator and Repressor Proteins by the Notch and NGF Signaling Pathways: Michael Caudy, Paul Castella, Anders Strom, Al Fisher and Keiko Nakao; Department of Cell Biology; Cornell Medical College; New York, N. Y., 10021.

Genetic analysis in Drosophila has revealed that a family of bHLH activator and repressor genes control the switch between neuronal and non-neuronal cell fates during fly neurogenesis. These genes are closely associated with the Notch signaling pathway: The Enhancer of split E(spl) bHLH repressor genes are the direct nuclear targets and effectors for the Notch pathway, and the proneural bHLH activator genes are targets for repression by the E(spl) repressor proteins. We have found that both bHLH activator and bHLH repressor proteins are post-translationally regulated by cell signaling pathways. In Drosophila, the proneural protein Achaete is posttranslationally inhibited by the Notch lateral inhibition pathway, in addition to its previously shown transcriptional repression by the E(spl) effector proteins of the Notch pathway. In the rat PC12 cell line we have found that the mammalian Hairy and Enhancer of split (HES) homologue, HES-1 is posttranslationally inhibited by the NGF signaling pathway.

Post-translational inhibition of proneural proteins by the Notch signaling pathway in Drosophila: The proneural protein Achaete is subject to multiple aspects of posttranslational inhibition by the Notch signaling pathway which independently affect the N-terminal and C-terminal domains of Achaete. Deletions of the N-terminal domain result in increased transcriptional activation activity of Achaete protein expressed in cultured cells and also in increased proneural activity of protein expressed in flies. For example, N-terminal deletions of Achaete give rise to tufts of adjacent bristles which are very similar to the tufts observed in clones of neurogenic mutant cells in which the Notch lateral inhibition pathway has been disrupted. Moreover, mutation of a single serine residue in that domain also gives a clear although weaker neurogenic phenotype. Thus, it appears that the N-terminal domain may be a target for posttranslational inhibition by the Notch signaling pathway. The C-terminal domain of Achaete functions as a strong transcription activation (TA) domain when fused to a heterologous GAL4 DNA-binding domain and expressed in cultured cells. However, this TA activity is strongly inhibited by co-expression of a constitutively active Notch protein. Thus, both the N-terminal and C-terminal domains of Achaete appear to be targets for posttranslational inhibition by the Notch signaling pathway. These observations are important because they may explain the previous observation that ectopic expression of the various proneural bHLH activator proteins of the Achaete-Scute Complex under heat shock or GAL4/UAS promoter systems never give rise to adjacent bristles and apparently are subject to posttranslational inhibition by the Notch lateral inhibition pathway (1-4). These observations are described in more detail in the abstract for the poster by K. Nakao.

Post-translational inhibition of the mammalian HES-1 bHLH repressor protein by the NGF signaling pathway in PC12 cells: HES-1 is the mammalian homologue of the Drosophila Hairy protein, and both of these "Hairy-related" proteins are well-characterized repressors of neuronal differentiation in cultured cells and in embryos (see references listed in 5). The HES-1 and Hairy proteins have consensus protein kinase C (PKC) phosphorylation sites in their DNA-binding domains (5). HES-1 is present in uninduced PC12 cells where it inhibits differentiation in the absence of NGF. Expression of a dominant negative form of HES-1 which inactivates the endogenous HES-1 by forming non-DNA-binding heterodimers with it results in a partial induction of neurite outgrowth and differentiation in the absence of NGF (5). Moreover, during NGF signaling, the endogenous HES-1 DNA binding activity decreases although the protein level does not decrease, indicating that DNA-binding activity of HES-1 is posttranslationally inhibited during NGF signaling. PKC(s) are known to be activated during NGF signaling, and phosphorylation of bacterially-expressed and purified HES-1 (which is not phosphorylated) by PKC in vitro strongly inhibits HES-1 DNA-binding activity. By contrast, PKC does not inhibit a mutant HES-1 protein lacking the PKC phosphorylation sites, which acts

as a constitutively active protein in vitro. Expression of this mutant HES-1 in PC12 cells results in constitutive block of NGF signaling. Together these results suggest that post-translational inhibition of HES-1 partially mediates and is essential for the induction of neurite outgrowth by NGF signaling in PC12 cells (5).

This may be relevant to Notch signaling. Previous work by others suggests that HES-1 is transcriptionally upregulated by Notch signaling (6). As a result, it is interesting to note that NGF signaling may modulate Notch signaling during neuronal differentiation We currently are testing this hypothesis.

Given i) the established role of HLH genes in controlling cell fate decisions and ii) the above evidence that both bHLH activator proteins and bHLH repressor proteins are posttranslationally regulated by the Notch and NGF neuronal cell signaling pathways, our working hypothesis is that HLH transcription factors may be common targets for and mediators of many cell signaling pathways controlling neuronal cell determination and differentiation.

Literature cited:

1) Rodriguez, I., et al, (1990) EMBO J. 9(11): 3583-3592.
2) Brand, M., et al., (1991) Development 119(1): 1-17.
3) Hinz, U., et al., (1994) Cell 76(1): 77-87.
4) Giebel, B., et al, (1997) Mech Dev 63 (1): 75-87.
5) Strom, et al., (1997): Genes and Development: 11: 3168-3181.
6) Jarriault, et al., (1995) Nature 377: 355-358.

Session 4

Functions of Notch in the definition and patterning of epithelia

Chairperson: Sarah Bray

The neurogenic genes *egghead* and *brainiac* define a novel signaling pathway essential for epithelial morphogenesis during *Drosophila* oogenesis

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Notch (*N*) and other neurogenic genes have been implicated in two distinct processes, lateral specification of cell fates, and epithelial development. Previous studies have suggested that the neurogenic gene *brainiac*(*brn*) is specifically required for epithelial development (Goode et al., 1996). *brainiac* encodes a novel, putative secreted protein that cooperates with *grk* TGFalpha to produce the follicular epithelium. In addition, we have shown that *egghead* (*egh*), a gene with phenotypes identical to *brn*, encodes for a novel, putative secreted or transmembrane protein (Goode et al., 1997).

By comparing the function of germline *egh* and *brn* to *N* during oogenesis, we have obtained direct evidence for the involvement of follicle cell Notch in epithelial maintenance, and the specificity of *brn* and *egh* in epithelial development during oogenesis. The most striking phenotype observed for all three genes is a loss of apical-basal polarity and accumulation of follicular epithelial cells in multiple layers around the oocyte. The spatio-temporal onset of this phenotype correlates with the differential accumulation of *egh* transcripts in the oocyte at stage 4 of oogenesis. In contrast to *N*, we find that *brn* and *egh* are essential for the organization, but not specification, of stalk and polar cells.

The expression patterns and functional requirements of *brn*, *egh*, and *N* lead us to propose that these genes mediate follicular morphogenesis by regulating germline-follicle cell adhesion. This proposal offers explanations for (1) the involvement of *egh* and *brn* in *N*-mediated epithelial development, but not lateral specification, (2) why *brn* and *egh* embryonic neurogenic phenotypes are not as severe as *N* phenotypes, and (3) how *egh*

and *brn* influence Egfr-mediated processes. The correlation between the differential expression of *egh* in the oocyte and the differential requirement for *brn*, *egh*, and *N* in maintaining the follicular epithelium around the oocyte, suggests that Egghead is a critical component of a differential oocyte-follicle cell adhesive system.

Goode, S., Morgan, M., Liang, Y-P., and Mahowald, A. P. (1996). *brainiac* encodes a novel, putative secreted protein that cooperates with *grk* TGFa to produce the follicular epithelium. *Dev. Biol.*, 178, 35-50.

Goode, S., Melnick, M.B., Chou, T.-B. and Perrimon, N. (1996). The role of Egghead during morphogenesis of the follicular cell epithelium. Development 122, 3863-3879.

Notch in the segmentation of Xenopus embryos

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One important process underlying the development of the vertebrate embryo is the segmentation of the paraxial mesoderm into somites.. To study this process, we have characterized*X-Delta-2* which encodes the second *Xenopus* homolog of *Drosophila Delta*, and*ESR-5* which encodes an *Enhancer of split-related* (*Esr*)-like basic helix-loop-helix (bHLH) protein. These genes showed a segmental expression in the presomitic mesoderm, corresponding to prospective somites (somitomeres). To test whether these genes are involved in establishing a segmental pattern prior to somitogenesis, we mis-expressed dominant-negative and wild-type forms of these genes in *Xenopus* embryos. Altering the function of any of these genes invariantly alters the pattern of somites without affecting their differentiation into myotomal cells. In addition we have assayed potential regulatory interactions between the genes through misexpression studies. The results from these studies indicate that a nascent segmental pattern is established in the presomitic mesoderm through a cascade of molecular interactions involving the Notch signaling pathway..

Restriction of Notch activation in *Drosophila* imaginal discs

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The Notch protein functions as a receptor in a cell-cell signalling pathway essential for cell fate decisions in many different organisms. Other components of the pathway are the ligands Delta (Dl) and Serrate (Ser), the intracellular transducer Suppressor of Hairless (Su(H)) and the nuclear proteins encoded by the *Enhancer of split* complex (Artavanis-Tsakonas et al., 1995). During the development of the wing imaginal disc in *Drosophila*, *Notch* is required for the correct specification of several cell types, such as the sensory organs, wing veins and the wing margin. In these processes normal Notch function requires a precise regulation of the places where Notch is activated. We are interested in the mechanisms that regulate restricted Notch activation during imaginal development, and a convenient place where this can be analysed at the cellular level is the formation of the wing margin. In this process, Notch activation is restricted to the dorsal and ventral cells that form the dorso-ventral boundary. These cells belong to two different lineage compartments, and participate in the organisation of the wing margin. Notch activity is required in these cells to activate the expression of several genes, such as *vestigial* (*vg*), *wingless* (*wg*) and *cut* (*ct*), which play important roles in the development of the wing and the wing margin (Irvine and Vogt, 1997). The active state of Notch, as visualised by the expression of E(spl) proteins, persists in the dorso-ventral boundary for most of the third larval instar (48 hours), suggesting that some mechanisms must exist to ensure restricted Notch activation (de Celis et al., 1996).

The activation of Notch at the dorso-ventral boundary requires interactions with the ligands Dl and Ser. These ligands have some surface specificity with Ser activating Notch in ventral cells and Dl in the dorsal ones. These effects are determined by the activity of the secreted protein Fringe (frg),which is expressed only in dorsal cells. It has been postulated that frg has a dual role, potentiating the activating effect of Dl and preventing Ser to activate Notch in frg-expressing cells (Irvine and Vogt, 1997). Other mechanisms participate in ensuring the correct activation of Notch at the dorso-ventral boundary. These include positive feedback on Notch transcription and regulation of Ser and Dl expression by both Notch and Notch-downstream genes. In addition, it has also been proposed that Notch activity is modulated by

other signal transduction pathways, such as wingless, that are also operative at the dorso-ventral boundary (Couso and Martinez-Arias, 1994; Hing et al., 1994; Axelrod et al., 1996).

In experiments in which Dl and Ser are ectopically expressed it has been shown that they are able to suppress Notch activity at the dorso-ventral boundary. Notch suppression by Dl and Ser could constitute a mechanism by which the polarity of signalling is directed from cells expressing the ligands to cells in which their expression is reduced or absent. To analyse this aspect of Notch signalling, we have develop a experimental system in which positive and negative effects of Notch ligands can be studied at the cellular level. This system combines GAL4 controlled gene expression with FLP/FRT mediated recombination to generate clones of marked cells where specific proteins are miss-expressed (de Celis and Bray, 1997). Using this system we have analysed: 1)negative effects of Dl, frg and Ser on wild type and mutant Notch proteins and 2)the relationships between Notch and wingless signalling. We find that in *Abruptex* (*Ax*) mutations (amino acid substitutions in the Notch EGF repeats 17-19; Kelley et al., 1987) there is ectopic activation of Notch at the dorso-ventral boundary, particularly in the dorsal side. This suggests that *Ax* mutations identify a domain in the Notch protein required for the restriction of Notch activation. Ectopic expression of Dl suppress Notch activity within Dl-expressing cells, both in wild-type and in *Ax* mutant backgrounds. In contrast, ectopic expression of frg and Ser is not able to suppress Notch activity in *Ax* backgrounds. These results indicate that Ax proteins have lost the ability to be inactivated by frg and Ser. Thus the EGF repeats affected in *Ax* mutations appear to be involved in the negative interactions between Ser/frg and Notch. Using our miss-expression assay we have also studied if Notch mutations interfere with wingless signalling, and the effects of the over-expression of several components of the wingless pathway on Notch signalling.

Artavanis-Tsakonas, S., Matsuno, K. and Fortini, M. E. (1995). *Notch* Signalling. Science **268**, 225-232.
Axelrod, J. D., Matsuno, K., Artavanis-Tsakonas, S. and Perrimon, N. (1996). Interaction between wingless and Notch signaling pathways mediated by dishevelled. Science **271**, 1826-1832.
Couso, J. P. and Martinez-Arias, A. (1994). *Notch* is required for *wingless* signaling in the epidermis of *Drosophila*. Cell **79**, 259-272.
de Celis, J. F. and Bray, S. (1997). Feedback mechanisms affecting Notch activation at the dorso-ventral boundary in the *Drosophila* wing. Development **124**, 3241-3251.
de Celis, J. F., Garcia-Bellido, A. and Bray, S. (1996). Activation and function of *Notch* at the dorsoventral boundary in the *Drosophila* wing imaginal disc. Development **122**, 359-369.
Hing, H. K., Sun, X. and Artavanis-Tsakonas, S. (1994). Modulation of wingless signaling by *Notch* in *Drosophila*. Mech. Dev. **47**, 261-268.
Irvine, K.D. and Vogt, T.F. (1997). Dorso-0ventral signalling in limb development. Current Opinion Cell Biol. **9** , 867-876.
Kelley, M. R., Kidd, S., Deutsh, W. A. and Young, M. W. (1987). Mutations altering the structure of epidermal growth factor-like coding sequences at the Drosophila *Noth* locus. Cell **51**, 539-548.

Session 5

Serrate, Fringe and Notch signalling

Chairperson: Gerry Weinmaster

Fleming, Robert J.

Molecular complementation and the requirement of a C-terminal valine demonstrate cooperative interactions between *Serrate* molecules in *Notch* signaling. Hukriede, N.A. and Fleming, R.J. Department of Biology, University of Rochester, Rochester, NY 14627.

NOTCH activation is essential to the establishment of the Drosophila wing margin and is mediated by both of its transmembrane ligands *Serrate (Ser)* and *Delta (Dl)*. It has recently been demonstrated that loss of the intracellular domains of either SER or DL results in the inability of the ligand to activate NOTCH and, in fact, generates a dominant-negative interference of NOTCH signals. Since both SER and DL require intracellular sequences for proper function, we examined the intracellular domains of *Ser*-like and *Dl*-like molecules for conserved homologies and found that most identified *Notch* family ligands end in a C-terminal valine. C-terminal valine residues have been shown to be required for extracellular cleavage events of membrane-bound ligands such as TGF-α, MCSF-1, and the c-KIT ligand (1). In the absence of the intracellular C-terminal valine, these ligands are not cleaved into their active, soluble forms. We deleted the C-terminal valine of SER (called SERV-), which causes SER to terminate in a methionine, and tested the effects of this molecule during development of the wing imaginal disc under the control of the *patched (ptc)* promoter. Under these conditions, the SERV- construct behaves in a dominant-negative fashion, disrupting margin-specific gene expression and cell proliferation in the ventral wing compartment. These effects are indistinguishable from the effects of the SERTM molecule (2), which lacks the entire intracellular domain. Thus, the C-terminal valine is important for *Ser* signaling. To further test this hypothesis, we constructed a second SER construct that removed the terminal methionine residue of the SERV- construct (called SERMV-) which results in this form again terminating in a valine (the wild type SER C-terminus ends as Val-Met-Val). Consistent with similar effects seen for TGF-α, the SERMV- form restores function to apparently wild type levels. These results strongly suggest that the presence of a C-terminal valine is required for normal SER-NOTCH interactions.

In contrast to the dominant-negative effects of SERV- under *ptc* expression, when the SERV- form is expressed in a wild type pattern under the *Ser* promoter, it results in increased CUT margin expression comparable to wild type SER under the same conditions. This paradoxical finding suggested that there may be some interaction between endogenous SER and the SERV- form. By co-expressing SERV- and other signaling compromised forms of SER under the *ptc* promoter, we have been able to demonstrate intermolecular complementation suggestive of cooperativity amongst SER molecules. Taken together with the necessity of the C-terminal valine, these findings suggest that SER may function as a dimer or other cooperative form in some NOTCH-mediated signaling processes and that these signals may share elements with other membrane-bound receptor ligands such as TGF-α.

1. **Bosenberg, M.W., Pandiella, A. and Massagué, J.** (1992). The cytoplasmic carboxy-terminal amino acid specifies cleavage of membrane TGF∝ into soluble growth factor. *Cell* **71**, 1157-1165.

2. **Sun, X. and Artavanis-Tsakonas, S.** (1996). The intracellular deletions of DELTA and SERRATE define dominant negative forms of the Drosophila Notch ligands. *Development* **122**, 2465-2474.

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MOLECULAR AND DEVELOPMENTAL STUDIES OF FRINGE ACTIVITY IN *DROSOPHILA*

Genetic and molecular studies of the *Drosophila fringe* gene indicate that it mediates interactions between dorsal and ventral cells during wing development. These interactions induce both cell proliferation and the specification of specialized cells at the edge of the wing, the wing margin. Our recent results have indicated that fringe functions by modulating the activity of the Notch signaling pathway.

Notch is activated specifically along the boundary between dorsal and ventral cells in the *Drosophila* wing, and this activation is essential for wing formation. Both of the two Notch ligands, Serrate and Delta, are also essential for normal wing development, however their ability to activate Notch is spatially restricted: Ser can activate Notch in ventral cells but not dorsal cells, while Delta preferentially activates Notch in dorsal cells. By a combination of expression and co-expression studies in the *Drosophila* wing, we demonstrated that Fringe is responsible for the differential responsiveness of dorsal and ventral wing cells to Serrate and Delta. Fringe is expressed specifically by dorsal cells, and it both inhibits a cell's ability to respond to Ser and potentiates a cells ability to respond to Delta. Our studies also demonstrated that Fringe acts cell autonomously, that is, the effects of Fringe on Notch signaling are restricted to Fringe-expressing cells.

Our published observations established that Fringe modulates Notch signaling in the *Drosophila* wing, however Fringe also plays essential roles in the development of many other tissues. Comparative studies of Fringe function in different tissues are being pursued to determine whether the understanding we have developed of Fringe activity in the wing actually reflects general principles of Fringe-dependent cell signaling, and we are focussing on the eye and the leg as two model systems for comparative studies. We have also initiated studies to explore the effects of Fringe on lateral inhibition during neurogenesis. Our results thus far are consistent with the proposal that Fringe functions generally as a modulator of Notch signaling. We have also collaborated with Dr. Thomas Vogt's lab to identify and characterize mammalian *fringe* genes. The expression profiles of mouse *fringe*-related genes suggest that they modulate Notch signaling in the mouse. Moreover, we have assessed the activity of mammalian *fringe* genes by expressing them in the *Drosophila* wing, and our results indicate that they can modulate signaling through the *Drosophila* Notch receptor.

Fringe defines a new protein family. We have used epitope-tagging to monitor the cellular distribution and post-translational modification of fringe. Intriguingly, Fringe protein is secreted into the media, but some Fringe also remains associated with the plasma membrane. Based on the cell autonomous effects of Fringe on Notch signaling, we postulate that membrane-associated Fringe is functional, while diffusible Fringe may be the non-functional by-product of a downregulatory process. We are employing immunolocalization and site-specific mutagenesis studies to investigate the mechanism and regulation of the activity and distribution of Fringe protein.

The role of *Fringe* genes in developmental decisions and their relationship to the Notch pathway

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In a wide range of organisms and contexts the Notch signal transduction pathway provides critical input for the execution of developmental programs. In *Drosophila*, the *fringe* gene encodes a secreted protein that modulates the activation of the Notch signal transduction pathway at the dorsal-ventral boundary of the wing imaginal disc. To examine the conservation of fringe function we have cloned *fringe*-related genes from butterfly, frog, zebrafish, mouse and human. We have focused on the three mammalian *fringe*-related family members: *Manic*, *Radical* and *Lunatic Fringe*. To test conservation of fringe function we have introduced mammalian *Fringe* genes into *Drosophila* and demonstrated that they can modulate the Notch pathway.

An important component of our current investigations of Fringe function centers on genetic analyses. The evolution of a family of mammalian *Fringe* genes has resulted in related proteins with differences in primary protein structure, secretion, and patterns of expression. In collaboration with Ken Irvine's lab, we are continuing to investigate in transgenic *Drosophila* the ability of various mammalian *Fringe* gene constructs to modulate the Notch pathway. Consistent with observations and activities in *Drosophila*, vertebrate *Fringe* gene family members exhibit a striking coordinated expression with Notch and its ligands. These expression patterns suggest an important role for *Fringe* family members in segmentation of the developing embryo and in cell fate decisions. To begin to test this hypothesis we have determined the genomic structures of the three *Fringe* family members and have determined their map location in the zebrafish (with P. Haffter), mouse and human genomes. We are currently exploring the possible allelism of *Fringe* gene family members with classical mouse mutants. The human *RADICAL* gene maps within the critical region of a complex human genetic disease and its candidacy is being investigated. To directly address function we have created *Radical Fringe* and *Manic Fringe* loss-of-function mutations in the mouse by gene-targeting. Homozygous mutants are currently being analyzed for developmental defects. Our preliminary analysis of the *Radical* mutant mice provides additional support for Fringe modulation of the Notch pathway. Lastly, we are addressing the issues of functional overlap among *Fringe* family members and participation in the Notch pathway by the construction of double mutant strains. Results of these studies will be presented.

The role of the Notch-signalling system during wing development in Drosophila melanogaster.

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The Notch signalling system has been shown to be involved in several steps during wing development. We have investigated the relation of N to other genes required for wing development, like *wingless*, *vestigial* and *scalloped*. Our results suggest, that *Notch* collaborate with changing partners in order to induce its target genes.
We further present evidence for a Su(H) independent signal transduction mechanism of some aspects of N-signalling.

RELATIONSHIPS BETWEEN THE MOLECULAR CLOCK LINKED TO SEGMENTATION DEFINED BY THE C-HAIRY1 MRNA OSCILLATIONS AND THE NOTCH-DELTA PATHWAY

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In vertebrate embryos, the most obvious metameric structures are the somites. They constitute the basis of the segmental pattern of the body and give rise to the axial skeleton, the dermis of the back and all striated muscles of the adult body. In the chick embryo, a somite pair is laid down every 90 min in a rostro-caudal progression, and a total of 50 somite pairs are formed during embryogenesis. Experiments performed in the mouse and in the frog have established the important role played by the Notch-Delta pathway in this process. This situation is in contrast to that reported in the fly in which these genes are not implicated in the segmentation of the embryonic axis. Conversely, numerous vertebrate homologues of the *Drosophila* segmentation genes have been identified but are not expressed during somitogenesis. This, therefore, has supported the view that segmentation arose independently in vertebrates and invertebrates.

We have identified and characterised *c-hairy1*, an avian homologue of the *Drosophila* segmentation gene, *hairy*. In *Drosophila*, *hairy* is a member of the pair-rule genes which are the first to reveal the prospective metameric body-plan of the fly. They are expressed in a series of stripes with alternate-segment periodicity, and are used in combination to establish the future segmental periodicity of the embryo. *c-hairy1* is strongly expressed in the presomitic mesoderm where its mRNA exhibits a cyclic posterior-to-anterior wave of expression whose periodicity corresponds to the formation time of one somite (90 min). This wave is not due to massive cell displacement along the antero-posterior axis, but arises from pulses of *c-hairy1* expression that are coordinated in time and space. Analysis of in vitro cultures of isolated presomitic mesoderm demonstrates that rhythmic *c-hairy1* mRNA production and degradation is an autonomous property of the paraxial mesoderm and does not result from caudal-to-rostral propagation of an activating signal. Blocking protein synthesis does not alter the propagation of *c-hairy1* expression, indicating that negative autoregulation of *c-hairy1* expression is unlikely to control its periodic expression. These results provide the first molecular evidence of a developmental clock linked to segmentation and somitogenesis of the paraxial mesoderm, and support the possibility that segmentation mechanisms used by invertebrates and vertebrates have been conserved.

The link between this segmentation clock and the Notch-Delta pathway remained, however, elusive. Both *Notch* and *Delta* genes are expressed all along the presomitic mesoderm and do not appear to exhibit the dynamic behaviour of *c-hairy1* expression. However, we have recently observed that a component of the Notch-Delta pathway, *Lunatic Fringe* is expressed in a rythmic fashion similarly to *c-hairy1*. Comparison of the expression domains of both genes suggest that they are similar. Blocking protein synthesis disrupts the dynamic expression of Lunatic Fringe while it has no effect on *c-hairy1*, indicating a different regulation mode for the two genes. *Lunatic Fringe* is thus an interesting candidate to act downstream of *c-hairy1* and could correspond to an effector of the segmentation clock. It could thus participate in the translation of the temporal oscillations of the clock into the periodic pattern of the somites by periodically modulating the reception of the *Delta* signal in the presomitic mesoderm .

Session 6

Functional multiplicity

Chairperson: Judith Kimble

Presenilin Function and Colocalization with Notch in *Drosophila*

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Studies in C. *elegans* and mice have demonstrated that Notch/lin-12 synthesis or function is impaired by loss-of-function mutations in members of the Presenilin/Sel-12 protein family. To assess the functional involvement of Presenilin in *Drosophila* Notch signaling, we have undertaken a genetic and molecular characterization of the fly *presenilin* gene. As determined by PCR tests and low stringency cDNA library screens, a single-copy *presenilin* gene is present in *Drosophila*. The coding region occupies ~2.4 kb of genomic DNA and alternative splicing generates two mRNA species, which differ with respect to a 14 amino acid insertion in the large hydrophilic loop region of the protein.

Antibodies raised against peptide antigens from the N-terminus and the loop region of fly Presenilin have been used to investigate the expression and subcellular localization of the protein in various tissues throughout development. Presenilin is widely expressed in all tissues examined, including embryonic tissues, larval imaginal discs, and developing ovarioles. Double staining experiments using antibodies against Notch and Presenilin reveal extensive colocalization of the two proteins at the plasma membrane and in the cytoplasm of certain cell types. Both proteins are also detected in vesicular structures, although there is apparently little or no overlap between the Notch-positive and Presenilin-positive vesicles as examined by confocal microscopy.

We have performed a systematic mutagenesis of the cytological region 77A-C, which harbors the *presenilin* gene, in order to isolate mutations in the gene. Phenotypic rescue of complementation groups recovered in this screen by different segments of genomic DNA containing the *presenilin* transcription unit has allowed us to identify putative loss-of-function mutations in the gene. The results of genetic interaction tests between these mutations and mutations in known Notch pathway components, such as *Notch, Delta, Suppressor of Hairless, deltex,* and *mastermind,* will be presented. To complement these studies, we have also used the yeast two-hybrid screening method to isolate ~100 cDNA clones that encode proteins capable of binding to *Drosophila* Presenilin. So far, these clones fall into groups corresponding to three separate genes, whose relevance to Presenilin function and Notch signaling is under further investigation.

The interaction of signaling pathways mediated by *Notch-1* and *Wnt-3a* regulates *Fgf-8* expression in the apical ectodermal ridge of developing chick limbs

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The vertebrate limb serves as an excellent model system to study the molecules involved in development and how these interact to establish complex signaling pathways. Perhaps the most important step in limb development is the formation of an organizer centre (termed the apical ectodermal ridge, or AER) at the distal tip of the developing limb bud. Whilst tissue manipulation experiments show that the AER plays a key role in controlling and driving limb outgrowth, we have only recently begun to understand these functions at the molecular level. Remarkably most of the known functions of the AER can be reproduced by members of the fibroblast growth factor family. For instance, *Fgf-8*, a gene which is normally expressed in the AER, is able to induce a complete additional limb if applied to the flank of the embryo and furthermore, is also able to maintain limb outgrowth following surgical removal of the AER. This suggests that establishing the correct temporal and spatial expression of *Fgf-8* is a determining step in normal limb development and that understanding how *Fgf-8* expression is induced is essential if we are to establish how limb development is realised.

We have recently shown that the AER is induced and positioned through the interaction of dorsal cells expressing *Radical fringe* and ventral cells that express *engrailed* but not *R-fng*. To further investigate the molecular pathways leading to *Fgf-8* expression, we examined other genes known to be expressed in the AER to determine whether they might be involved in the induction of *Fgf-8*. Transcripts of *Wnt-3a* and *Notch-1* appear prior to *Fgf-8* expression and AER formation. As limb outgrowth proceeds, transcripts for these genes become restricted to the newly formed AER. These results suggest a potential interaction between *R-fng*, *Notch-1* and *Wnt-3a* and that this may be important for regulating *Fgf-8* expression.

In our discussion we will focus on the relationship between the *Wnt-3a* and *Notch-1* signalling pathways during vertebrate AER formation. Our data indicate that the interaction between these two pathways is important during AER formation to refine the initially broad and diffuse expression of *Fgf-8*. Whilst the exact molecular mechanisms by which this is achieved have yet to be established, it is clear that the appropriate restriction of *Fgf-8* is essential during vertebrate embryogenesis. Indeed, perturbations in the expression of this highly potent growth factor, leads to a range of severe phenotypes throughout the embryo. It is likely that similar patterns of reciprocal regulation between distinct signalling pathways will be seen during the formation of other organiser centres elsewhere in the embryo.

-Rodriguez-Esteban, C., Schwabe, J.W.R., De La Peña, J., Foys, B., Eshelman, B. and Izpisúa Belmonte, J. C. (1997). *Radical fringe* positions the apical ectodermal ridge at the dorsoventral boundary of the vertebrate limb. **Nature 386:360-366.**

-Kengaku, M., Capdevila, J., Rodriguez-Esteban, C., De La Peña, J., Johnson, R.L., Izpisúa Belmonte, J.C. and Tabin, C.J. (1997). WNT3a regulates AER formation and utilizes an intracellular signaling pathway distinct from the dorsoventral signal WNT7a during chick limb morphogenesis. **Science, in press.**

-Rodriguez-Esteban, C., Schwabe, J.W.R., Kengaku, M., De La Peña, J., Wettstein, D., Kintner, C., Tabin, C. and Izpisúa Belmonte, J.C. (1997). The interaction of signaling pathways mediated by *Notch-1* and *Wnt-3a* regulates *Fgf-8* expression in the apical ectodermal ridge of developing chick limbs. Submitted.

Physical interactions between Notch, Delta and Wingless in embryos and cultured cells. Cedric S. Wesley and Michael W. Young. Laboratory of Genetics, The Rockefeller University, 1230 York Avenue, New York, NY 10021 USA.

In vitro analyses of deletions affecting different segments of the extracellular domain of N have shown that Dl, and Serrate (Ser) (the only other identified ligand of N), bind N in the region of EGF-like repeats 11 and 12 (1). A single amino acid substitution in this region can produce an embryonic lethal phenotype (2). However, these two repeats are not sufficient for wild type N function: Loss of the remaining extracellular sequence blocks formation of embryonic cuticle (10), and single amino acid substitutions affecting the 2nd (*nd*^{3}), 14th (*spl*), 24th (*Ax*^{9}, *Ax*^{59b}, *Ax*^{59d}), 25th (*Ax*^{1}), 27th (*Ax*^{71d}), 29th (*Ax*^{16}, *Ax*^{E2}), or 32nd (*N*^{ts1}) EGF-like repeats, or the lin12/Notch repeats (*l(1)N*^{B}) produce lethality or aberrant *Notch* function (3, 4). As most of the latter mutations alter the structure of N EGF-like repeats similar to those forming the Dl/Ser binding site, we explored the possibility that these extracellular regions mediate interactions with alternative ligands.

The initial methodology employed was that of biopanning (5). A library of *D. melanogaster* embryonic cDNA was established in a filamentous phage producing vector. *Drosophila* proteins were expressed as phage coat protein fusions. Bacteriophages were screened for selective binding to N receptors displayed by live cultured insect cells (*Drosophila* S2 cells). We reasoned that presentation of N on S2 cells might promote physiological conformation of the cysteine rich receptor, and that such a screen might simulate the native environment for cell surface ligand-receptor interaction.

The biopanning screen enriched for phagemids expressing *Delta*, *Serrate*, *wingless*, *big brain*, *pecanex*, *fringe*, *Stubble*, and *Notch* itself. The strongest selection was for the maternal-effect neurogenic gene *pecanex* (6). *pecanex* -expressing phagemids constituted 25% of the enriched library; representation of these phagemids was increased almost 100,000 fold. *wingless* phagemids and phagemids expressing *Delta*, an established N ligand, were recovered with similar frequencies, but much less often than *pecanex*. The recovery of *wingless* was of special interest because earlier work indicated genetic interactions between *Notch* and *wingless* (7), and prior molecular studies showed a physical interaction between N and Dishevelled (Dsh), a protein that influences *wingless* signaling (8).

Immunoprecipitations revealed that the Wingless protein (Wg), Dl, and a truncated form of the N receptor can be isolated as a multimeric complex during embryogenesis. Wg also bound to S2 cells that expressed either a truncated N receptor, resembling that produced *in vivo* (9), or Dl. Although the truncated form of N is deficient for the Dl binding domain, Wg induced adhesion of these cells to Dl-expressing S2 cells.

1. I. Rebay, R. J. Fleming, R. G. Fehon, L. Cherbas, P. Cherbas, S. Artavanis-Tsakonas, *Cell* **67**, 687 (1991).
2. J. F. de Celis, R. Barrio, A. del Arco, A. Garcia-Bellido, *Proc. Natl. Acad. Sci. USA* **90**, 4037 (1993).
3. M. R. Kelley, S. Kidd, W. A. Deutsch, M. W. Young, *Cell* **51**, 539 (1987).
4. D. Lyman, M. W. Young, *Proc. Natl. Acad. Sci. USA* **90**, 10395 (1993); T. Xu, L. A. Caron, R. G. Fehon, S. Artavanis-Tsakonas, *Development* **115**, 913 (1992).
5. J. K. Scott and G. P. Smith, *Science* **249**, 386 (1990); J. D. Marks, W. H., Ouwehand, J. M. Bye, R. Finnern, B. D. Gorick, D. Voak, S. J. Thorpe, N. C. Hughes-Jones, G. Winter, *Bio/Technology* **11**, 1145 (1993).
6. S. G. LaBonne, I. Sunitha, A. P. Mahowald, *Dev. Biol.* **136**, 1 (1989).
7. J. P. Couso, and A. Martinez-Arias, *Cell* **79**, 259 (1994). K. Brennan, R.Tateson, K. Lewis, and A. Martinez-Arias, *Genetics* **147**, 177 (1997).
8. J. D. Axelrod, K. Matsuno, S. Artavanis-Tsakonas, N. Perrimon, *Science* **271**, 1826 (1996).
9. C. S. Wesley, M. W. Young, (in preparation).
10. T. Lieber, S. Kidd, E. Alcamo, V. Corbin, M. W. Young, *Genes Dev.* **7**, 1949 (1993).

Interactions between Notch and Wingless involve a Su(H) independent Notch signalling event in Drosophila

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Genetic analysis indicates that, in addition to its well known role in the process of cell fate suppression known as "lateral inhibition", Notch mediates other functions during the patterning of tissues in both vertebrate and invertebrates (1). This type of analysis in *Drosophila* has led to the suggestion that during the assignation of cell fates, Notch plays more than one role and that each of these different functions might implemented by different signalling pathways.

One of these is revealed through interactions with *wingless*, a segment polarity gene which mediates cell interactions (2). Mutations in *Notch* dramatically enhance losses of *wingless* function during the development of wings and legs, and specific mutations can be found in *Notch* which mimic and interact with *wingless* mutations during the establishment of neural precursors (3). Furthermore, loss of Notch function during embryogenesis results in segment polarity mutant phenotypes similar to those caused by mutations in wingless (2).

In the course of a structure-function analysis of the Notch molecule, we have identified a function of Notch during the establishment of neural precursors which is: 1) independent of Suppressor of Hairless; 2) mediated by different parts of the molecule than those required for lateral inhibition; and 3) modulated by the activity of the Sgg/Zw3 kinase. This function can be modulated by Wingless protein. In this context, it might be important our observation that the activity of Notch affects the amount of Armadillo, a *Drosophila* homologue of Beta-catenin, which plays a key role in wingless signalling (4, 5). A detailed analysis of this function of Notch has led us to suggest that, in *Drosophila*, Notch acts as a receptor for Wingless and participates in the wingless signalling event. Specifically, our results suggest that wingless signalling is a two step mechanism: 1) Wingless suppresses a repression of cell fate established by Notch in a Su(H) independent manner and 2) Wingless drives Notch into a receptor complex in which it implements a positive signalling event.

A range of interactions between Wingless and Notch support this hypothesis. We have observed that Wingless binds to full length Notch in S2 cells in a manner comparable to the way it binds to members of the Frizzled family of receptors. This interaction appears to require specific regions of Notch which are different from those involved in its interaction with Delta and assays of interactions between various Notch and Wingless molecules in the developing wing support the direct and functional nature of these interactions. In addition, our studies have uncovered a function for Notch in tissue polarity in which it interacts with Frizzled and Dishevelled. These interactions are consistent with a function for Notch in wingless signalling.

1. Artavanis Tsakonas, S., Matsuno, K. and Fortini, M.(1995). Notch signalling. Science 268, 225-232.

2. Couso, J.P. and Martinez Arias, A. (1994). *Notch* is required for *wingless* signalling in the epidermis of *Drosophila* . Cell. 79, 259-272

3. Brennan, K., Tateson, R., Lewis, K. and Martinez Arias, A. (1997). A functional analysis of *Notch* mutations in *Drosophila*. Genetics. 147, 177-188

4. Brennan, K., Tateson, R., Zecchini, V and Martinez Arias, A. (1997) A Suppressor of Hairless independent function of Notch that regulates Shaggy/Zeste White 3 activity in *Drosophila* Submitted

5. Brennan, K., Rolfe, S., Zecchini, V., Klein, T., Wilder, E. and Martinez Arias, A. (1997) Direct interactions between Wingless and Notch in *Drosophila* Submitted

POSTERS

Different response of Notch1 and Notch2 molecules to cytokine induced differentiation through the NCR domain.

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Mammalian Notch family members are highly conserved molecules containing the previously described Notch domains. Notch functions to inhibit differentiation in a wide variety of tissues and organisms. However the individual role of different Notch molecules (Notch1-4) co-expressed in the same tissue remains unclear.
The process of hematopoiesis involves continuous cell-fate decisions, permitting both lineage commitment and progressive maturation of blood cells. Different Notch molecules are expressed in hematopoietic progenitor cells and there is now functional evidence that Notch1 plays a role in myeloid and lymphoid differentiation.
We have studied the effects of activated Notch1 and Notch2 molecules on differentiation of the myeloid progenitor cell line, 32D. We have found that expression of an activated Notch1-IC molecule specifically inhibits differentiation in response to G-CSF. In contrast, 32D cells expressing activated Notch2-IC differentiated in the presence of G-CSF but remain undifferentiated when stimulated with GM-CSF. Cells expressing hybrid Notch1/Notch2 molecules and deletion mutants indicate that the previously undefined NCR (Notch Cytokine Response) domain confers the specificity of this cytokine response. Hybrid molecules containing the Notch1 NCR region (N2-cdc/N1-NCR) functions as the native Notch1 molecule, whereas Notch2 NCR hybrid molecules (N1-cdc/N2-NCR) function as the native Notch2 molecule regardless of the cdc10/ankyrin repeats they contain.
In addition, deletions in the NCR region of Notch1 abolish the inhibitory effect of Notch1 on G-CSF induced differentiation, whereas the equivalent Notch2 NCR deletions eliminate Notch2 specificity, resulting in an inhibitory function in G-CSF. Studies to further characterize the NCR domain are in progress.

A SUPPRESSOR OF HAIRLESS INDEPENDENT FUNCTION OF NOTCH DURING NEUROGENESIS THAT REGULATES SHAGGY ACTIVITY IN DROSOPHILA.

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The *Notch* gene of *Drosophila* encodes a large, single-span transmembrane protein that is required for many cell fate decisions in development. Its best understood role is in a process known as "lateral inhibition". This process selects a single cell to form a sense organ, from groups of cells, known as proneural clusters, that are all capable of forming sense organs. The cell that will form the sense organ prevents its neighbours from doing likewise by emitting an inhibitory signal. The receptor for this signal is encoded by the *Notch* gene and the signal is transduced to the nucleus of the receiving cells by the Suppressor of Hairless protein. The loss of function of either these genes results in the production of supernumerary sense organs.

To determine the regions of the protein that are required for this function we have carried out a detailed genetic analysis of a range of *Notch* alleles which have been physically mapped. Complementary to this analysis we have also examined the phenotypes generated by the over expression of deliberately deleted Notch proteins. In addition to identifying the regions of the Notch protein required for lateral inhibition, these analyses have highlighted a previously uncharacterised function of Notch. It appears that this function is required for the definition of the proneural clusters.

Further analysis of this function has indicated that it does not require the function of the Suppressor of Hairless protein and that it does require the function of the Shaggy protein. This suggests that the Notch protein is involved in signal transduction mechanism that is separate and distinct from lateral inhibition.

As the Shaggy kinase is a component of the Wingless signalling cascade, these results suggest that this previously uncharacterised function of Notch is involved in Wingless signalling. In keeping with this hypothesis we have found that the Wingless protein can interact with Notch both *in vitro* and *in vivo*. In addition this interaction requires the regions within the extracellular domain of the Notch protein that were identified by the genetic analysis as being important for this previously uncharacterised function of Notch. Consequently we believe that the Notch protein is involved in the transduction of the Wingless signal into the cell.

PRONEURAL GENE SELF-STIMULATION IN NEURAL PRECURSORS IS ESSENTIAL FOR SENSE ORGAN DEVELOPMENT AND IS REGULATED BY NOTCH SIGNALING.

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To learn on the acquisition of neural fate by ectodermal cells, we have analyzed a very early sign of neural commitment in *Drosophila*, namely, the specific accumulation of *achaete-scute* complex (AS-C) proneural proteins in the cell that becomes a sensory organ mother cell (SMC). We have characterized an AS-C enhancer that directs expression specifically in SMCs. This enhancer promotes Scute protein accumulation in these cells, an event essential for sensory organ development in the absence of other AS-C genes. Interspecific sequence comparisons and site-directed mutagenesis show the presence of several conserved motifs necessary for enhancer action, some of them binding sites for proneural proteins. These and other data indicate that the enhancer mediates *scute* self-stimulation, although only in the presence of additional activating factor(s) that most likely interact with conserved motifs reminiscent of NF-κB binding sites. Cells neighbouring the SMC do not acquire the neural fate because the *Notch* signaling pathway effectors, the *Enhancer of split* bHLH proteins, block this proneural gene self-stimulatory loop, possibly by antagonizing the action on the enhancer of the NF-κB-like factor(s) and/or the proneural proteins. These data suggest a mechanism for SMC commitment.

Differential activity of members of the E(spl) family of transcriptional regulators

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A common consequence of Notch signaling in *Drosophila* is the transcriptional activation of the *E(spl)* genes, which encode a family of seven closely related bHLH transcriptional repressors. Different E(spl) proteins can functionally substitute for each other, raising the question of whether any specialization exists within the family. We have expressed each individual E(spl) gene using the GAL4-UAS system in order to analyse their effect in a number of cell fate decisions taking place in the wing imaginal disk. We have focused on sensory organ precursor determination, wing vein determination and wing margin specification. All of the E(spl) proteins affect the first two processes in the same way, namely they antagonize neural precursor and vein fates. Yet, the efficacy of this antagonism is quite distinct. For example, mβ has the strongest vein suppression effect, whereas m8 and m7 are the most active bristle suppressors. Reduction in Notch signaling leads to loss of wing margin and surrounding wing blade tissue (notching). While overexpression of E(spl) mγ and mδ suppresses this phenotype, other E(spl) proteins have no effect, and m7 and m8 enhance wing notching. Selective activity of E(spl) proteins probably reflects their preference for distinct target gene sites or target proteins.

Requirement for dynamin during Notch signalling in Drosophila neurogenesis

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Singling out of a unique neural precursor from a group of equivalent cells, during Drosophila neurogenesis, involves Notch-mediated lateral signalling. During this process, activation of the Notch signalling pathway leads to repression of neural development. Disruption of this signalling pathway results in the development of an excess of neural cells. The loss of activity of dynamin, which is encoded by the gene *shibire* and leads to inhibition of endocytosis, results in a similar phenotype. We have investigated the requirement of *shibire* function for Notch signalling during the segregation of sensory bristles on the notum of the fly. Overexpression of different constitutively active forms of Notch in *shibire* mutant flies indicates that *shibire* function is not necessary for transduction of the signal downstream of Notch, even when the receptor is integrated in the plasma membrane. However, when wild-type Notch is activated by its ligand Delta, dynamin is required in both signalling and receiving cells for normal singling out of precursors. This suggests an active role of the signalling cell for ligand-mediated receptor endocytosis in the case of transmembrane ligands. We discuss the possible implications of these results for normal functioning of Notch-mediated lateral signalling.

Notch Is Expressed in Adult Brain, is Co-Expressed with Presenilin-1, and is Altered in Alzheimer Disease

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In C. Elegans, the Notch family member lin-12 has a genetic interaction with sel-12, a homologue of the Alzheimer related Presenilin genes in humans. We reasoned that, if Notch has a role in presenilin function in Alzheimer disease, it would have to be expressed in adult brain and colocalized with presenilins. Using double immunofluorescence and confocal microscopy, in situ hybridization, RT-PCR, and Western blotting techniques we have demonstrated the continued expression of Notch1, Notch2, and Jagged in the adult human and rodent brain. Notch immunoreactivity co-localizes in neurons that contain presenilin 1 immunoreactivity. Moreover, Notch1 levels appear to be increased about 2 fold ($p=0.007$) in the Alzheimer hippocampus, as assessed by both immunohistochemical and Western blot analysis. These results support the conclusions that Notch maintains a role in differentiated neuronal populations, and are consistent with the possibility that Notch/presenilin interactions are relevant in the development of Alzheimer disease.

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Identification of genes repressed in response to Notch signalling

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Key targets of activated Notch in *Drosophila* are the genes of the *Enhancer of split Complex* [*E(spl)*-C] which are transcribed in response to Notch signalling. Seven genes within the E(spl)-C encode basic-helix-loop-helix proteins that repress transcription in conjunction with the Groucho co-repressor protein to promote some cell fates adopted by cells containing high Notch activity. The E(spl) proteins are involved with many different cell fate decisions mediated by Notch signalling during development raising the question; which types of genes need to be downregulated in response to Notch signalling to facilitate cell fate decisions?

To determine how the *E(spl)* proteins influence cell fates we have begun by investigating their DNA-binding characteristics and effects on transcription, using complementary *in vitro* and *in vivo* assays. Our results identify the optimal binding site for these proteins *in vitro* as a palindromic 12 base pair sequence that we have called the ESE-box which contains a specific version of the E-box (CACGTG) and differs from the previously described binding site for the E(spl) proteins known as the N-box (CACNAG). The optimal *in vitro* binding site is a target for the E(spl)bHLH proteins *in vivo* where we show it confers repression on a heterologous promoter, confirming that these proteins function as transcriptional repressors in the developing organism. Simple changes to this site lead to dramatic changes in the profile of transcription factors regulating reporter gene expression *in vivo* indicating that target recognition by bHLH transcription factors is indeed very complex. The results from these experiments also raise the possibility that proneural proteins and E(spl) proteins could compete for binding sites under certain circumstances. p We are currently screening the *Drosophila* genome for genes repressed by E(spl) proteins and anticipate that a knowledge of the character of these genes will lead to a greater understanding of the events that occur in cells to mediate cell fate decisions in response to Notch signalling.

Notch3 mutations cause CADASIL, an hereditary adult onset arteriopathy responsible for stroke and dementia

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Notch signalling pathway has been previously involved in various developmental contexts and in cancer diseases. Last year, we established that *Notch3* mutations cause CADASIL, a recently identified autosomal dominant adult onset arteriopathy responsible for stroke and dementia in humans. Key features of CADASIL include recurrent subcortical ischaemic events, migraine attacks and vascular dementia, in association with diffuse white matter abnormalities on neuroimaging. CADASIL is underlaid by a non atherosclerotic non amyloid angiopathy involving mainly the media of small cerebral arteries. Histopathological analysis shows a prominent thickening of the arterial media and major lesions of vascular smooth muscle cells which eventually disappear. Ultrastructural examination of the arteries shows abnormal patches of granular osmiophilic material within the vascular smooth muscle cell basal membranes. CADASIL patients carry strongly stereotyped mis-sense mutations, located within the EGF-like repeats, in the extracellular domain of Notch3, leading to either a loss or gain of a cysteine residue and therefore to an unpaired number of cysteine residues within a given EGF domain. In addition mutations are strongly clustered within 2 exons encoding for the first five EGF-like repeats. CADASIL mutations may result in an abnormal folding or dimerisation of the Notch3 receptor or an impairement of interaction with its ligand or aberrant interaction with another protein.

These findings point out the unsuspected role of the Notch3 signalling pathway in the blood vessel biology in adult tissues. Interestingly mutations in one Notch ligand, Jagged1, are responsible for Alagille syndrome, which includes in some patients severe arterial lesions, in addition to various developmental defects. We are currently focusing on the dissection of the Notch3 signalling pathway and on the understanding of its function in the blood vessel physiology. Preliminary results of Notch3 expression analysis using in situ hybridization reveal that Notch3 is strongly expressed in vessels. We are currently investigating if Notch3 is expressed in smooth muscle cells and/or in endothelial cells, and which genes of the Delta/Serrate ligands family is coexpressed within the arterial wall and may interact with Notch3.

Notch/RBP-J signaling prevents C2C12 cells myogenesis and activates transcription of a gene that inhibits expression of MyoD

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When C2C12 myoblast cells are transferred into medium containing low serum, they are aligned with each other and fused to myotubes. Stimulation by Notch ligand or expression of the Notch intracellular region prevents this myogenic differentiation. The intracellular region of Notch directly binds to the DNA-binding protein, RBP-J and activates transcription of genes carrying the recognition motifs of RBP-J in their promoter regions. We have demonstrated that transcriptional activation of RBP-J by the Notch intracellular region is responsible for suppression of myogenesis. Here we report a useful system in which both inhibition of myogenesis and transcriptional activation through the RBP-J recognition motifs are observed by Notch-ligand interaction. We have established stable cell lines expressing mouse Delta1, one of Notch ligands (D10 cells). C2C12 cells co-cultured with D10 cells does not differentiate in medium containing low serum. To determine whether this suppression is caused by the same mechanism as the Notch intracellular region, the transcriptional activities through the RBP-J recognition motifs were measured. The RBP-J-dependent transcriptional activity in C2C12 cells was augmented by the contact with D10 cells but not with parent cells, suggesting that ligand-induced Notch signal which leads to myogenic suppression is also mediated by RBP-J.

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ACTIVATION OF NOTCH1 BY ITS LIGAND, JAGGED1, INHIBITS GRANULOCYTIC DIFFERENTIATION AND PERMITS EXPANSION OF IMMATURE MYELOID PROGENITORS.

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Interactions between hematopoietic and stromal cells in the hematopoietic microenvironment contribute to the regulation of proliferation and differentiation of hematopoietic progenitors during hematopoiesis. Here we provide evidence that signaling between the Notch ligand, Jagged1, on stromal cells and Notch1 on hematopoietic cells can influence hematopoietic differentiation and the maintenance of immature progenitors. We have previously shown that expression of an activated intracellular form of Notch1 in 32D myeloid progenitors inhibits G-CSF-induced granulocytic differentiation, but permits expansion of undifferentiated cells. In the current studies we show that activation of a full-length form of Notch1 (FLN1) by the Notch ligand, Jagged1, results in the same phenotypic effects as expression of the activated intracellular form of Notch1. 32D cells expressing FLN1 differentiated in response to G-CSF comparably to parental 32D cells and control retroviral-expressing clones; after 6-7 days of culture 40-50% of the cells were mature ganulocytes. However, when cultured in the presence of G-CSF and various forms of Jagged1, FLN1-expressing 32D clones showed a marked decrease in granulocytic differentiation and increase in proliferation of undifferentiated progenitors compared to control clones. When cultured with G-CSF and the stromal cell line, HS-27a, which endogenously expresses Jagged1, 40-60% of the FLN1-expressing cells remained undifferentiated and less than 20% were mature granulocytes after 5-6 days of culture. In contrast, 70-80% of control cells were mature granulocytes and less than 5% remained undifferentiated under these conditions. The total number of undifferentiated cells and proliferative potential were also significantly greater for the FLN1 clones compared to control clones: after 6 days, cultures of FLN1 cells had maintained undifferentiated cells corresponding to 90% of the original number of cells plated (compared to 5% for control clones) and replating efficiency of single cells was >250% (compared to 9%). Differentiation patterns of FLN1 and control clones in the presence of G-CSF and stromal lines not expressing Jagged1 were comparable to differentiation in G-CSF alone. We also tested the effects of Jagged1 expressed as a soluble extracellular protein by COS cells and as a small peptide corresponding to the unique DSL (Delta/Serrate/Lag) domain of Notch ligands. Addition of these forms of Jagged1 to G-CSF-stimulated cultures produced the same functional effects as culture with HS-27a, which expresses Jagged1 as a membrane-bound protein. Addition of control proteins (COS supernatants) and peptides did not produce these effects. These results, considered together with the known expression of Jagged1 in a subset of normal bone marrow stromal cells and Notch1 in normal hematopoietic progenitors as well as the high evolutionary conservation of Notch/Notch ligand structure and function, leads us to speculate that signaling through the Notch pathway may play an important role in mediating cell-fate determination and self-renewal of multipotent progenitors during hematopoiesis.

Notch signal transduction: Extracellular regulation of an activating intracellular proteolytic processing event.

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We are investigating the nature of the inhibitory role played by the extracellular domain of mouse Notch 1. It has been established that the intracellular domain of Notch molecules can mimic gain of function allele activities. Deletion construct analyses have also shown that in the absence of ligand binding, discrete extracellular domains inhibit Notch signaling transduced by the intracellular domain (Rebay et al., *Cell* 74: 319-329, 1993; Lieber at al., *Genes & Dev.* 7:1949-1965, 1993). However, how ligand activation of the full length receptor alleviates inhibition by the extracellular domain thus allowing the Notch signal to be propogated to the nucleus remains unresolved. We are studying what role the extracellular domain might play in regulating an intracellular proteolytic processing event, initially typified for an extracellularly truncated form of Notch: ΔE (Kopan et al., *PNAS* 93: 1683-1687, 1996). Cleavage at this site results in the release of the intracellular fragment (NICD, Notch intracellular domain) from its transmembrane tether. We now have evidence that ligand activation of the full length receptor results in the production of a NICD-like intracellular fragment. The NICD fragment translocates to the nucleus where it associates with $RBP_{J\kappa}$ (a mammalian Su(H) homolog) and activates members of the Hes family of transcriptional repressor genes (Jarriault et al, *Nature* 377:355-358, 1995). To test the inhibitory mechanism utilized by the extracellular domain to regulate this process we have generated chimeric receptors between Notch and other cell surface receptors which allow us to manipulate the oligomerization state of these molecules. Our data suggests a model whereby changes in the oligomerization state of an extracellular domain results in inhibition or promotion of NICD-type cleavage. Changes in the oligomerization state of these chimeric receptors which promote processing are shown to positively correlate with the ability to activate a Hes-1 promoter reporter construct. In addition, we have begun to look at other changes in the topology of the Notch receptor in order to identify the precise physical mechanism responsible for regulating NICD production..

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INHIBITION OF CORTICAL NEUROGENESIS BUT NOT GLIOGENESIS BY ACTIVATED NOTCH1 IN VIVO Nicholas Gaiano[2], Ying Peng[1], Daniel Turnbull,[3] Gord Fishell,[2] and Jeffrey S. Nye[1]*. [1]Molec. Pharmacol. and Pediatrics, Northwestern Univ. Med. School, Chicago, IL 60611; Dev. Genetics, Skirball Institute and Cell Biology[2] and Radiology[3], NYU Medical Center, New York, NY 10016.

The Notch/lin-12/glp-1 family of proteins mediate signals that regulate cell fate decisions during development. Notch signals appear to play a critical role in determining the number and type of cells that emerge from precursors. In vertebrates, excess Notch signals suppress neurogenesis in mammalian EC cells, Xenopus embryos and the retina, while reduced Notch signaling increases the number of neurons. To learn the role of Notch signals in cortical neurogenesis, we have studied the progeny of cortical ventricular zone cells following infection durind early or mid-neurogenesis in the mouse with retroviral vectors that deliver the Notch1-intracellular domain (Notch1-IC) along with tau-β-galactosidase to detect the infected cells. After infection in early neurogenesis (E9.5) progeny of infected precursors show a marked reduction in the numbers of neurons which develop compared to control infections. After infections during mid-neurogenesis (E14.5), Notch1-IC virus inhibits virtually all cells of the ventricular zone (VZ) from differentiating as neurons. However, the number of cells differentiating as glia appears unnaffected. Similarly, postnatal cortical sub-ventricular zone cells that differentiate into glia are unnaffected by infection with Notch1-IC vectors. These data suggest that there is a window of susceptibility to Notch signals in the maturation of precursors to cortical neurons and glia.

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Genes and genetic hierarchies involved in establishing myogenic identity.
Shahragim Tajbakhsh, Didier Rocancourt, Giulio Cossu[2] and Margaret Buckingham.

Skeletal muscles in the vertebrate body are derived from epithelial somites which respond to environmental signals to form a dorsal epithelial dermomyotome (dermis, muscle) and ventral mesenchymal sclerotome (axial skeleton, ribs). Although somites contribute to some head muscles, the remainder are derived from paraxial head and prechordal mesoderms. Gene inactivation studies of the myogenic regulatory factors (MRFs) have placed Myf5 and MyoD genetically upstream of myogenin and MRF4 where the combined activities of both Myf5 and MyoD are essential for establishing skeletal muscle fibres and myoblasts in the mouse embryo. Using a *Myf5* allele containing the *nlacZ* reporter gene, we have shown that β-galactosidase$^+$ muscle progenitors are present in *Myf5* null embryos, however, they migrate aberrantly and change their fate. This finding demonstrates that Myf5 (and MyoD) are directly implicated in confering myogenic identity. In a parallel study, we have evaluated the role that *Pax3* plays within the muscle genetic hierarchy by analysing *Pax3* mutant (*splotch*), *Myf5-nlacZ* null and *splotch/Myf5* double mutant mice. Strikingly, skeletal muscles and their precursor myoblasts are lacking in the body of *splotch/Myf5* double null embryos whereas head muscles appear normal indicating that, in the absence of Pax3 and Myf5, MyoD cannot rescue myogenesis in the body; head myogenesis does not appear to be programmed by Pax-3 or its homologue Pax-7.

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