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**An *amuse bouche* of stem cell regulation:
Underlying principles and mechanisms from adult *Drosophila* intestinal stem cells**

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Abstract

Stem cells have essential functions in the development and maintenance of our organs. Improper regulation of adult stem cells and tissue homeostasis can result in cancers and age-dependent decline. Therefore, understanding how tissue specific stem cells can accurately renew tissues is an important aim of regenerative medicine. The *Drosophila midgut* harbors multipotent adult stem cells that are essential to renew the gut in homeostatic conditions and upon stress-induced regeneration. It is now a widely used model system to decipher regulatory mechanisms of stem cell biology. Here we review recent findings on how adult intestinal stem cells differentiate, interact with their environment, and change during aging.

Introduction

Adult tissues rely largely on the activity of stem cells to maintain tissue homeostasis and orchestrate a regenerative response to injury. Adult stem cell proliferation, self-renew, and lineage differentiation are coordinated by numerous signaling pathways through epigenetic, transcriptional, post-transcriptional and post-translational mechanisms. Functional changes of stem cell properties during aging have been associated with a failure of these regulatory mechanisms.

Given its relative simplicity along with precise genetic tools, the midgut of *Drosophila melanogaster* and its adult intestinal stem cells (ISCs) have proven to be an excellent model system to decipher stem cell lineage decisions, proliferation control during homeostasis and stress, and age-related functional decline. Here, we present an *amuse bouche*, where we briefly summarize the major advances in this field with a focus on recent publications; it is meant as both an overview and a prelude for the reader towards a deeper exploration of many of the creative studies cited herein.

ISC identity and cell lineage decisions

Previous studies have characterized the ISC lineage and division properties allowing for differentiation and stem cell self-renewal. Comparable to the mammalian intestine and lung epitheliums, the *Drosophila* ISCs can produce two differentiated cell types: hormone-producing enteroendocrine cells (EEs) and absorptive enterocytes (ECs) through dividing enteroendocrine precursors (EEPs) and directly differentiating enteroblasts (EBs), respectively [1–4]. Here we highlight recent findings advancing our understanding of cell fate and lineage decisions in ISCs (**Figure 1**).

Spindle orientations and asymmetric vs symmetric stem cell fate decisions

Adult stem cells undergo routine renewal as well as regenerative repair, a process that often requires expansion of the stem cell pool. While previous studies proposed a role for spindle orientation in asymmetric/symmetric fate acquisition [5], recent work has clarified this further: Jun Kinase (JNK) signaling directly controls the spindle regulatory components Kif1a, Wdr62, and Mud[6]. Planar spindle orientations correlate with symmetric ISC fate acquisition and are more prevalent during regeneration, adaptive resizing, and aging [6]. As cell fate acquisition in the two ISC daughter cells depends largely on Notch/Delta signaling, future studies will determine how JNK-driven planar ISC divisions may alter ISC fate via Notch/Delta signaling and how they relate to studies indicating a contribution of Bmp signaling on symmetric ISC divisions [7].

Maintenance of ISC identity and early lineage decisions

ISC lineage decisions rely heavily on Notch signaling promoted by its ligand Delta (DI). We cover current models for functions of Notch signaling and open questions on its role in the lineage in **BOX 1**. Independent of DI/Notch signaling, additional control helps maintain ISC identity. A role for the cohesin component, Rad21, in ISC maintenance has been established, likely through its contributions to chromatin structure and regulation of differentiation genes [8]. Tramtrack69, a transcriptional repressor, is also required to maintain ISC identity; its inactivation converts ISCs to neural stem cell-like state [9]. In addition to control at the transcriptional level, recent studies also uncovered a role of post-transcriptional regulation, whereby P-bodies in ISCs sequester and block the translation of mRNAs encoding differentiation genes such as Pdm1, expressed at low levels in ISCs [10]. These findings highlight the necessity of transcriptional and post-transcriptional coordination required to preserve stem cell identity.

How to build an enterocyte

Recent work has better defined how lineage differentiation of ISCs is directed towards EC or EE terminal cell fates (**Figure 1**). EBs can remain dormant in the tissue before being "activated" to differentiate. Activation leads to EB cell growth, endoreplication, and morphological changes that include lamellipodia formation and expression of septate junction components, such as Tsp2A and Mesh required for EB integration in the epithelium [11–13]. Previous studies indicated that EB differentiation requires coordinated activity of numerous transcription factors, including Esg, Sox21a, GATAe, and Pdm1 (reviewed in [14]). New findings demonstrate that an early event in the EB differentiation is the expression of the transcription repressor, Klumpfuss (Klu), which inhibits EE determinant genes [11,15]. In addition, Sox100B has a direct role activating transcription of Sox21a in EBs, essential for EC fate acquisition [16–19]. Further EB morphological changes and growth are promoted by the zinc finger transcription factor Zfh2 [19]. Future studies will be needed to understand the regulatory interplay between Klu, Zfh2, Sox100B, and previously identified transcription factors.

Interestingly, there is an emerging role for organelle activity and metabolic state in specific steps of differentiation (**Figure 1**). Peroxisome function, which is induced upon injury, promotes repair and EC differentiation by impinging on endocytosis and late endosome maturation. Defective peroxisomes halt differentiation of EBs by altering late endosomes and blocking JAK/STAT activation required for downstream Sox21a expression [20]. Defects in the metabolism of mitochondria also inhibit EB growth and EC differentiation via activation of FOXO which prevents mTor signaling [21]. How other metabolic processes impinge on cell states will be an important future direction of study.

Another critical aspect of EB differentiation is the shift from the mitotic cell cycle in ISCs, to the endocycle in differentiating EBs, resulting in polyploid ECs (**Figure 1**). Recent studies suggest an

important role of the Cdk1 inhibitory kinase, Myt1, which inhibits Cyclin A in EBs to promote G2 arrest and a switch to endocycles [22]. In addition, Klu is also proposed to inhibit the mitotic cell cycle in EBs through binding to and repressing *CycE* and *CycB* [15]. Alteration of nucleotide metabolism has also been suggested to be critical for EC differentiation, likely through control of endocycle [23].

It was recently established that EB fate is also under the control of cell death pathways. In routine homeostatic conditions, EBs are subjected to both cell death-promoting caspase activity and inhibition of this process through the EB-specific expression of the caspase inhibitor, Diap1 [24]. A balance of EB survival versus apoptosis is accomplished by Notch signaling priming EBs for cell death and EGFR signaling promoting cell survival. This mechanism ensures that in healthy, homeostatic tissue, excess numbers of EBs are culled, whereas in regenerative conditions, EB survival is enhanced [24]. Additional non-apoptotic functions of the initiator caspase Dronc also promote EB differentiation [25,26].

These studies bring to light the complexity of regulation of the ISC to EC differentiation process, integrating intrinsic transcription and post-transcriptional regulation as well as tissue-level information.

How to keep an enterocyte

Once differentiated, EC nuclear organization and program of transcription are actively preserved. The Non-STOP Identity Complex (NIC), a deubiquitylase that targets histone H2B-Ub, and the transcription factor Hey, appear to be important for maintenance of the EC state, with their inactivation resulting in de-repression of *Delta*, a gene normally expressed only in ISCs [27,28]. The inactivation of Hey in the EC favors the expression of the lamin, LamDmO, which is usually only expressed in ISCs. Conversely, EC-specific LamC is reduced in these conditions, ultimately modifying the EC transcriptional profile. Similarly, inactivation of Non-STOP leads to a downregulation of EC genes and upregulation of non-EC programs. Thus, the differentiated cell state is actively maintained through chromatin regulation. Additional studies are required to understand the influence of changes to chromatin, their impact on lineage choices, and fate restriction.

EE fate and diversity

Several factors regulating ISC differentiation towards EE fate have been established and are also described more extensively elsewhere [14,29]. The transcriptional program leading to EE differentiation relies on the bHLH factor Scute that primes ISCs toward EE fate (**Figure 1** and **BOX 1**). Upon cell division, the EE primed ISC will give rise to an EE precursor cell (EEP) along with a renewed ISC (see **BOX 1** for additional details). Single-cell RNA-seq data has provided additional evidence that EE cells originate through distinct EEPs [11,30]. It was recently suggested that EE priming in progenitor

cells is epigenetically regulated by Polycomb complex proteins [31]. The inactivation of *Polycomb* leads to downregulation of EE genes, which is likely indirect given Polycomb's well-characterized role in transcriptional repression.

Single cell RNA sequencing of the whole gut or an EE-enriched cell population illustrated the functional diversity of EE cells [11,30]. 10 major subtypes of EEs were identified with strong regional specialization. The differentiation of most of the characterized subtypes relies on the expression of 14 different transcription factors and arise from distinct sub-lineages with differential requirements for Notch signaling [30,32]. Each subtype is associated with the expression of 2 to 5 peptide hormones as well as specific hormone receptors. While some functions of EE peptide hormones are known [14], undoubtedly future studies will elucidate additional activities of these molecules.

Fine-tuning ISC proliferation

Many signaling pathways have been shown to regulate ISC proliferation in homeostasis and in response to tissue injury. Although some cell-autonomous factors directly regulate ISC divisions, non-cell autonomous signals are important to control stem cell proliferation in response to tissue and organismal needs. Jak-Stat and EGFR pathways are the primary mitogenic regulators driving ISCs to increase proliferative capacity, though ISCs receive important input from other pathways including Wnt/Wg, BMP/TGF β Hippo, JNK, and p38 among others (for a more detailed review, see [14,29]). How these different pathways are integrated in ISCs to balance cell proliferation rates is still somewhat elusive. We discuss here recent advances in our understanding of cell-autonomous and non-cell-autonomous regulation (**Figure 2**).

Cell-autonomous regulation of proliferation

Recent studies indicate that the trafficking and degradation of signaling receptors are exquisitely regulated in ISCs (**Figure 2**). Upon tissue damage, internalization of the BMP receptor, Tkv, in ISCs requires Awd, a facilitator of dynamin endocytic function; this promotes Mad activation driving ISC return to quiescence [33]. Similarly, endocytosis of the Wnt receptor (Fz3) and EGFR is regulated by the RalA GTPase, which is essential for the regenerative response of ISCs after damage [34,35]. Likewise, under homeostatic conditions, EGFR protein levels are tightly regulated in ISCs and limited through SH3PX1-dependent autophagic degradation [36]. EGFR protein levels are also controlled indirectly by the chromatin remodelers, Kismet and Trithorax-related, that promote expression of Cbl, an E3-ligase that degrades EGFR, thereby restraining ISC proliferation during homeostasis [37]. Identifying other regulators of receptor and ligand intra- and extracellular dynamics will be necessary to fully understand ISC proliferative responses in homeostasis and upon injury.

Genetic screens have identified genes with striking phenotypes in controlling ISC proliferation (**Figure 2**). Interestingly, the transcription factor Lola, limits ISC proliferation by downregulation of cell cycle regulators and seems to act downstream of Hippo/Wts, yet independently of the canonical component Yki [38]. In addition, the loss of activity in progenitor cells of Spen, involved in RNA processing and transcriptional repression, results in a large excess of ISCs, which requires Insulin signaling [39]. Finally, a novel role on ISC proliferation was found for Adenosine receptor (AdoR) signaling that stimulates Ras [40]. Acting during homeostasis, AdoR signaling is amplified after tissue damage due to enhanced extracellular adenosine levels [40]. Further knowledge of stem cell-intrinsic regulatory programs governing cell cycle and proliferative status will be informative.

NON-cell autonomous regulation

Enterocytes play primary roles in sensing epithelial damage and produce mitogens, including Unpaireds (Upds) and EGFs to activate ISC proliferation non-cell-autonomously. How tissue damage is sensed in ECs to induce a regenerative response has been further investigated in the last few years.

One important stress signal in ECs is reactive oxygen species (ROS; **Figure 2**). Tissue damage induces ROS via Nox and Duox enzymes activating the Ask1-MJ3-p38 pathway [41]. However, ROS can also activate JNK signaling, whose downstream effects in ECs rely at least in part on the transcription factor Ets21c [42]. Ets21c induces Upd3 expression in ECs, which mediates Jak/STAT-dependent proliferation in adjacent ISCs. ROS production can also arise due to defects in mitochondrial metabolism activating JNK signaling in ECs [43]. In addition, a recent study demonstrates that in response to JNK signaling caused by tumor growth, EGFs are cleaved and activated by rhomboid to upregulate EGFR signaling in ISCs [44]. JNK, therefore, is a broad sensor of numerous types of tissue damage and can also be activated by alteration in pyruvate metabolism [45]. Strikingly, gut epithelial ROS can signal to nearby other tissues: In response to damage-induced ROS, tracheal cells promote stem cell proliferation via the FGF/FGFR pathway [46,47]. ROS produced in dying ECs cells can also recruit hemocytes, which themselves produce ROS leading to JNK activation in nearby ECs [48].

In addition to tissue damage *per se*, diverse cellular defects in ECs cause a stress response that non-autonomously stimulates ISC proliferation (**Figure 2**). Functional septate junctions (SJ) are essential sensors of EC health; their perturbation leads to activation of Yki and Upd3 expression [12,49–51]. Mechanistically, the SJ component, Tsp2A, promotes endocytosis and lysosomal degradation of aPKC [12]. Since aPKC antagonizes Hippo, its accumulation upon Tsp2A knockdown results in activation of Yki. SJs likely have sensor functions during stress, aging, and defective trafficking or autophagy, conditions in which loss of SJ proteins occur [12,44,52,53]. Conversely, autophagy has an additional role in preventing ISC overproliferation: autophagic degradation of Dach5, an activator of

Yki, is required to prevent Yki-Upd3-driven overproliferation of ISCs in response to ROS generated by commensal bacteria contexts [53].

Overall, these new studies demonstrate how ISCs integrate cell-intrinsic and extrinsic cues from neighboring cells in the tissue to fine-tune proliferation allowing both homeostatic cell replacement and regenerative responses. In addition to coordination within the gut epithelium, it is now appreciated that longer-range signaling from the other tissues including the hemocytes, brain, and gonads. In particular, mating provokes physiological and behavioral changes in females that are mediated by Sex peptide, Juvenile Hormone and Ecdysone allowing an increase in nutrient intake required to fulfill the energy-demanding egg production (further detailed in **Figure 3**).

Stem cell long-term maintenance and aging

Despite the adult fly only living 6 to 8 weeks, the midgut shows significant age-related changes in tissue organization and cellular function during this time. At the tissue level, aging promotes stem cell overproliferation and results in dysplasia. Studies of aging and its impact on *Drosophila* ISCs have been recently comprehensively reviewed [54]. Here, we highlight the newest findings in this area of study.

DNA damage and genome instability

Previous work has demonstrated increased marks for oxidative DNA damage and DNA breaks during aging intestinal stem cells [55]. A recent study also showed an aged-related increase in the level of O-GlcNac, a nutrient-driven post-translational modification of proteins associated with oxidative stress [56]. This O-GlyNacylation in part mediates DNA damage upregulation in ISCs upon oxidative stress and high sugar diet, therefore linking nutrient uptake and glucose metabolism to DNA damage [56].

In addition to increased marks of DNA damage in ISCs during aging, our previous studies found a rise in somatic mutations in the gut [57]. Notably, a functional consequence of this is that 10-15% of aged male flies acquire tumor-like neoplasia due to the spontaneous mutation of the X-linked tumor suppressor *Notch* [57]. Recently, we have expanded our characterization of the genome alterations affecting aged intestinal stem cells [58,59]. Whole-genome sequencing of male neoplasia reveal that structural variants and point mutations occur genome-wide [58]. In addition to structural variants and point mutations, de novo insertion of transposable elements (TEs) are also detected genome-wide as well as within the *Notch* locus, likely responsible for neoplasia formation in some instances [59]. Our finding suggested tissue-specific variation in TE subclass mobility, with some TEs being more mobile in the gut than the brain or germline [59]. Altogether, this demonstrates that the ongoing DNA damage in ISCs during aging can have significant genomic consequences and alter tissue homeostasis.

Nuclear organization and epigenetic regulation during aging

The nuclear organization and chromatin structure were shown to be altered upon aging in different model systems [60]. ECs were shown to undergo alteration of chromatin organization with changes in histone H3K9me3 and HP1 [61]. Similarly, as mentioned above, Hey and NON-Stop promote genomic organization in ECs and their expression in ECs decreases during aging, correlating with changes to Lamin structures [27,28].

How the chromatin landscape and nuclear organization changes in stem cells during aging has only begun to be investigated. Recent ATAC-seq data suggests that aging leads to mild alteration of chromatin accessibility at promoters enriched for binding motifs of Polycomb interactors. The authors suggest that changes in ISC H3K27me2 levels, catalyzed by the Polycomb Repressive Complex 2 (PRC2), lead to a bias toward EE fate by upregulation of EE-specific gene expression [31]. Further characterization of chromatin states in young and aged stem cells would be required to establish the full extent of epigenetic alterations in ISC and differentiated cells.

Metabolic alteration with age

Loss of proteostasis has been associated with aging in numerous tissues and organisms [60]. Upon induction of protein aggregates thought to disrupt proteostasis, ISCs undergo cell cycle arrest, which depends on Keap1-Nrf2/CncC and the cyclin-dependent kinase inhibitor, Dacapo. Interestingly, this "proteostatic checkpoint" is defective in aged ISCs. Overexpression of Nrf2/CncC pathway improves age-related increase in cell proliferation and decline in gut barrier function [62].

As mention above, mitochondria have important roles in ISCs; their quality control during aging is essential in *Drosophila* ISCs [63] as well as in a wide variety of cell types in many species [60]. Mitochondrial metabolism is crucial for ISC proliferation and differentiation [21,64,65]. Upon tissue damage, ISC proliferation requires an intensification of mitochondrial activity [66]. In aged ISCs, mitochondrial Ca²⁺ levels as well as ATP production are reduced marking a switch from mitochondrial respiration to aerobic glycolysis [66]. This Warburg-like metabolic reprogramming drives ISC hyperproliferation and resembles the metabolic rewiring of oncogene-transformed cells [66]. Additional studies will be required to understand how changes in mitochondria and their metabolic function during aging feedback on cell-cell signaling pathways.

Conclusions future questions:

Studies over the last 15 years using the *Drosophila* adult midgut and ISCs as a model have uncovered fundamental underlying principles of stem cells and their interactions with the external environment and other tissues. Through candidate approaches, genetic screening, and scRNA-seq analyses, the field now has a solid framework of the transcriptional regulators control of lineage decisions and proliferation. Nevertheless, future studies adapting additional genomic techniques in this system will undoubtedly provide a better characterization of epigenetic landscapes and their functional relevance in the ISC lineage. The last few years have also demonstrated the versatility of the gut to restore homeostasis after tissue damage and adapt the tissue to organismal needs. This process requires a complex integration of signals in the different cell types and tissues to tune stem cell proliferation and adapt cell differentiation and turnover. Recent advances in live-imaging technologies will contribute to the understanding of the dynamic nature of cell death, cell proliferation and tissue remodeling in real time [67,68]. Future studies will also tease apart the precise role that stem cell proliferation has on aging phenotypes in this tissue. As many age-related alterations in stem cells are rescued by the inhibition of stem cell proliferation, the extent to which aging phenotypes are primary defects or secondary ones, due to deregulated proliferation program needs to be clarified. Importantly, aging is also characterized by intestinal dysbiosis. The interaction with diverse microbiota influences intestinal stem cell function and tissue regeneration [69–71]. While we only alluded to this here, a large body of literature focuses on the effects of commensal and pathogenic bacteria on metabolism and immunity of the gut and organism [72,73]. The insight gained on processes controlling adult stem cells using the *Drosophila* midgut will provide important first principles and testable hypotheses for addition studies in other model organisms and human pluripotent cell culture systems.

Conflict of interest statement

The authors declare no conflicting interests.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest

**of outstanding interest

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Figure 1: Lineage regulators of intestinal stem cell differentiation towards enterendocrine and enterocyte cells

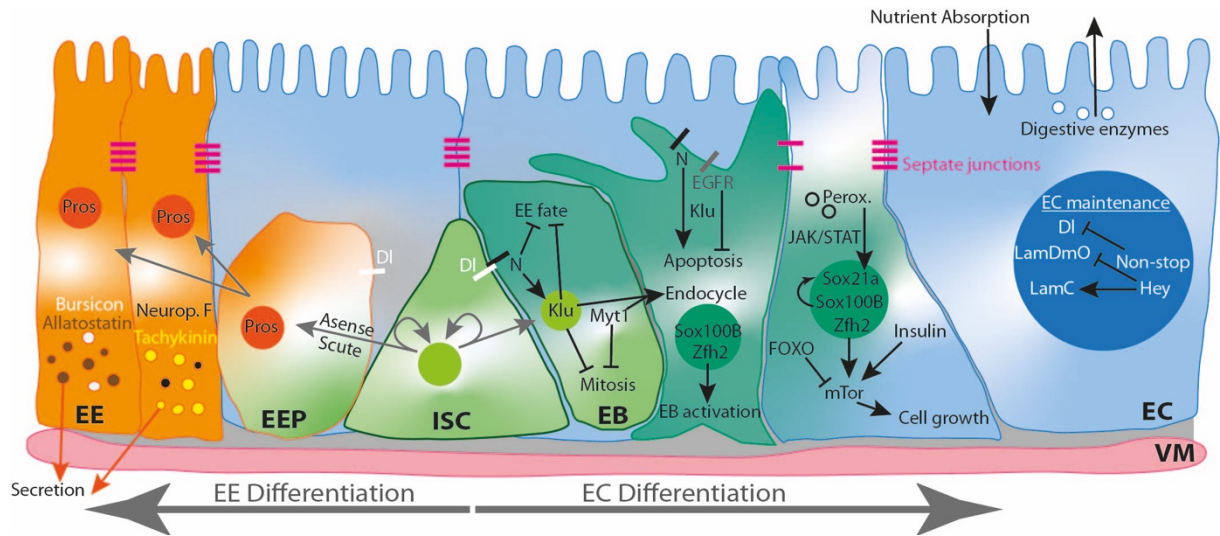
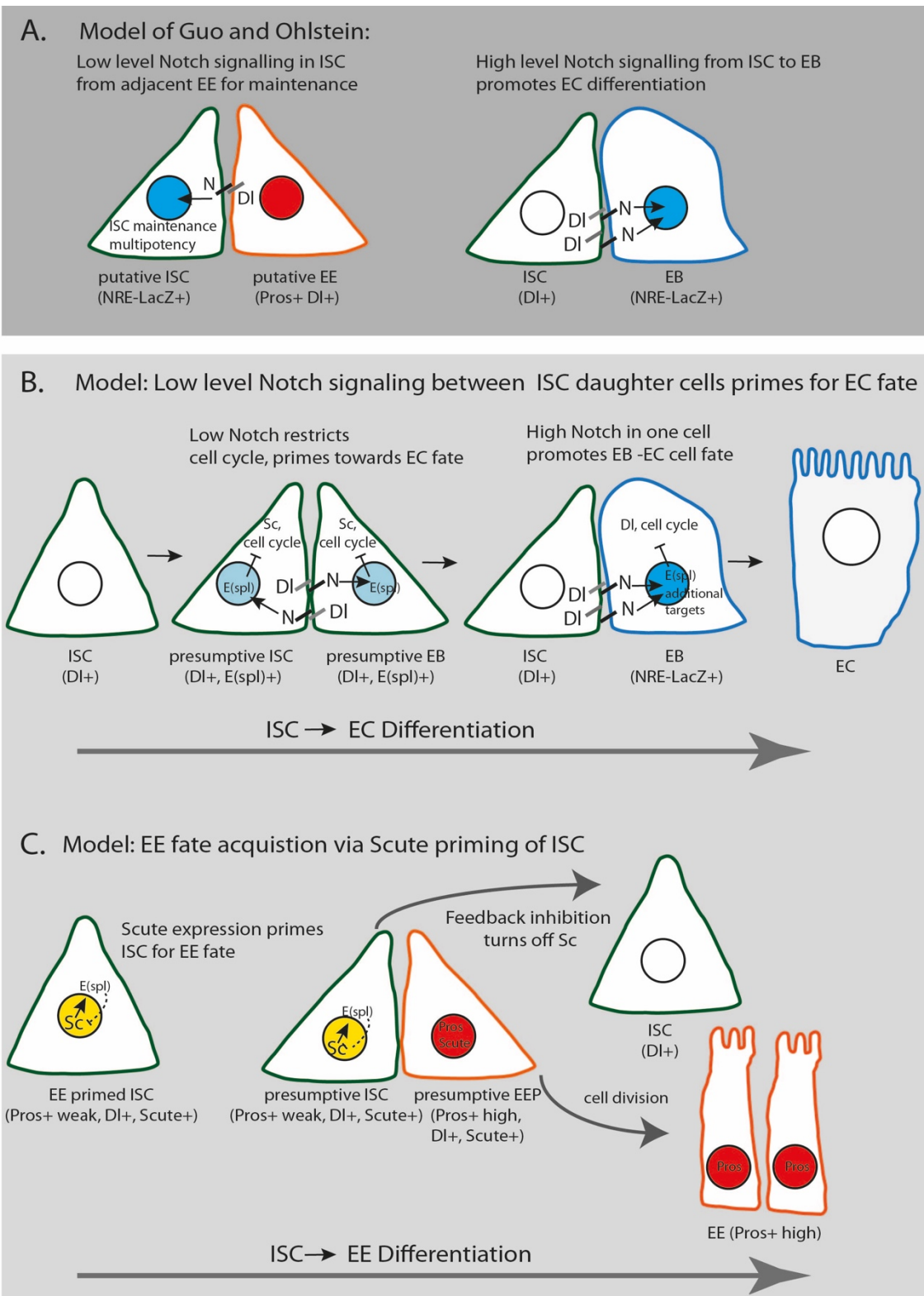


Figure 1: Lineage regulators of intestinal stem cell differentiation towards enterendocrine and enterocyte cells

Intestinal stem cells (ISCs) are essential to maintain tissue homeostasis, they mostly divide asymmetrically to self-renew and produce a differentiating progenitor. Intestinal stem cells are multipotent as they can generate two differentiated cell types: absorptive enterocytes (ECs) representing ~80% of the progeny and hormone secreting enteroendocrine cells (EEs), ~20%. The differentiation is achieved through enteroblasts (EBs) and dividing enteroendocrine precursors (EEPs), respectively. The transcriptional programs driving EE and EC differentiation had been partially described previously (please see Miguel-Aliaga et al., 2018 for more details). Here we focused on recent advances, please see the main text for further details.

BOX 1: Models of Notch signaling and cell fate decisions in the ISC lineage.



BOX1: Models of Notch signaling and cell fate decisions in the ISC lineage.

Studies over the years have implicated different levels of Notch signaling in regulating the ISC lineage [74–76]. A complete understanding of how Notch signaling acts has been challenging, in part due to previous mis-conceptions about the cell lineage. Indeed, evidence now suggests that a dividing enteroendocrine precursor cell (EEP) is made by the stem cell and further divides once to make 2 EE cells. Notch signaling was previously thought to be off in ISCs, though recent data suggest that low levels of Notch signaling and activation of a subset of target genes may have essential functions in ISCs to regulate their maintenance, proliferation, and lineage decisions.

Earlier studies proposed that a low level of Notch signal was activated in ISCs coming from Delta (DI) ligand in adjacent EEs or EE precursor cells (**panel A**) [75]. Support for this model came from genetic experiments in which the overexpression of the proneural transcription factor, *asense* (*ase*), was used to prime all ISCs for the EE cell fate. The authors detected pairs of cells, in which one cell expressed the Notch ligand, Delta as well as the transcription factor Prospero (DI+ Pros+) and the other adjacent cell had an activated reporter for Notch-driven transcription (NRE-LacZ), suggesting signaling from the DI+ cell to its neighbor. The ISC was presumed to be the cell with Notch transcription activity (NRE-LacZ+). The adjacent cell expressing both the stem cell marker DI+ and the EE associated transcription factor Pros (DI+ Pros+) was considered to be an EE (see **panel A**). The authors proposed a model in which Notch signaling is activated in ISCs by DI ligand in adjacent EEs (or EEPs) and promotes ISC survival and multipotency. The genetic experiments supporting these findings are consistent with those of Chen and colleagues, though the interpretations of the underlying role of Notch and in which cell it is activated, differ [4,11,30].

Indeed, more recent studies interrogated the identity of DI+ Pros+ cells using lineage tracing and single cell analyses and support the notion that in wild-type contexts, these are EE-primed ISCs or EEPs [3,4,11,30]. A careful dissection in ISCs and their daughters of the gene regulatory network previously described during lateral inhibition in peripheral nervous system, suggested an alternative model for low level of Notch activation and its function (**panel B**): Notch signaling occurring between the two ISC daughter cells leads to low level activation of bHLH *E(spl)-C* target genes (not detectable by NRE-LacZ reporters) [77]. bHLH *E(spl)-C* proteins with their corepressor, Groucho, act to repress cell cycle genes [77]. We propose that this low-level Notch activation may also be essential to maintain these ISCs in an EC-primed state by repression of the proneural transcription factors *scute*, and likely *asense*, consistent with reports of high levels of Scute upregulation when *Notch* is inactivated [4,78].

How then is an EE cell produced? Elegant studies demonstrated that Scute becomes expressed in a subset of ISCs, likely implicating a Scute auto-feedforward loop [4]. When a sufficient level of Scute is present, the ISC becomes EE-primed and will divide and produce an ISC and an EEP, which upon further cell division will make EE cells (**panel C**).

Many questions remain unanswered here: How exactly is the choice between ISC and EEP made? Can ISCs convert into EEPs in some contexts, leading to their direct production of EEs and loss? Some EE cell sub-types require Notch signaling - does this signaling occur directly between EE cells? If not, are there multiple divisions of EEPs in these sub-lineages, some having Notch-dependent binary decisions? How may Notch cell fate decisions between daughter cells be coupled with the cell division angle? Additional studies with live imaging and fate markers will determine the potential role of Pros segregation during ISC division as well as other factors implicated in EE fate choice such as Slit-Robo signaling, Numb, and mechanical input [3,4,11,14,30]. Future work will undoubtedly reveal additional surprises of cell fate control in the ISC lineage.

Figure 2. Cell autonomous and Non-cell autonomous control of proliferation in the midgut.

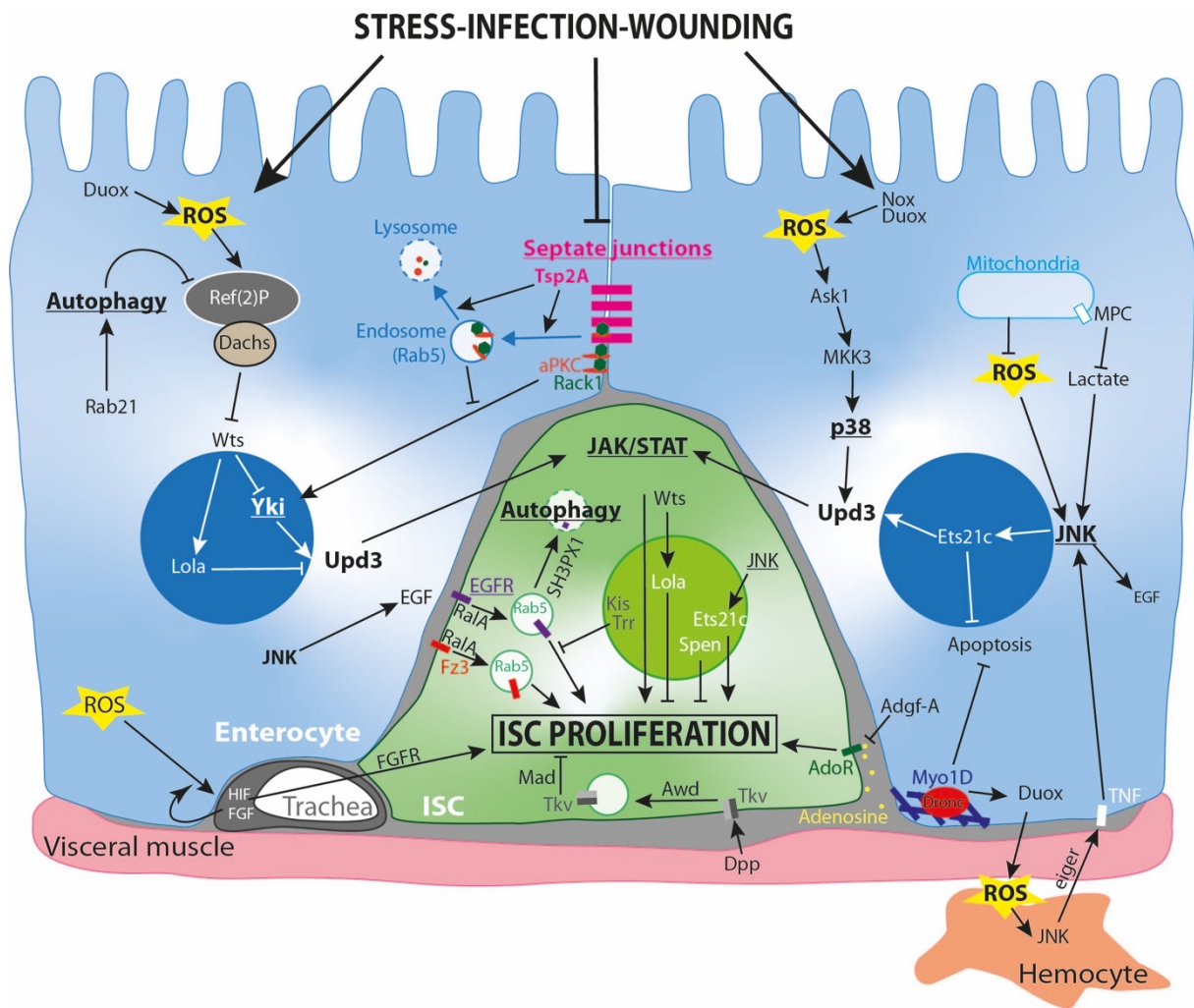


Figure 2. Cell autonomous and Non-cell autonomous control of proliferation in the midgut.

In homeostatic conditions, stem cell proliferation is adjusted to the needs of the tissue. Upon tissue stress or damage, stem cell proliferation is triggered as a regenerative response. To balance proliferation and quiescence, ISCs integrate JAK/STAT, JNK, WNT/Wg, Hippo, EGFR, FGFR, AdoR and BMP/Dpp signaling pathways, cues of which are provided cell-autonomously and non-cell-autonomously. This scheme represents some sensing mechanisms driving mitogenic ligands and interactions recently identified. For simplicity, the arrows can represent direct protein-protein interactions, transcriptional control or genetic interactions. Please see the main text for references.

Figure 3: Steroid and peptide hormone regulation of gut physiology and stem cell proliferation

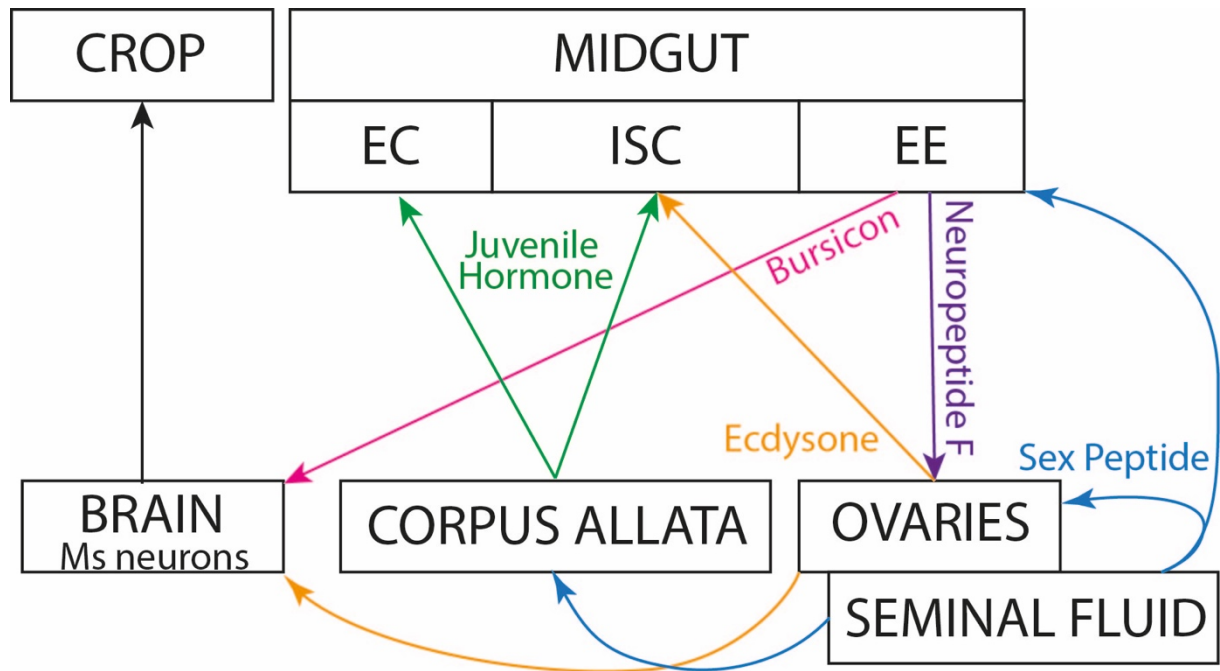


Figure 3: Steroid and peptide hormone regulation of gut physiology and stem cell proliferation

After mating, Sex Peptide (SP) in the male seminal fluid induces a fast response in females responsible for an increase in Ecdysone production by the ovary and an increase in Juvenile hormone production by the corpus allata through a neuronal relay [79,80]. Both SP and Ecdysone directly promote ISC proliferation and gut expansion [81,82]. Mechanistically, Ecdysone induces the expression of EGFR pathway genes (*spi*, *krn*, *rho*) via *Eip75B* [83] and promotes the Pri mediated post-translational modification of the mitogenic factor Shavenbaby [84]. Ecdysone also stimulates food intake through myosuppressin (Ms) expressing neurons mediated crop enlargement [85]. In addition, the enteroendocrine cell (EE) pool increases upon mating. EE secretion of the neuroendocrine peptide Bursicon drives: (1) Ms neuron activation responsible for food intake [85], revealing a brain-gut-brain axis regulated by Ecdysone; and (2) lipid metabolism and energy catabolism in the Enterocytes and in the fat body [80,86]. Through different pathways, enteroendocrine cells modulate organismal metabolism [87]. EE cells feedback to the ovaries and promote germline stem cells division via production of neuropeptide F in response to SP signaling [79]. In males as well midgut metabolic changes are linked to sperm production and food intake regulation [88].