

# Development and growth control



**D**uring 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 of these signalling molecules has to be tightly regulated since deregulation of their activity might cause uncontrolled growth and cancer. Understanding how the activity of these molecules is controlled and how cells of different tissues or organs translate the activity of their pathways into activation or repression of the cell cycle machinery in a context-dependent manner are currently two of the most intriguing questions in developmental and cancer biology. Growth and fate specification must also be tightly coupled to generate a shaped and sized structure. Uncoupling these two processes has disastrous consequences in development and disease. In the last few years, much has been learned about the regulation of growth and fate specification. However, very little is known about how these processes are coupled. In multicellular organisms, stable subdivision into adjacent tissues and organs relies on the acquisition of distinct cell affinities. These are defined either by the differential expression of cell adhesion molecules or by interactions between adjacent cell populations that lead to cell repulsion. Intermingling of cells between adjacent tissues and organs has catastrophic effects on patterning and growth. The wing primordium of *Drosophila*, 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 these processes at a genetic, cellular and molecular level during development.

Our laboratory works on the following research lines: 1) Regulation of the activity of signalling molecules, 2) Coordinated control of cell cycle progression, tissue growth and fate specification, and 3) Molecular characterisation of cell affinity differences between adjacent tissues. In particular, the following topics have been addressed during 2008:

## A wingless and Notch double-repression mechanism regulates G1-S transition in the *Drosophila* wing

The control of tissue growth and patterning is orchestrated in various multicellular tissues by the coordinated activity of the signalling molecules Wnt/Wingless and Notch, and mutations in these pathways can cause cancer. The role of these molecules in the control of cell proliferation and the crosstalk between their corresponding pathways remain poorly understood. Cross-talk between Notch and Wingless (Wg) has been proposed to be responsible for organising the growth and patterning of the *Drosophila* wing primordium.

Héctor Herranz, with the help of Lidia Pérez in the lab, has revised the role of Wg and Notch in the control of cell proliferation and has presented evidence that a Wg and Notch double-repression mechanism controls G1-S transition in the presumptive wing primordium (Figure 1). These molecules exert their function by regulating the expression of the *dMyc* proto-oncogene and the *bantam* micro-RNA, which positively modulate the activity of the E2F transcription factor. Thus, in this cellular context Notch acts as a repressor of cell cycle progression and Wg has a permissive role in alleviating Notch-mediated repression of G1-S progression in wing cells. This work clarifies and simplifies the role of Notch and Wg in cell cycle control in the *Drosophila* wing and provides a suitable model by which to analyse the function of Notch and Wg signalling pathways in the regulation of the cell cycle machinery.

There is evidence in mammals that Notch acts as a tumour suppressor gene or as an oncogene, depending on the cellular context. The cellular context should then modulate the differential response of the cells to changes in Notch activity, in some cells

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leading to hyper-proliferation, in others to quiescence. How this context and the differential response are defined at the molecular level remains unclear. *Drosophila*, again, provides a very suitable model system to address this issue. Although our results highlight that Notch is involved in the inhibition of G1-S progression in wing discs, in other developmental contexts Notch exerts a proliferative function. Interestingly, in both cases, Notch acts through Rbf and E2F to positively or negatively control the G1-S transition. We therefore speculate that the molecular context is then defined by the effectors available, like *dMyc* and *bantam*, or by the presence of nuclear factors that act as a switch in the ultimate activation/inactivation of E2F via Rbf.

#### Notch signalling coordinates tissue growth and wing fate specification in *Drosophila*

During the development of a given organ, tissue growth and fate specification are simultaneously controlled by the activity of a

discrete number of signalling molecules. The wing primordium contains the progenitors of both the adult body wall and the wing (Figure 2b). The developmental decision between wing and body wall is made early in development and it is defined by the opposing activities of the two secreted signalling molecules Wg and the EGFR ligand Vein (Vn) in the most ventral and dorsal sides of the wing primordium, respectively (Figure 2a). Notch activity has been proposed to participate in this process since loss of this signalling molecule during this developmental stage leads to the failure to induce wing fate with a concomitant duplication of body wall structures (Figure 2b). With the corresponding wing and body wall molecular markers, Neus Rafel in the lab is further analysing the role of Notch in this process, not only in the adult fly but also in the developing wing primordium. Her data indicate that growth of the wing primordium mediated by the activity of Notch is required for wing fate specification (Figure 2a).

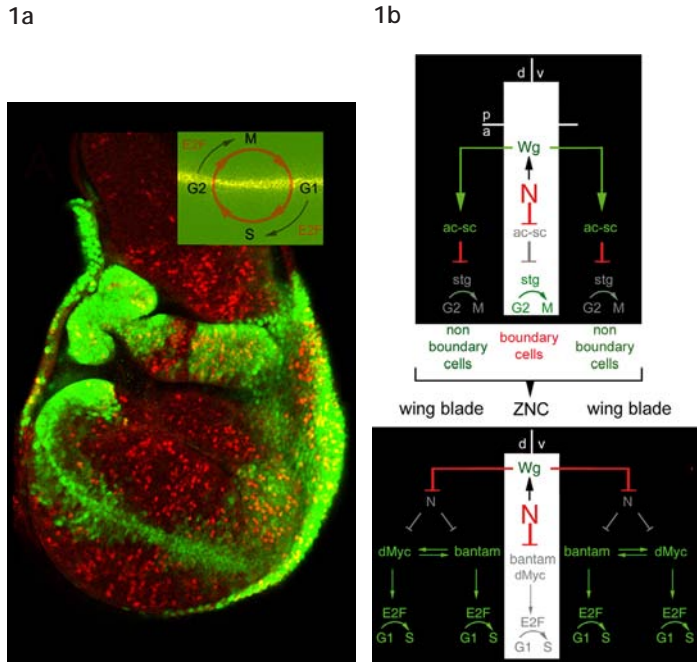


Figure 1. (a) Wing imaginal disc labelled to visualise cells in S-phase (red) and Notch activation (green). (b) Regulation of G1-S and G2-M transition in the *Drosophila* wing is controlled by the activity of Notch (N) and Wingless (Wg) through bantam micro-RNA and dMyc proto-oncogene.

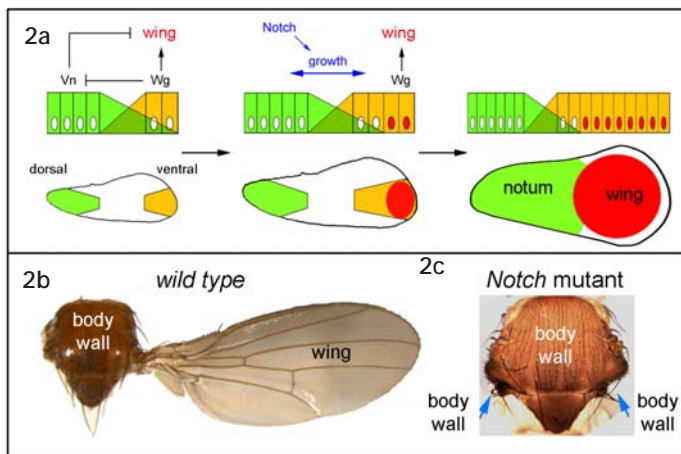


Figure 2. (a) Subdivision of the wing primordium into wing and body wall is mediated by the opposite activities of the signalling molecules Wingless (Wg) and Vein (Vn), respectively. Growth induced by the Notch activity pulls the Wg and Vn sources apart, thus facilitating wing fate specification. (b, c) Adult wing and body wall of wild-type (a) or mutant fly lacking Notch activity (b). Note in b lack of wings and duplication of body wall structures.

Expression of Wg in the most ventral part of the wing disc specifies the wing field at the same time as restricting Vn expression to the most dorsal part. Vn is required to block responsiveness of body wall cells to Wg. Thus, the relative concentration of the diffusible proteins Wg and Vn experienced by disc cells directs their wing versus body wall fate. In the early wing primordium, Vn reaches every wing cell, thereby blocking responsiveness to Wg and repressing wing fate specification. Growth induced by Notch activity pulls the sources of Wg and Vn apart, most ventral cells do not sense sufficient Vn levels so Wg is able to induce wing fate. These results highlight a critical role of Notch in linking tissue growth and fate specification in the developing wing primordium.

Growth promoted by Notch has also been shown to be directly involved in the specification of the eye within the *Drosophila* eye-antenna primordium, a process that also depends on the opposing activities of two secreted signalling molecules, in this case Dpp (a TGF- $\beta$  family member) and Wg. Thus, in both eye and wing primordium, Notch elegantly coordinates tissue growth and eye/wing specification by modulating the response of cells to the activities of signalling molecules. These results also suggest that the same mechanism may be commonly used in animal development to coordinate tissue growth and fate specification.

The evolution of wings was crucial in the process of adaptation, allowing insects to escape predators or colonise new niches. It has recently been shown that loss and recovery of wings has occurred during the course of evolution. This finding would suggest that wing developmental pathways are conserved in wingless insects and are being re-used. On the basis of our results, we speculate that adaptative changes in animal size modulates the cellular response to signalling molecules like Wg, thus helping to drive some of these extraordinary reversible transitions.

### A permissive role of Notch in maintaining the DV affinity boundary of the *Drosophila* wing

Cell affinities can contribute to organising cells into tissues and organs. *Drosophila* limbs and the vertebrate central nervous system are subdivided into adjacent populations that do not mix. These cell populations are called compartments. Cell interactions mediated by Notch receptor have been implicated in the specification of compartment boundaries (Figure 3). However, the contribution of Notch to this process remains controversial. The instructive role of Notch and the transcriptional requirement of the pathway have been questioned in the last few years.

Given the central role of Notch in making developmental boundaries in vertebrates and invertebrates, Isabelle Becam is re-evaluating its contribution and its signalling pathway in the maintenance of an affinity difference between dorsal (D) and ventral (V) compartments in the *Drosophila* wing. Her data indicate that unrestricted, low levels of Notch are sufficient for the formation of a stable DV affinity boundary. Cleavage of the Notch protein, release of the intracellular domain and a transcriptional function of Notch via the Suppressor of Hairless

transcription factor is required and sufficient in this process. Isabelle's data support a permissive role of Notch in maintaining the DV affinity boundary. This contrasts with the instructive role of Notch in executing the organising activity of this boundary. The DV compartment boundary organises the growth and pattern of the wing primordium. Restricted and high levels of Notch activity mediate this organising activity in a Su(H)-dependent manner. Unrestricted activation of Notch has disastrous consequences on the growth and patterning of the wing primordium.

## SCIENTIFIC OUTPUT

### Publications

Becam I and Milán M. A permissive role of Notch in maintaining the DV affinity boundary of the *Drosophila* wing. *Dev Biol*, 322(1), 190-98 (2008)

Bejarano F, Luque CM, Herranz H, Sorrosal G, Rafel N, Pham TT and Milán M. A gain-of-function suppressor screen for genes involved in dorsal-ventral boundary formation in the *Drosophila* wing. *Genetics*, 178(1), 307-23 (2008)

Herranz H and Milán M. Signalling molecules, growth regulators and cell cycle control in *Drosophila*. *Cell Cycle*, 7(21), 3335-37 (2008)

Herranz H, Pérez L, Martín FA and Milán M. A Wingless and Notch double-repression mechanism regulates G1-S transition in the *Drosophila* wing. *EMBO J*, 27(11), 1633-45 (2008)

Quijano C, Tomancac P, López-Martí J, Suyama M, Bork P, Milán M, Torrents D and Manzanares M. Selective maintenance of *Drosophila* tandemly arranged duplicated genes during evolution. *Genome Biol*, 9(12), R176 (2008)

Rafel N and Milán M. Notch signalling coordinates tissue growth and wing fate specification in *Drosophila*. *Development*, 135(24), 3995-01 (2008)

### Research networks and grants

#### *Ajuts a grups emergents*

Agency for Administration of University and Research Grants (AGAUR), 2005-SGR-00118 (2006-2009)

Principal investigator: Marco Milán

#### *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 (2008-2012)

Principal investigator: Marco Milán

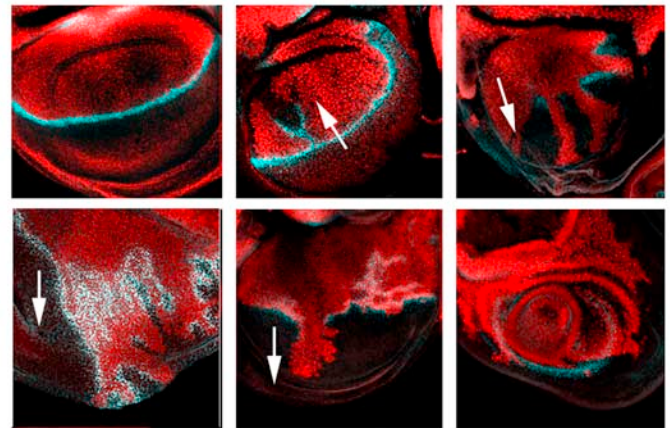


Figure 3. The interface between dorsal (red) and ventral (black) cells behaves as an affinity boundary but also as an organiser centre. This affinity boundary is violated (see white arrows) when the DV boundary (blue) is not properly established.

### Collaborations

#### *Growth control in the Drosophila wing*

Francisco Martín, Centro de Biología Molecular, Universidad Autónoma de Madrid/CSIC (Madrid, Spain)

### Awards

EMBO Young Investigator Programme Award, European Molecular Biology Organization (2008-2010)