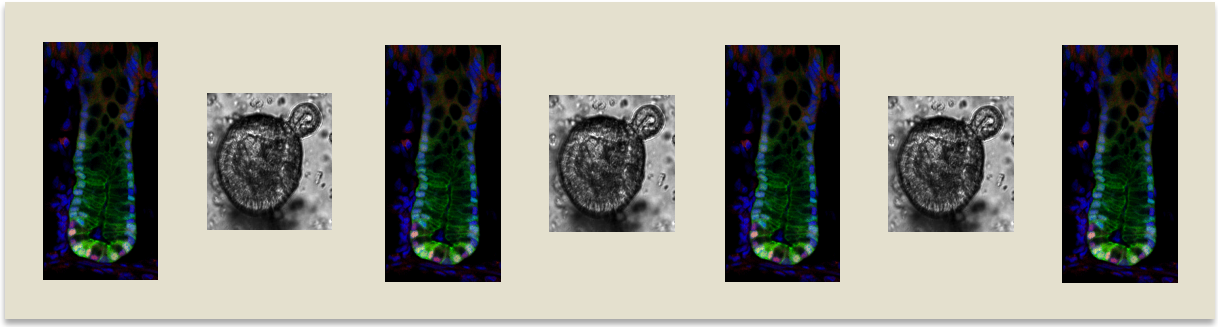

Intestinal Stem Cells
New concepts and methods

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Notch signals and intestinal stem cells: from crypt homeostasis to colon cancer

In adult organisms, tissues are maintained and repaired by stem cells, which divide and differentiate to generate more specialized progeny. The mechanisms that control the balance between self-renewal and differentiation promise fundamental insights into the origin and design of multi-cellular organisms. A tissue that is particularly suited to approach these questions is the intestinal epithelium, as it consists of a monolayer of epithelial cells that endlessly divide, migrate while differentiating and are replaced to ensure continuous and fast cell renewal throughout adult life. In this tissue, somatic stem cells represent crucial elements that govern tissue remodeling and homeostasis and the combined work of several laboratories has provided evidence for the existence of at least two different stem cell populations in the small intestinal crypt. Our lab focuses on the Notch signaling pathway as a new promising marker to study gut stem cell physiology. Our observations indicate that the Notch1 receptor is expressed in both crypt stem cell populations, implying that it can mark either one of the two *in vivo* and providing a precious tool to dissect the hierarchy between these different stem cells.

Notch signaling plays an evolutionarily conserved role in metazoans. Extensive molecular and genetic analyses established that the developmental role of Notch signals is to link cell fate choices of one cell to those of a cellular neighbor. The precise identity of the cells in which Notch signaling is active is still unclear, mainly due to the lack of reliable tools to investigate Notch expression *in vivo*. For this purpose, we have recently developed and characterized a novel roster of unique transgenic mice that permit to assess Notch expression and activation *in vivo* in an unprecedented fashion. Thanks to these novel mice, we have been able to formally show that the expression of the Notch1 receptor and of its transcriptional target Hes1 identifies crypt stem cells. We are now using these mouse models to dissect the hierarchy between stem cell populations in the small intestine, while expanding our knowledge on colonic stem cells, that remain poorly studied. In addition, we study the dynamic behavior of stem cells during regeneration upon injury.

The critical role played by Notch signaling in intestinal renewal and differentiation, as well as in tumourigenesis in this tissue, is exemplified by the vast number of reports on this topic appeared in recent years (i.e. (Fre et al., 2011a; Fre et al., 2011b; Fre et al., 2005; Fre et al., 2009; Pellegrinet et al., 2011; van Es et al., 2005)). The Notch signaling pathway has emerged as an essential regulator of intestinal homeostasis; indeed Notch signals can

control the segregation of each mature lineage from undifferentiated progenitor cells, and they are instrumental for maintaining the proliferating intestinal cell pool. When Notch signaling is inhibited, all crypt cells cease to proliferate and differentiate into secretory cells (van Es et al., 2005). Reciprocally, we have shown that expression of a constitutively active form of the Notch receptor in the developing intestinal epithelium dramatically impairs cell differentiation and increases the proportion of dividing cells (Fre et al., 2005). The role of Notch in promoting intestinal proliferation requires Wnt signals, whereas it specifies cell fate independently of Wnt. Importantly, our work has shown that Notch acts in synergy with the Wnt pathway to induce intestinal adenomas (Fre et al., 2009). Our results are consistent with a model supporting a role for Notch in expanding a potentially malignant cell population and hence increasing the chances of a tumorigenic event. It is widely accepted that the primary events in tumourigenesis are linked to stem cell transformation: the process of tumor development is thought to initially affect normal stem cells or closely related early progenitors. In order to establish the identity of Notch1-expressing cells within a tumor and to follow their fate *in vivo,* we have started to use our novel mouse lines to perform lineage tracing of Notch-expressing tumor cells. Our preliminary results show that only a small fraction of tumor cells expresses a N1cre-driven reporter. These experiments will allow us to highlight *in vivo* which and how many cells within a tumor present Notch activity.

In addition, we plan to examine the dynamic behavior of stem cells during regeneration upon injury. This latter hypothesis will be directly tested upon tissue injury in different experimental contexts, both *in vivo* and *in vitro*. We have started to analyze the behavioral responses of marked crypt stem cells to local injuries generated by two-photon laser ablation experiments ex vivo in intestinal organoids (Sato et al., 2009). The regeneration dynamics of ablated crypts will allow us to visualize the dynamic behavior of marked cells upon local injuries and to compare it to the responses observed after broad crypt loss in the whole tissue upon whole-body irradiation. We would like to develop a method to automatically track single cells in time-lapse videos, in order to perform computational modeling of stem cell behavior in organoids and to quantify the extent of regeneration from Notch1+ stem cells during homeostasis and injury conditions.

The study of stem cell replacement dynamics and perturbations of their physiological state upon tissue damage (irradiation causing crypt loss or ablation of specific crypt cells) can reveal the presence and uncover the plasticity of quiescent stem cell populations that do not otherwise actively participate to normal tissue homeostasis. This information will be relevant for gaining insights into the current debate regarding the existence of a reservoir of quiescent stem cells that would be activated only in case of tissue damage.

Broadly, our research aims at understanding the molecular features of adult stem cells, as well as the mechanisms by which Notch signaling controls the fate of specific cell populations during regeneration and tumourigenesis. We believe that the identification of common organizational principles of tissue architecture is an essential step in order to develop safe and efficacious applications of stem cells in regenerative medicine.

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