

Gut stem cells, a story of snails, flies and mice

Marc Amoyel

Intestinal stem cells (ISCs) replenish and regenerate several types of cells in the gut, both during normal homeostasis and in response to various insults such as infections. Although gut structure and complexity vary across phyla, two functional categories of differentiated cell types are always present: absorptive cells and those of the secretory lineage. A series of studies in *Drosophila* and mouse published in *The EMBO Journal*, including one in this issue, identifies conserved roles for the Snail family of zinc finger transcription factors in regulating self-renewal and differentiation of ISCs (Korzelius *et al*, 2014; Loza-Coll *et al*, 2014; Horvay *et al*, 2015).

See also: **K Horvay *et al*** (May 2015),
J Korzelius *et al* (December 2014) and
MA Loza-Coll *et al* (December 2014)

The mouse gut is made up of enterocytes (ECs) and several types of secretory intestinal cells, enteroendocrine (EE), Paneth and goblet cells, while *Drosophila* guts only contain ECs and absorptive EE cells. In both species, the decision to commit to absorptive (EC) or secretory (EE) fate depends on the activity of the conserved Notch (N) signalling pathway: low N during differentiation leads to EE fate, while high N specifies EC fate (Ohlstein & Spradling, 2007).

The *Drosophila* gene *escargot* (*esg*) is a member of the Snail family, along with *snail* and *wormiu* (Nieto, 2002), and has been used as ISC marker. In addition to ISCs, *Esg* is also found in enteroblasts (EBs), the daughter cells of ISC that remain undifferentiated under non-injured conditions. The

mouse genome also encodes three Snail family members: *Snai1* (or *Snail*), *Snai2* (or *Slug*) and *Snai3* (or *Smuc*) (Nieto, 2002). Strikingly, mouse *Snai1* is also expressed in intestinal stem cells known as crypt base columnar cells (CBCs) and their undifferentiated progeny, which are transit amplifying cells that have not yet chosen a final fate (Horvay *et al*, 2011).

Two groups have now established the central role of *Esg* in regulating *Drosophila* ISC maintenance (Korzelius *et al*, 2014; Loza-Coll *et al*, 2014). Loss of *Esg* in the stem cells leads to their differentiation, resulting in the depletion of the ISC pool. As a consequence, the regenerative ability of the gut following infection is lost with dire consequences on survival (Korzelius *et al*, 2014). Similarly, loss of *Esg* in the EBs also leads to their rapid differentiation (Korzelius *et al*, 2014; Loza-Coll *et al*, 2014). Thus, *Esg* maintains the undifferentiated state both in the stem cells and in their multipotent daughters. Similarly, in the mouse gut, conditional knockout of *Snail* leads to the loss of CBCs (Horvay *et al*, 2015). Further analysis showed that CBCs die by apoptosis after *Snail* ablation, indicating that although *Snail* and *Esg* play roles in maintaining ISCs, they may do so in different ways. As in the case of flies, the regenerative ability of mouse guts lacking *Esg* is severely impaired following insult, in this case radiation. However, loss of *Snai1* does not appear to affect the transit amplifying population in the mouse. Moreover, to further emphasise the role of *Esg/Snai1* in maintaining ISCs, over-expression of *Esg/Snai1* in mouse and fly ISCs leads to excessive stem cell accumulation and a lack of differentiated cells (Korzelius *et al*, 2014; Horvay *et al*, 2015). This also results in an inability of the

gut to respond to challenges, as the prevention of differentiation means that cells lost to damage are not replenished. Finally, expressing *Esg* in ECs leads to partial de-differentiation into stem cells (Korzelius *et al*, 2014).

How does *Snai1/Esg* act in stem cells to specify their fate? This family of transcription factors is thought to act mainly by repressing expression of its targets (Nieto, 2002). The two fly groups used DamID and expression profiling to identify promoters bound by *Esg* and genes whose expression depends on *Esg*. Cross-referencing these lists led to the identification of candidate genes likely to be the direct targets of *Esg*, and they fell into one of two classes: genes downregulated or upregulated by *Esg* (Korzelius *et al*, 2014). The former reflects *Esg*'s expected repressor function. Many of the genes required for differentiation into ECs and EEs are repressed by *Esg*, including *pdm* (also called *nubbin*), which encodes a POU and homeodomain transcription factor that the authors show can trigger EC fate, as well as the EC marker *Myo31-DF* (also called *Myo1a*), the cell proliferation inhibitor *tribbles* and many other differentiation markers, such as immune genes and gut enzymes. This wide set of *Esg*-bound genes indicates that *Esg* acts in a very pleiotropic manner to inhibit several aspects of differentiation at once. Similar results in mouse muscle, where *Snai1* prevents the binding of *MyoD* on the enhancers of differentiation genes, establish *Esg* repression of a wide range of differentiation genes as a paradigm for its function (Soleimani *et al*, 2012). The second class of targets were unexpectedly upregulated by *Esg*. They contain *Esg*-binding regions and encode components of several signalling pathways required for ISC self-renewal. This

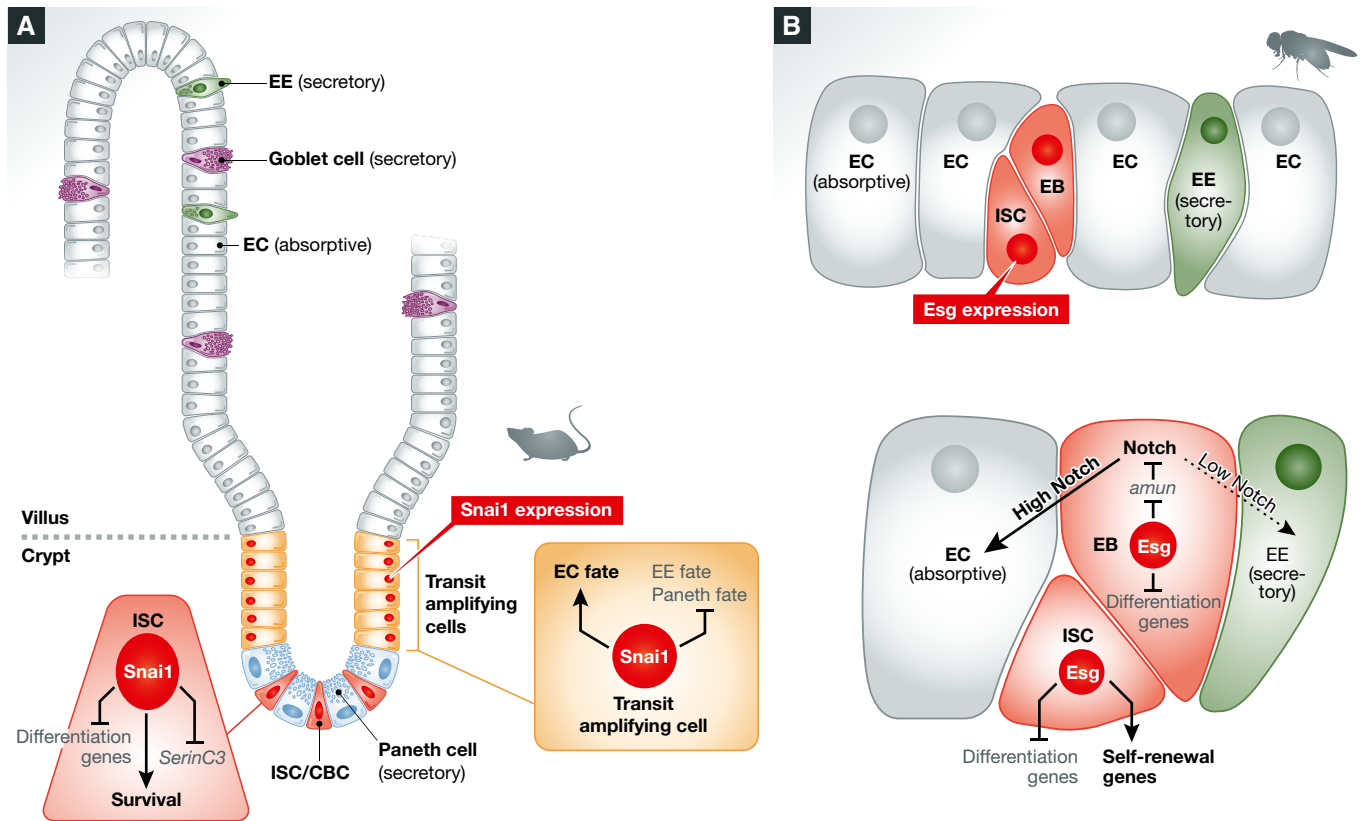


Figure 1. Model of Snai1 action in the mouse gut and of Esg in fly ISCs.

(A) Snai1 is expressed in CBCs and transit amplifying cells. It regulates CBC survival and differentiation of transit amplifying cells to the secretory or absorptive lineage. (B) Esg is found in ISCs and EBs in the fly gut, where it acts to repress differentiation genes. Moreover, it may activate self-renewal genes in ISCs. Finally, *amun* is a target of Esg in EBs, where it affects N signalling to determine EC versus EE fate.

suggests that, in addition to acting as a repressor of differentiation genes, Esg may play a role as a transcriptional activator in specifying ISC maintenance. Although surprising, there is evidence that other members of the Snail family can act as activators of transcription, depending on the enhancer context (Rembold *et al*, 2014). Horvay *et al* performed genome-wide expression analysis to identify Snail-regulated genes in CBCs. They found that Snai1 directly regulates expression of the apoptosis inhibitor *SerinC3* transcriptionally, and speculate that this may explain the death of CBCs lacking Snai1.

In addition to its roles in maintaining ISCs, Loza-Coll and Horvay also identified a requirement for Esg/Snai1 in regulating the fate of differentiating cells in the intestine (Loza-Coll *et al*, 2014; Horvay *et al*, 2015). *esg*-deficient cells in the gut gave rise to many more EEs than normal, and *Snai1* mutants had many more EEs and Paneth cells. In both cases, this increase was at the expense of the EC lineage. Conversely,

over-expression of Snail led to a decrease in EE and Paneth cells, suggesting that Snai1 and Esg bias cells towards absorptive fates at the expense of secretory fates. Loza-Coll *et al* (2014) explored the relationship between Esg and N, a known inhibitor of secretory fate specification. They found that N activity was reduced in the absence of Esg and that this was sufficient to explain the bias in cell fate. Using DamID, they identified *amun*, a known modulator of N signalling, as transcriptional target being repressed by Esg. They observed that *amun* derepression led to the inhibition of N, which was responsible for the EE accumulation observed in Esg knockdowns. Thus, by regulating multiple targets, Esg can play many roles both in stem cell maintenance and in biasing daughter cell fate.

Altogether, these studies provide insight into the cell-intrinsic actions of a transcription factor that controls stem cell self-renewal. However, stem cells reside in niches and depend on niche-derived signals for their continued maintenance, and the

relationship between these extrinsic maintenance cues and intrinsic ones like Snail genes is an enduring question. Horvay *et al* (2011) have previously shown that Wnt signalling is required for both Snai1 expression and its nuclear localisation in CBCs, suggesting a direct link between extrinsic niche signalling and Snail activity within the stem cells. The mechanisms controlling *esg* transcription in the fly gut are not known; however, it is tempting to postulate a similar regulation by niche signals. Moreover, Korzelius *et al* (2014) suggest that differentiation factors such as Pdm1 are responsible for repressing *esg* in differentiated cells, pointing to the existence of stable genetic states in which either stem cell or differentiation genes are expressed.

Finally, these studies add to the growing body of work that implicates epithelial-mesenchymal transition (EMT)-regulating transcription factors in stem cell biology. EMT is characterised by loss of epithelial organisation and adhesion, and adoption of mesenchymal features; it is thought to be

essential for metastasis to occur. EMT factors, including the Snail family and two others, Twist and Zeb, are expressed in many different stem cells. For instance, Snail is present in mammalian epidermal stem cells, Snai2 in mammary and haematopoietic stem cells (Perez-Losada *et al*, 2002; Guo *et al*, 2012; De Craene *et al*, 2014), while both Snai1 and Snai2 are required to prevent differentiation in muscle progenitors (Soleimani *et al*, 2012). In flies, cyst stem cells (CySCs) in the testis require both Esg and the Zeb homologue, Zfh1, for self-renewal (Leatherman & Dinardo, 2008; Voog *et al*, 2014) while neuroblasts require Worniu to prevent differentiation (Lai *et al*, 2012). Sequencing data from Korzelius *et al* indicate that *snail*, *worniu*, *Zfh1* and its paralogue *Zfh2* are all expressed in ISCs. However, the relationship between the roles of these genes in self-renewal and EMT is not clear. Indeed, fly and mouse ISCs express E-cadherin (Snippert *et al*, 2010; Korzelius *et al*, 2014), which is normally repressed during EMT, as do testis CySCs, which require E-cadherin for self-renewal (Voog *et al*, 2008). Nonetheless, Korzelius *et al* do find that genes required for septate junctions are repressed by Esg. Recent work suggests that the levels of the EMT transcription factor Twist required to regulate proliferation and 'stemness' are lower than those required to induce EMT (Beck *et al*, 2015). This could apply also to other EMT transcription factors and could be a safeguard mechanism to ensure that stem cells that acquire too much of a self-renewal transcription factor are detached from their niche due to decreased adhesion, thereby reducing their exposure to self-renewal factors.

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