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Lineage selection and plasticity in the intestinal crypt

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We know more about the repertoire of cellular behaviours that define the stem and progenitor cells maintaining the intestinal epithelium than any other renewing tissue. Highly dynamic and stochastic processes define cell renewal. Historically the commitment step in differentiation is viewed as a ratchet, irreversibly promoting a given fate and corresponding to a programme imposed at the point of cell division. However, the emerging view of intestinal self-renewal is one of plasticity in which a stem cell state is easily reacquired. The pathway mediators of lineage selection are largely known but how they interface within highly dynamic populations to promote different lineages and yet permit plasticity is not. Advances in understanding gene regulation in the nervous system suggest possible mechanisms.

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Introduction

The sheet of cells that comprises the small intestinal epithelium is indented to create glandular crypts in which cell proliferation is restricted and from which all cell types are generated. Absorptive enterocytes and secretory (Goblet and enteroendocrine) cells actively migrate from crypts while undergoing a phenotypic maturation that is accompanied by a restricted number of transient cell divisions (Figure 1). The most morphologically undifferentiated cells are located at or near the crypt base where they interface with long-lived differentiated secretory Paneth cells. These undifferentiated cells are maintained by robust levels of active Wnt signalling, characterised by expression of Lgr5 (a R-spondin receptor) and contain much of, and arguably all, the steady-state stem cell activity as shown by lineage tracing. The colonic epithelium has similar organisation but lacks both villi and Paneth cells.

There are differences in the properties of cells in the crypt base which are recognised by heterogeneous expression of markers and that arises from both the geography of the lower crypt and the availability of Paneth cells for cell-cell interaction. Together these factors create a nuanced biology; undifferentiated cells immediately above the Paneth cell region (at, or around, cell position 4 from the crypt base) tend to express different markers than those within it. The cells within these different zones have been proposed as alternative candidates for the stem cell population. Position specific heterogeneity in marker expression and in properties such as quiescence has previously been interpreted as indicative of relatively stable sub-populations moving unidirectionally through discrete cellular intermediates from multipotent stem cells to committed progeny. However, recent evidence for plasticity challenges this interpretation and suggests that normal cell fates are easily altered and stemness regained.

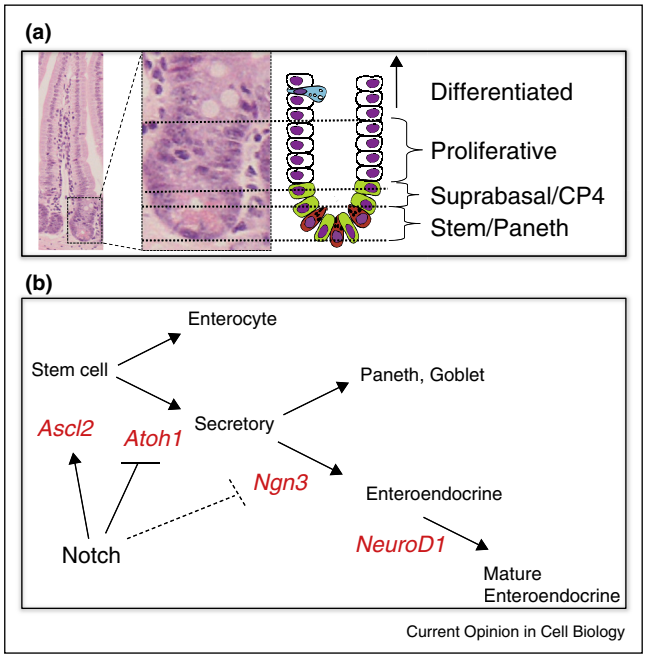
Intestinal lineage specification by Notch and the bHLH proteins

Historically attempts to explain how multiple phenotypically distinct cell types arise within the crypt have assumed the creation of lineage-restricted progenitors that can be distinguished by different transcription factor profiles [1,2]. Commitment has been viewed as a series of binary decisions, the first directing absorptive versus a 'pan' secretory fate, followed by further diversification into the four principal secretory types [3].

Several key bHLH 'proneural' proteins play distinct and crucial roles in early lineage specifications as well as differentiation events in the crypt, and their expression and activity are spatially and temporally regulated (Figure 1). A large part of this regulation appears to be via the Notch signalling pathway [4–7].

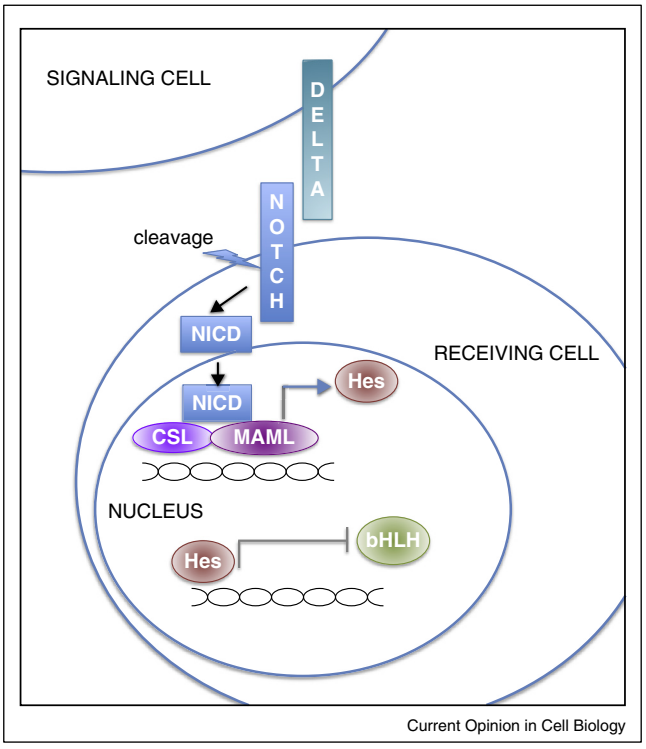
Ultimately Notch signalling regulates the stem versus secretory fate decision as well as further fate choice and differentiation events in the crypt [8,9]. Expression of the proneural bHLH transcription factor Ascl2 is associated with stemness and is absolutely required for intestinal stem cell maintenance. Active Notch is required for Ascl2 expression and its loss results in precocious crypt cell differentiation [8,10]. The proneural protein Atoh1 acts as a master regulator of fate specification of the secretory lineage [2,11]. Ascl2 expression is maintained by active

Figure 1



Organisation and lineage control in the intestine. **(a)** H&E section of intestine showing crypt-to-villus axis. Expanded view of crypt shown alongside a schematic showing the location of the different functional zones. (b) Schematic of classical view of bHLH transcription factor-driven control of fate choice and differentiation in the intestine, and a simplified view of their regulation by Notch signalling. However, complex interaction between cells, potential oscillating expression of bHLHs, and a clear ability to move back up the hierarchy towards stemness, points strongly to a great deal of potential for plasticity, rather than cells following a linear pathway as depicted here.

Figure 2



Schematic of the Notch signalling pathway. In brief, activation of the Notch membrane receptor requires binding by a member of the membrane-bound ligand Delta family (primarily Delta-like, Dll 1 and 4 and Jag 1 in the crypt) [9]. Binding of ligand to the receptor leads to release of the Notch intracellular domain (ICD) by protein cleavage. NICD translocates to the nucleus and associates with the CSL complex (CBF-1/RBP-J, Su(H), Lag1), displacing transcriptional repressors. This complex now associates with transcriptional co-regulators of the MAML family, resulting in upregulation of multiple downstream targets including Hes (Hairy/Enhancer of Split) proteins. Notch signalling via Hes proteins act to potentiate stem cell maintenance and inhibit secretory via regulation of bHLH transcription factors. For many more details see [5].

Notch signalling that also acts to suppress Atoh1. Expression of Atoh1 is cell-autonomously inhibited by Hes proteins and in the absence of Notch signalling, crypt stem cells precociously differentiate into secretory goblet cells [7,12].

The spatial organisation of cells expressing Notch ligand and receptor in the crypt evokes a classic lateral inhibition scenario for control of stem versus secretory fate (Figure 2). Stem cells towards the crypt base found preferentially adjacent to Delta-expressing Paneth cells, express Notch receptor [13•,14], and are maintained in an undifferentiated state by constant Notch signalling and suppression of Atoh1 [7,9,15,16], As migrating cells lose contact with Paneth cells and the high Notch signalling they confer, they become poised between secretory and non-secretory fate. Lineage selection may then arise by stochastic variation in Delta expression leading some cells to express higher levels than others. This initial stochastic imbalance in Delta expression becomes reinforced allowing only a subset of cells (Delta high, Atoh high) rising up the crypt to become committed to a secretory fate while the rest become absorptive enterocytes.

This regulation and functional organisation readily explains a binary fate in a supra-Paneth cell poised population but fits less well with a subsequent downstream cascade of secretory lineage choices specified after a series of cell divisions each progressing unidirectionally towards a more restricted fate. Moreover, recent evidence derived from regenerating systems casts doubt both on the existence of stable populations of progenitors and the irreversibility of lineage specification.

Plasticity

For many years it has also been known that intestinal regeneration following damage is not solely a function of surviving stem cells expanding to restore homeostasis (Figure 3) [17]. Following radiation induced injury the clonogenic fraction of crypt cells is elevated suggesting that these might correspond to the abundant and immature absorptive cells present within the early transit-amplifying