Marco Milán

Development and growth control

During the development of a given tissue or organ, growth and fate specification are controlled in a coordinated manner by the activity of a discrete number of signalling molecules and their corresponding pathways to give rise to a well-formed structure with a particular size, shape and pattern. The activity or expression of these signalling molecules has to be tightly regulated since deregulation of their activity might cause uncontrolled growth and cancer. The Drosophila *wing primordium, a highly proliferative epithelium that arises as a group of 30-40 cells in the embryonic ectoderm and proliferates during five days to reach a final size of around 50,000 cells, is a highly suitable model system to analyse the mechanisms used to regulate the expression and activity of signalling pathways at a genetic, cellular and molecular level.*

Genetic and epigenetic mechanisms regulating hedgehog expression in the Drosophila *wing*

Drosophila limb primordia are subdivided into adjacent territories called compartments, cell populations that do not mix during development. Short-range cell interactions between adjacent compartments lead to the restricted expression or activity of organising molecules to the compartment boundaries. These organise the growth and pattern of the developing limb primordia.

Two orthogonal compartment boundaries behave as signalling centres and organise the growth and pattern of the developing wing primordium. Activation of the receptor Notch along the dorsal-ventral (DV) compartment boundary and the long-range activity of the signalling molecule Dpp, a member of the BMP/TGF-β family, expressed along the anterior-posterior (AP) compartment boundary, execute the organising activities of these signalling centres. The formation of the AP compartment boundary in *Drosophila* limbs and the restricted expression of Dpp along this boundary rely on asymmetric signalling of the secreted molecule Hedgehog (Hh) from P to A cells. This asymmetry is generated by the complementary activities of Engrailed/Invected and Cubitus interruptus (Ci) transcription factors in P and A cells, respectively. In P cells, the homeodomain proteins Engrailed (En) and Invected induce hh and repress ci expression, the essential downstream component of the Hh signalling pathway. Ci represses hh expression in A cells and, at the same time, confers the capacity to respond to Hh coming from P cells, thereby inducing Hh target gene expression. Thus, P cells express Hh while A cells respond to it.

The Polycomb (PcG) and the Trithorax (TrxG) group of proteins form the basis of a cellular memory system that maintains the transcriptional state of their target genes heritable during development. The genes controlled by the PcG/TrxG system have PcG response elements (PREs), to which these proteins bind and either keep the gene permanently repressed (PcG) or active (TrxG). Some PREs have been shown to maintain the initial transcriptional state of a nearby reporter gene through several rounds of mitosis during development, and as such they have been termed Cellular Memory Modules (CMMs). Interestingly, a 3.4-kb fragment situated upstream of the hh transcription start site behaves as a PRE and exhibits CMM activity. In A cells, PcG

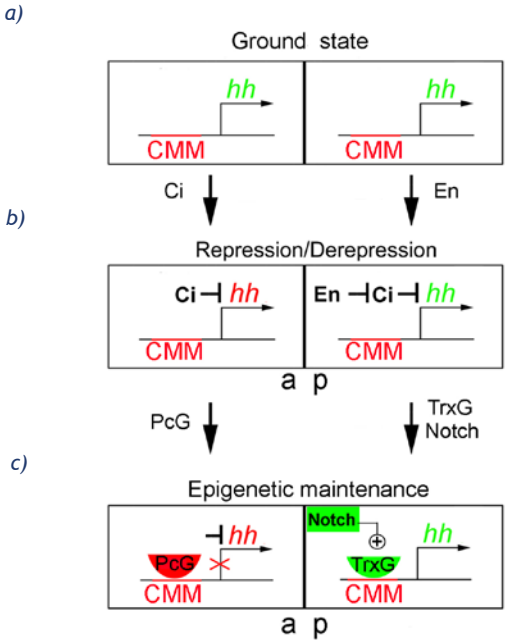

Figure 1. ***Regulation of hh expression in the*** **Drosophila** ***wing: an integrative model****. (a) The ground state in all wing cells is hh transcriptional activation. (b) Ci represses hh expression in anterior cells, while an En/Ci double-repression mechanism maintains hh expression in the posterior compartment. (c) The PcG proteins, presumably through the hh CMM, help to maintain the inactive transcriptional state of hh in anterior cells. In posterior cells, Notch, together with the TrxG proteins and through the hh CMM, contributes to the maintenance of the active transcriptional state of hh.*

genes are involved in maintaining the repressive transcriptional state of hh while TrxG genes maintain the active transcriptional state of hh once it is ectopically induced. However, the role of PcG and TrxG genes in maintaining hh expression in its endogenous expression domain, namely the P compartment, has not been studied to date.

Fernando Bejarano in the lab has analysed the contributions of En, Ci and the PcG/TrxG system in the regulation of hh expression in P cells of the *Drosophila* wing (Figure 1). First, his data indicate that the initial transcriptional state of hh is a direct consequence of the presence or absence of Ci. It is well known that Ci represses hh expression in A cells, while En activity represses Ci expression in P cells. He has demonstrated that in the absence of Ci and En activities, wing disc cells express hh. Thus, En is required in P cells

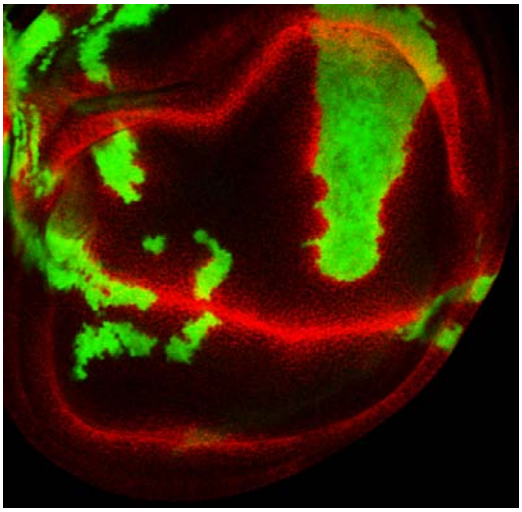

Figure 2. ***A non-autonomous role of Notch.*** *In* Drosophila *wing discs, removal of Notch in a group of cells (in green) induces the activation of the Notch pathway (in red) in nearby wild-type cells.*

Research Group Members

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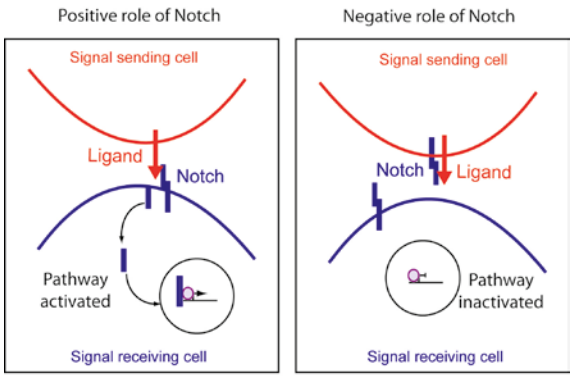

Figure 3. ***Two opposite roles of Notch receptor.*** *While binding of the ligand to the Notch receptor in the neighbouring cell induces the activation of the pathway, binding of this receptor to the ligand in signal-sending cells represses the activation of the pathway in the signal-receiving cells.*

to relieve Ci-mediated repression of hh. He has also presented evidence that *TrxG* genes are involved in the maintenance of hh expression in P cells. Interestingly, epigenetic maintenance of hh expression is positively regulated by the activity of Notch, whose activation is spatially restricted in the wing primordium. He has demonstrated that Notch is required, together with TrxG genes, to maintain hh expression and this is achieved through the previously defined hh CMM. To our knowledge, this is the first study in which Notch has been implicated in regulating the activity of a particular PRE (Bejarano and Milán, 2009).

A role of Notch receptor in ligand cis-inhibition in Drosophila

Notch and its ligands mediate short range cell interactions that play a conserved role in inducing cell fate specification. Several regulatory mechanisms have been described to ensure robust polarised signalling from signal-sending to signal-receiving cells. High levels of ligand expression activate Notch in nearby cells and exert a cell-autonomous dominant negative effect on Notch activity. This regulatory process is called cis-inhibition and it helps to restrict Notch activation to signal-receiving cells. By combining genetic mosaics in the *Drosophila* wing primordium (Figure 2) by means of cell culture assays, Isabelle Becam, in collaboration with Alfonso Martínez-Arias' lab in Cambridge (UK), has presented evidence that Notch promotes the clearance of Serrate ligand from the cell surface and exerts an inhibitory effect on the activity of Serrate expressed in the same cell (Figure 3). These regulatory mechanisms are independent of Notch- mediated transcription and are executed by the extracellular domain of Notch. Isabelle has shown that this process is required to block Serrate-mediated activation of Notch in the signal-sending cell population and helps to restrict Notch activation to signal-receiving cells. All together, her results, in concert with the previous results on ligand-mediated Notch cis-inhibition, indicate that mutual inhibition between ligand and receptor in signal-sending cells helps to block Notch activity in these cells and to restrict receptor activation in signal-receiving cells.

Scientific output

Publications

Bejarano F and Milán M. Genetic and epigenetic mechanisms regulating hedgehog expression in the *Drosophila* wing. *Dev Biol*, **327**(2), 508-15 (2009)

Research networks and grants

Compartments, organizing molecules and growth control in Drosophila
EMBO Young Investigator Programme (2008-2010)
Principal investigator: Marco Milán

Establishment and maintenance of compartment boundaries in the Drosophila *wing imaginal disc*
Spanish Ministry of Science and Innovation, BFU2007-64127/BMC (2007-2010)
Principal investigator: Marco Milán

From genes to shape: analysis of morphogenesis in Drosophila *and vertebrates*
Spanish Ministry of Science and Innovation, CSD2007-00008 (2008-2010)
Principal investigator: Marco Milán

Collaborations

A role of receptor Notch in ligand cis-inhibition in Drosophila
Alfonso Martínez-Arias, University of Cambridge (Cambridge, UK)

Lafora disease in Drosophila
Joan Guinovart, IRB Barcelona (Barcelona, Spain)

miRNAs and growth control
Stephen M Cohen, Temasek Life Sciences Laboratory (Singapore)