



HAL
open science

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

Benjamin Boumard, Allison J Bardin

► To cite this version:

Benjamin Boumard, Allison J Bardin. An amuse bouche of stem cell regulation: Underlying principles and mechanisms from adult *Drosophila* intestinal stem cells. *Current Opinion in Cell Biology*, 2021, 73, pp.58-68. 10.1016/j.ccb.2021.05.007 . hal-03442869

HAL Id: hal-03442869

<https://hal.science/hal-03442869>

Submitted on 23 Nov 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Benjamin Boumard¹ and Allison J. Bardin¹

¹ Institut Curie, PSL Research University, CNRS UMR 3215, INSERM U934, Stem Cells and Tissue Homeostasis Group, Paris, France.

author for correspondence (allison.bardin@curie.fr)

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

Acknowledgements

We apologize to our colleagues whose relevant work we could not cite due to space constraints. We thank members of the Bardin lab for input on the manuscript. AB receives salary support from the CNRS, BB from ENS de Lyon and the Fondation pour la Recherche Médicale (FDT202001010957). The Bardin lab supported by grants from the Worldwide Cancer Research Foundation (AB, 20-0147); Fondation pour la Recherche Médicale (AB, EQU202003010251), as well as funding from the program “Investissements d’Avenir” launched by the French Government and implemented by ANR, references: ANR SoMuSeq-STEM (AB), ANR ChronoDamage (AB), Labex DEEP (ANR-11-LBX-0044) and IDEX PSL (ANR-10-IDEX-0001-02 PSL).

REFERENCES

1. Micchelli CA, Perrimon N: **Evidence that stem cells reside in the adult *Drosophila* midgut epithelium.** *Nature* 2006, **439**:475–479.
2. Ohlstein B, Spradling A: **The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells.** *Nature* 2006, **439**:470–474.
3. He L, Si G, Huang J, Samuel ADT, Perrimon N: **Mechanical regulation of stem-cell differentiation by the stretch-activated Piezo channel.** *Nature* 2018, doi:10.1038/nature25744.
4. Chen J, Xu N, Wang C, Huang P, Huang H, Jin Z, Yu Z, Cai T, Jiao R, Xi R: **Transient Scute activation via a self-stimulatory loop directs enteroendocrine cell pair specification from self-renewing intestinal stem cells.** *Nat Cell Biol* 2018, doi:10.1038/s41556-017-0020-0.
5. Goulas S, Conder R, Knoblich JA: **The Par Complex and Integrins Direct Asymmetric Cell Division in Adult Intestinal Stem Cells.** *Cell Stem Cell* 2012, **11**:529–540.
6. Hu DJ-K, Jasper H: **Control of Intestinal Cell Fate by Dynamic Mitotic Spindle Repositioning Influences Epithelial Homeostasis and Longevity.** *Cell Rep* 2019, **28**:2807-2823.e5.
**** The authors identify here mechanisms controlling spindle orientation and stem cell fate in dividing intestinal stem cells. Spindle repositioning in condition of stress and aging promotes symmetric fate and stem cell accumulation.**
7. Tian A, Wang B, Jiang J: **Injury-stimulated and self-restrained BMP signaling dynamically regulates stem cell pool size during *Drosophila* midgut regeneration.** *Proc Natl Acad Sci* 2017, **114**:E2699–E2708.
8. Khaminets A, Ronnen-Oron T, Baldauf M, Meier E, Jasper H: **Cohesin controls intestinal stem cell identity by maintaining association of Escargot with target promoters.** *eLife* 2020, **9**.
9. Li Z, Guo X, Huang H, Wang C, Yang F, Zhang Y, Wang J, Han L, Jin Z, Cai T, et al.: **A Switch in Tissue Stem Cell Identity Causes Neuroendocrine Tumors in *Drosophila* Gut.** *Cell Rep* 2020, **30**:1724-1734.e4.
10. Buddika K, Huang Y-T, Butrum-Griffith A, Norrell SA, O'Connor AM, Patel VK, Rector SA, Slovan M, Sokolowski M, Kato Y, et al.: **Intestinal progenitor P-bodies maintain stem cell identity by suppressing pro-differentiation factors.** *BioRxiv* 2020, doi:10.1101/2020.06.27.175398.
11. Hung R-J, Hu Y, Kirchner R, Liu Y, Xu C, Comjean A, Tattikota SG, Li F, Song W, Ho Sui S, et al.: **A cell atlas of the adult *Drosophila* midgut.** *Proc Natl Acad Sci* 2020, **117**:1514–1523.
*** In the study, the authors performed single-cell sequencing of the whole gut. They could identify new transcription factors involved in progenitor cell identity and differentiation, as well as new enteroendocrine cell hormones and their expression diversity.**
12. Xu C, Tang H-W, Hung R-J, Hu Y, Ni X, Housden BE, Perrimon N: **The Septate Junction Protein Tsp2A Restricts Intestinal Stem Cell Activity via Endocytic Regulation of aPKC and Hippo Signaling.** *Cell Rep* 2019, **26**:670-688.e6.
**** In this study, the authors uncover the regulating mechanism by which septate junctions in the enterocytes are affecting stem cell proliferation. By promoting aPKC degradation, the septate junction protein Tsp2A limits Yorkie-dependent cytokine expression in the enterocytes.**
13. Antonello ZA, Reiff T, Ballesta-Illan E, Dominguez M: **Robust intestinal homeostasis relies on cellular plasticity in enteroblasts mediated by miR-8-Escargot**

switch. *EMBO J* 2015, **34**:2025–2041.

14. Miguel-Aliaga I, Jasper H, Lemaitre B: **Anatomy and Physiology of the Digestive Tract of *Drosophila melanogaster***. *Genetics* 2018, **210**:357–396.

15. Korzelius J, Azami S, Ronnen-Oron T, Koch P, Baldauf M, Meier E, Rodriguez-Fernandez IA, Groth M, Sousa-Victor P, Jasper H: **The WT1-like transcription factor Klumpfuss maintains lineage commitment of enterocyte progenitors in the *Drosophila* intestine**. *Nat Commun* 2019, **10**:4123.

*The authors identified an enteroblast specific transcription factor and characterized its functions in lineage commitment downstream of Notch signaling. Klumpfuss suppresses enteroendocrine gene expression and promotes enterocyte differentiation.

16. Doupé DP, Marshall OJ, Dayton H, Brand AH, Perrimon N: ***Drosophila* intestinal stem and progenitor cells are major sources and regulators of homeostatic niche signals**. *Proc Natl Acad Sci* 2018, doi:10.1073/pnas.1719169115.

17. Jin Z, Chen J, Huang H, Wang J, Lv J, Yu M, Guo X, Zhang Y, Cai T, Xi R: **The *Drosophila* Ortholog of Mammalian Transcription Factor Sox9 Regulates Intestinal Homeostasis and Regeneration at an Appropriate Level**. *Cell Rep* 2020, **31**:107683.

18. Meng FW, Rojas Villa SE, Biteau B: **Sox100B Regulates Progenitor-Specific Gene Expression and Cell Differentiation in the Adult *Drosophila* Intestine**. *Stem Cell Rep* 2020, **14**:226–240.

19. Rojas Villa SE, Meng FW, Biteau B: **zfh2 controls progenitor cell activation and differentiation in the adult *Drosophila* intestinal absorptive lineage**. *PLOS Genet* 2019, **15**:e1008553.

20. Du G, Xiong L, Li X, Zhuo Z, Zhuang X, Yu Z, Wu L, Xiao D, Liu Z, Jie M, et al.: **Peroxisome Elevation Induces Stem Cell Differentiation and Intestinal Epithelial Repair**. *Dev Cell* 2020, doi:10.1016/j.devcel.2020.03.002.

* This study shows a surprisingly specific effect of peroxisome function on intestinal stem cell differentiation. Peroxisome elevation promotes progenitor cell differentiation via a JAK/STAT dependent upregulation of Sox21a transcriptional signaling

21. Zhang F, Pirooznia M, Xu H: **Mitochondria regulate intestinal stem cell proliferation and epithelial homeostasis through FOXO**. *Mol Biol Cell* 2020, **31**:1538–1549.

22. Willms RJ, Zeng J, Campbell SD: **Myt1 Kinase Couples Mitotic Cell Cycle Exit with Differentiation in *Drosophila***. *Cell Rep* 2020, **33**:108400.

23. Arnautov A, Lee H, Haase KP, Aksenova V, Jarnik M, Oliver B, Serpe M, Dasso M: **IRBIT Directs Differentiation of Intestinal Stem Cell Progeny to Maintain Tissue Homeostasis**. *iScience* 2020, doi:10.1016/j.isci.2020.100954.

24. Reiff T, Antonello ZA, Ballesta-Illán E, Mira L, Sala S, Navarro M, Martinez LM, Dominguez M: **Notch and EGFR regulate apoptosis in progenitor cells to ensure gut homeostasis in *Drosophila***. *EMBO J* 2019, doi:10.15252/embj.2018101346.

** Here the authors elegantly describe how apoptosis is regulated in enteroblasts to control the progenitor cell pool. They identify the antagonistic regulation by Notch and EGFR of the pro-apoptotic transcription factors Klumpfuss and Lozenge, which are also involved in enterocyte differentiation.

25. Arthurton L, Nahotko DA, Alonso J, Wendler F, Baena-Lopez LA: **Non-apoptotic caspase activation preserves *Drosophila* intestinal progenitor cells in quiescence**. *EMBO Rep* 2020, **21**.

26. Lindblad JL, Tare M, Amcheslavsky A, Shields A, Bergmann A: **Non-apoptotic enteroblast-specific role of the initiator caspase Dronc for development and homeostasis of the *Drosophila* intestine**. *Sci Rep* 2021, **11**:2645.

27. Erez N, Israitel L, Bitman-Lotan E, Wong WH, Raz G, Cornelio-Parra DV, Danial S,

Flint Brodsky N, Belova E, Maksimenko O, et al.: **A Non-stop identity complex (NIC) supervises enterocyte identity and protects from premature aging.** *eLife* 2021, **10**:e62312.
** This study reports the role of a complex containing Non-stop in maintaining the enterocyte specific gene signature by ensuring chromatin accessibility. Upon aging, the levels of the complex subunits decline and the enterocyte fate is altered.

28. Flint Brodsky N, Bitman-Lotan E, Boico O, Shafat A, Monastirioti M, Gessler M, Delidakis C, Rincon-Arango H, Orian A: **The transcription factor Hey and nuclear lamins specify and maintain cell identity.** *eLife* 2019, **8**:e44745.

29. Gervais L, Bardin AJ: **Tissue homeostasis and aging: new insight from the fly intestine.** *Curr Opin Cell Biol* 2017, **48**:97–105.

30. Guo X, Yin C, Yang F, Zhang Y, Huang H, Wang J, Deng B, Cai T, Rao Y, Xi R: **The Cellular Diversity and Transcription Factor Code of Drosophila Enteroendocrine Cells.** *Cell Rep* 2019, **29**:4172-4185.e5.

* Using single cell sequencing of sorted cells, the authors describe here the variety of hormone combination expressed by different enteroendocrine cells as well as the specific transcription factors implicated in their differentiation and regulation.

31. Tauc HM, Rodriguez-Fernandez IA, Hackney JA, Pawlak M, Ronnen Oron T, Korzelius J, Moussa HF, Chaudhuri S, Modrusan Z, Edgar BA, et al.: **Age-related changes in polycomb gene regulation disrupt lineage fidelity in intestinal stem cells.** *eLife* 2021, **10**:e62250.

32. Beehler-Evans R, Micchelli CA: **Generation of enteroendocrine cell diversity in midgut stem cell lineages.** *Development* 2015, **142**:654–664.

33. Tracy Cai X, Li H, Safyan A, Gawlik J, Pyrowolakis G, Jasper H: **AWD regulates timed activation of BMP signaling in intestinal stem cells to maintain tissue homeostasis.** *Nat Commun* 2019, **10**.

34. Johansson J, Naszai M, Hodder MC, Pickering KA, Miller BW, Ridgway RA, Yu Y, Peschard P, Brachmann S, Campbell AD, et al.: **RAL GTPases Drive Intestinal Stem Cell Function and Regeneration through Internalization of WNT Signalosomes.** *Cell Stem Cell* 2019, **24**:592-607.e7.

35. Naszai M, Yu Y, Fernandez AR, Sandilands E, Johansson J, Norman JC, Sansom OJ, Bryant D, Cordero JB: **RAL GTPases mediate EGFR/MAPK signalling-driven intestinal stem cell proliferation and tumorigenesis upstream of RAS activation.** *BioRxiv* 2020, doi:10.1101/2020.10.07.329607.

36. Zhang P, Holowatyj AN, Roy T, Pronovost SM, Marchetti M, Liu H, Ulrich CM, Edgar BA: **An SH3PX1-Dependent Endocytosis-Autophagy Network Restrains Intestinal Stem Cell Proliferation by Counteracting EGFR-ERK Signaling.** *Dev Cell* 2019, **49**:574-589.e5.

** The authors show how the sorting nexin SH3PX1 promotes autophagic degradation of EGFR in intestinal and thus limits proliferation. They extensively describe the endocytosis-autophagy trafficking pathway regulating EGFR degradation or recycling.

37. Gervais L, van den Beek M, Jossierand M, Sallé J, Stefanutti M, Perdigoto CN, Skorski P, Mazouni K, Marshall OJ, Brand AH, et al.: **Stem Cell Proliferation Is Kept in Check by the Chromatin Regulators Kismet/CHD7/CHD8 and Trr/MLL3/4.** *Dev Cell* 2019, **49**:556-573.e6.

** In this work, the authors reported the function of chromatin regulators Kismet and Trithorax-related in regulating stem cell proliferation. Using DamID and RNA sequencing technique they show a direct binding and transcriptional control of EGFR signaling by these factors.

38. Hao X, Wang S, Lu Y, Yu W, Li P, Jiang D, Guo T, Li M, Li J, Xu J, et al.: **Lola regulates Drosophila adult midgut homeostasis via non-canonical hippo signaling.** *Elife*

2020,

39. Andriatsilavo M, Stefanutti M, Siudeja K, Perdigoto CN, Boumard B, Gervais L, Gillet-Markowska A, Al Zouabi L, Schweisguth F, Bardin AJ: **Spen limits intestinal stem cell self-renewal**. *PLoS Genet* 2018, **14**:e1007773.
40. Xu C, Franklin B, Tang H-W, Regimbald-Dumas Y, Hu Y, Ramos J, Bosch JA, Villalta C, He X, Perrimon N: **An in vivo RNAi screen uncovers the role of AdoR signaling and adenosine deaminase in controlling intestinal stem cell activity**. *Proc Natl Acad Sci* 2019, doi:10.1073/pnas.1900103117.
41. Patel PH, Pénalva C, Kardorff M, Roca M, Pavlović B, Thiel A, Teleman AA, Edgar BA: **Damage sensing by a Nox-Ask1-MKK3-p38 signaling pathway mediates regeneration in the adult *Drosophila* midgut**. *Nat Commun* 2019, **10**.
* This study precisely dissects the role and genetic interactions of the Ask1-p38 signaling pathway in enterocytes upon ROS production in response to different epithelial stress.
42. Mundorf J, Donohoe CD, McClure CD, Southall TD, Uhlirova M: **Ets21c Governs Tissue Renewal, Stress Tolerance, and Aging in the *Drosophila* Intestine**. *Cell Rep* 2019, **27**:3019-3033.e5.
43. Dai Z, Li D, Du X, Ge Y, Hursh DA, Bi X: ***Drosophila* Caliban preserves intestinal homeostasis and lifespan through regulating mitochondrial dynamics and redox state in enterocytes**. *PLoS Genet* 2020, **16**:e1009140.
44. Ngo S, Liang J, Su Y-H, O'Brien LE: **Disruption of EGF Feedback by Intestinal Tumors and Neighboring Cells in *Drosophila***. *Curr Biol* 2020, doi:10.1016/j.cub.2020.01.082.
* The authors describe here how intestinal tumor cells and their neighboring cells fuel tumor proliferation through activation of the EGFR pathway in two different feed-forward mechanisms.
45. Wisidagama DR, Thummel CS: **Regulation of *Drosophila* Intestinal Stem Cell Proliferation by Enterocyte Mitochondrial Pyruvate Metabolism**. *G3 GenesGenomesGenetics* 2019, doi:10.1534/g3.119.400633.
46. Perochon J, Yu Y, Aughey GN, Medina AB, Southall TD, Cordero JB: **Dynamic adult tracheal plasticity drives stem cell adaptation to changes in intestinal homeostasis in *Drosophila***. *Nat Cell Biol* 2021, doi:10.1038/s41556-021-00676-z.
47. Tamamouna V, Rahman MM, Petersson M, Charalambous I, Kux K, Mainor H, Bolender V, Isbilir B, Edgar BA, Pitsouli C: **Remodelling of oxygen-transporting tracheoles drives intestinal regeneration and tumorigenesis in *Drosophila***. *Nat Cell Biol* 2021, doi:10.1038/s41556-021-00674-1.
48. Amcheslavsky A, Lindblad JL, Bergmann A: **Transiently “Undead” Enterocytes Mediate Homeostatic Tissue Turnover in the Adult *Drosophila* Midgut**. *Cell Rep* 2020, **33**:108408.
* This study reports an apoptosis-induced proliferation in the intestine resembling the mechanism previously described in the wing disc. Prior to death enterocytes promote intestinal stem cell proliferation through ROS generation, hemocyte recruitment and JNK signaling.
49. Izumi Y, Furuse K, Furuse M: **Septate junctions regulate gut homeostasis through regulation of stem cell proliferation and enterocyte behavior in *Drosophila***. *J Cell Sci* 2019, **132**:jcs232108.
50. Chen H-J, Li Q, Nirala NK, Ip YT: **The Snakeskin-Mesh Complex of Smooth Septate Junction Restricts Yorkie to Regulate Intestinal Homeostasis in *Drosophila***. *Stem Cell Rep* 2020, doi:10.1016/j.stemcr.2020.03.021.
51. Izumi Y, Furuse K, Furuse M: **The novel membrane protein Hoka regulates septate junction organization and stem cell homeostasis in the *Drosophila* gut**. *J Cell Sci* 2021,

134.

52. Resnik-Docampo M, Koehler CL, Clark RI, Schinaman JM, Sauer V, Wong DM, Lewis S, D'Alterio C, Walker DW, Jones DL: **Tricellular junctions regulate intestinal stem cell behaviour to maintain homeostasis.** *Nat Cell Biol* 2016, doi:10.1038/ncb3454.

53. Nagai H, Tatara H, Tanaka-Furuhashi K, Kurata S, Yano T: **Homeostatic Regulation of ROS-Triggered Hippo-Yki Pathway via Autophagic Clearance of Ref(2)P/p62 in the Drosophila Intestine.** *Dev Cell* 2021, **56**:81-94.e10.

** In this study, the authors identified an important role of the autophagic pathway in enterocytes in the sensitization to reactive oxygen species produced in response to bacteria. Accumulation of Ref(2)P antagonizes the Hippo pathway and non-cell autonomously promotes stem cell proliferation.

54. Rodriguez-Fernandez IA, Tauc HM, Jasper H: **Hallmarks of aging in Drosophila intestinal stem cells.** *Mech Ageing Dev* 2020, **190**:111285.

55. Park J-S, Lee S-H, Na H-J, Pyo J-H, Kim Y-S, Yoo M-A: **Age- and oxidative stress-induced DNA damage in Drosophila intestinal stem cells as marked by Gamma-H2AX.** *Exp Gerontol* 2012, **47**:401–405.

56. Na H, Akan I, Abramowitz LK, Hanover JA: **Nutrient-Driven O-GlcNAcylation Controls DNA Damage Repair Signaling and Stem/Progenitor Cell Homeostasis.** *Cell Rep* 2020, **31**:107632.

57. Siudeja K, Nassari S, Gervais L, Skorski P, Lameiras S, Stolfi D, Zande M, Bernard V, Frio TR, Bardin AJ: **Frequent Somatic Mutation in Adult Intestinal Stem Cells Drives Neoplasia and Genetic Mosaicism during Aging.** *Cell Stem Cell* 2015, **17**:663–674.

** Using whole-genome Illumina and Nanopore sequencing techniques, the authors clearly address here the prevalence and tissue specificity of somatic transposition. Transposable elements insert in open chromatin and genic regions, but their insertion frequency does not correlate with their expression levels.

58. Riddiford N, Siudeja K, van den Beek M, Boumard B, Bardin AJ: **Evolution and genomic signatures of spontaneous somatic mutation in Drosophila intestinal stem cells.** *BioRxiv* 2020, doi:10.1101/2020.07.20.188979.

59. Siudeja K, Wurmser A, Stefanutti M, Lameiras S, Bardin AJ: **Unraveling the features of somatic transposition in the Drosophila intestine.** *EMBO J* 2021,

60. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G: **The Hallmarks of Aging.** *Cell* 2013, **153**:1194–1217.

61. Jeon H-J, Kim Y-S, Kim J-G, Heo K, Pyo J-H, Yamaguchi M, Park J-S, Yoo M-A: **Effect of heterochromatin stability on intestinal stem cell aging in Drosophila.** *Mech Ageing Dev* 2018, **173**:50–60.

62. Rodriguez-Fernandez IA, Qi Y, Jasper H: **Loss of a proteostatic checkpoint in intestinal stem cells contributes to age-related epithelial dysfunction.** *Nat Commun* 2019, **10**.

63. Koehler CL, Perkins GA, Ellisman MH, Jones DL: **Pink1 and Parkin regulate Drosophila intestinal stem cell proliferation during stress and aging.** *J Cell Biol* 2017, **216**:2315–2327.

64. Schell JC, Wisidagama DR, Bensard C, Zhao H, Wei P, Tanner J, Flores A, Mohlman J, Sorensen LK, Earl CS, et al.: **Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism.** *Nat Cell Biol* 2017, doi:10.1038/ncb3593.

65. Bensard CL, Wisidagama DR, Olson KA, Berg JA, Krah NM, Schell JC, Nowinski SM, Fogarty S, Bott AJ, Wei P, et al.: **Regulation of Tumor Initiation by the Mitochondrial Pyruvate Carrier.** *Cell Metab* 2019, doi:10.1016/j.cmet.2019.11.002.

66. Morris O, Deng H, Tam C, Jasper H: **Warburg-like Metabolic Reprogramming in Aging Intestinal Stem Cells Contributes to Tissue Hyperplasia.** *Cell Rep* 2020,

33:108423.

67. Koyama LAJ, Aranda-Díaz A, Su Y-H, Balachandra S, Martin JL, Ludington WB, Huang KC, O'Brien LE: **Bellymount enables longitudinal, intravital imaging of abdominal organs and the gut microbiota in adult *Drosophila***. *PLoS Biol* 2020, **18**:e3000567.

68. Martin JL, Sanders EN, Moreno-Roman P, Jaramillo Koyama LA, Balachandra S, Du X, O'Brien LE: **Long-term live imaging of the *Drosophila* adult midgut reveals real-time dynamics of division, differentiation and loss**. *eLife* 2018, **7**:e36248.

69. Ferguson M, Petkau K, Shin M, Galenza A, Fast D, Foley E: **Differential effects of commensal bacteria on progenitor cell adhesion, division symmetry and tumorigenesis in the *Drosophila* intestine**. *Development* 2021, doi:10.1242/dev.186106.

70. von Frieling J, Faisal MN, Sporn F, Pfefferkorn R, Nolte SS, Sommer F, Rosenstiel P, Roeder T: **A high-fat diet induces a microbiota-dependent increase in stem cell activity in the *Drosophila* intestine**. *PLoS Genet* 2020, **16**:e1008789.

71. Zhou J, Boutros M: **JNK-dependent intestinal barrier failure disrupts host-microbe homeostasis during tumorigenesis**. *Proc Natl Acad Sci* 2020, **117**:9401–9412.

72. Grenier T, Leulier F: **How commensal microbes shape the physiology of *Drosophila melanogaster***. *Curr Opin Insect Sci* 2020, **41**:92–99.

73. Lesperance DN, Broderick NA: **Microbiomes as modulators of *Drosophila melanogaster* homeostasis and disease**. *Curr Opin Insect Sci* 2020, **39**:84–90.

74. Perdigoto CN, Schweisguth F, Bardin AJ: **Distinct levels of Notch activity for commitment and terminal differentiation of stem cells in the adult fly intestine**. *Development* 2011, **138**:4585–4595.

75. Guo Z, Ohlstein B: **Bidirectional Notch signaling regulates *Drosophila* intestinal stem cell multipotency**. *Science* 2015, **350**:aab0988–aab0988.

76. Ohlstein B, Spradling A: **Multipotent *Drosophila* Intestinal Stem Cells Specify Daughter Cell Fates by Differential Notch Signaling** | *Science*. *Science* 2007, **315**:988–992.

77. Guo X, Huang H, Yang Z, Cai T, Xi R: **Division of Labor: Roles of Groucho and CtBP in Notch-Mediated Lateral Inhibition that Controls Intestinal Stem Cell Differentiation in *Drosophila***. *Stem Cell Rep* 2019, **12**:1007–1023.

* [The function of the transcriptional co-repressors Groucho and CtBP in stem cells and enteroblasts is investigated here. It refines our understanding of the Notch dependent expression of E\(spl\)-C genes regulating proliferation and differentiation.](#)

78. Bardin AJ, Perdigoto CN, Southall TD, Brand AH, Schweisguth F: **Transcriptional control of stem cell maintenance in the *Drosophila* intestine**. *Development* 2010, **137**:705–714.

79. Ameku T, Yoshinari Y, Texada MJ, Kondo S, Amezawa K, Yoshizaki G, Shimada-Niwa Y, Niwa R: **Midgut-derived neuropeptide F controls germline stem cell proliferation in a mating-dependent manner**. *PLoS Biol* 2018, **16**:e2005004.

80. White MA, Bonfini A, Wolfner MF, Buchon N: ***Drosophila melanogaster* sex peptide regulates mated female midgut morphology and physiology**. *Proc Natl Acad Sci* 2021, **118**:e2018112118.

81. Reiff T, Jacobson J, Cognigni P, Antonello Z, Ballesta E, Tan KJ, Yew JY, Dominguez M, Miguel-Aliaga I: **Endocrine remodelling of the adult intestine sustains reproduction in *Drosophila***. *eLife* 2015, **4**:e06930.

82. Zipper L, Jassmann D, Burgmer S, Görlich B, Reiff T: **Ecdysone steroid hormone remote controls intestinal stem cell fate decisions via the PPAR γ -homolog Eip75B in *Drosophila***. *eLife* 2020, **9**:e55795.

* [In this study, the authors describe the intestinal stem cell role of Eip75B downstream of](#)

Ecdysone reception. It demonstrates that a systemic hormone can promote stem cell proliferation.

83. Ahmed SMH, Maldera JA, Kronic D, Paiva-Silva GO, Pénalva C, Teleman AA, Edgar BA: **Fitness trade-offs incurred by ovary-to-gut steroid signalling in *Drosophila***. *Nature* 2020, **584**:415–419.

** In this work, the authors report the mating-dependent regulation of intestinal stem cell proliferation and growth of the gut by ovary-produced steroids. The intestinal expansion is shown to be essential to support the reproductive need in nutrients at the expense of tissue health as it also promotes tumorigenesis.

84. Al Hayek S, Alsawadi A, Kambris Z, Boquete J, Bohère J, Immarigeon C, Ronsin B, Plaza S, Lemaitre B, Payre F, et al.: **Steroid-dependent switch of *OvoL/Shavenbaby* controls self-renewal versus differentiation of intestinal stem cells**. *EMBO J* 2020, doi:10.15252/embj.2019104347.

85. Hadjieconomou D, King G, Gaspar P, Mineo A, Blackie L, Ameku T, Studd C, de Mendoza A, Diao F, White BH, et al.: **Enteric neurons increase maternal food intake during reproduction**. *Nature* 2020, **587**:455–459.

* In this study, the authors report a Brain-Gut-Brain axis regulating food intake after mating. They identified a subset of neuron innervating the crop and responding to hormones produced by the ovaries and the enterodocrine cells.

86. Scopelliti A, Bauer C, Yu Y, Zhang T, Kruspig B, Murphy DJ, Vidal M, Maddocks ODK, Cordero JB: **A Neuronal Relay Mediates a Nutrient Responsive Gut/Fat Body Axis Regulating Energy Homeostasis in Adult *Drosophila***. *Cell Metab* 2019, **29**:269-284.e10.

87. Zhou X, Ding G, Li J, Xiang X, Rushworth E, Song W: **Physiological and Pathological Regulation of Peripheral Metabolism by Gut-Peptide Hormones in *Drosophila***. *Front Physiol* 2020, **11**:577717.

88. Hudry B, de Goeij E, Mineo A, Gaspar P, Hadjieconomou D, Studd C, Mokochinski JB, Kramer HB, Plaçais P-Y, Preat T, et al.: **Sex Differences in Intestinal Carbohydrate Metabolism Promote Food Intake and Sperm Maturation**. *Cell* 2019, **178**:901-918.e16.

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

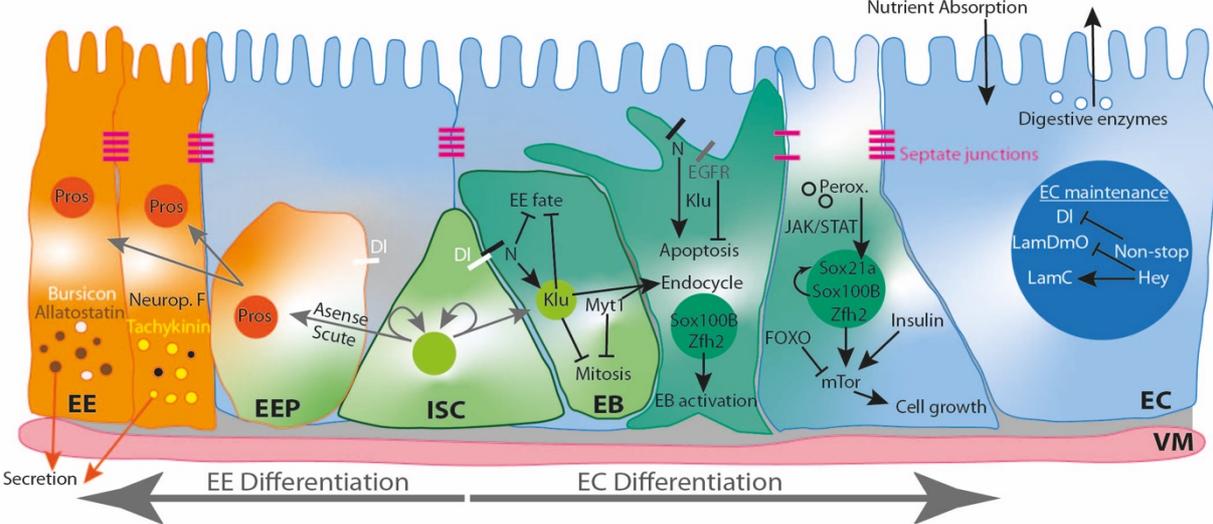
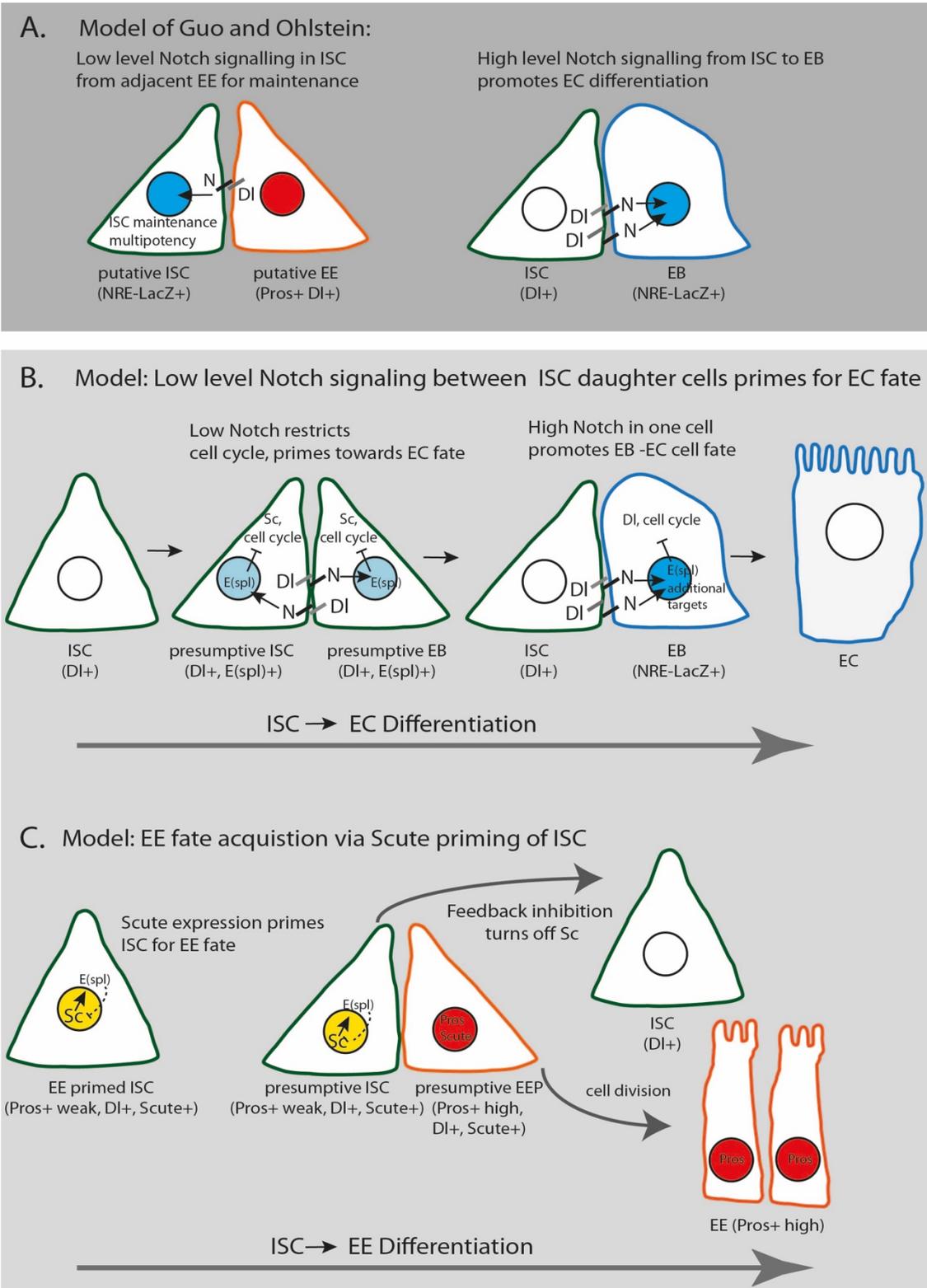


Figure 1: Lineage regulators of intestinal stem cell differentiation towards enterendocrine and enterocyte cells

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.

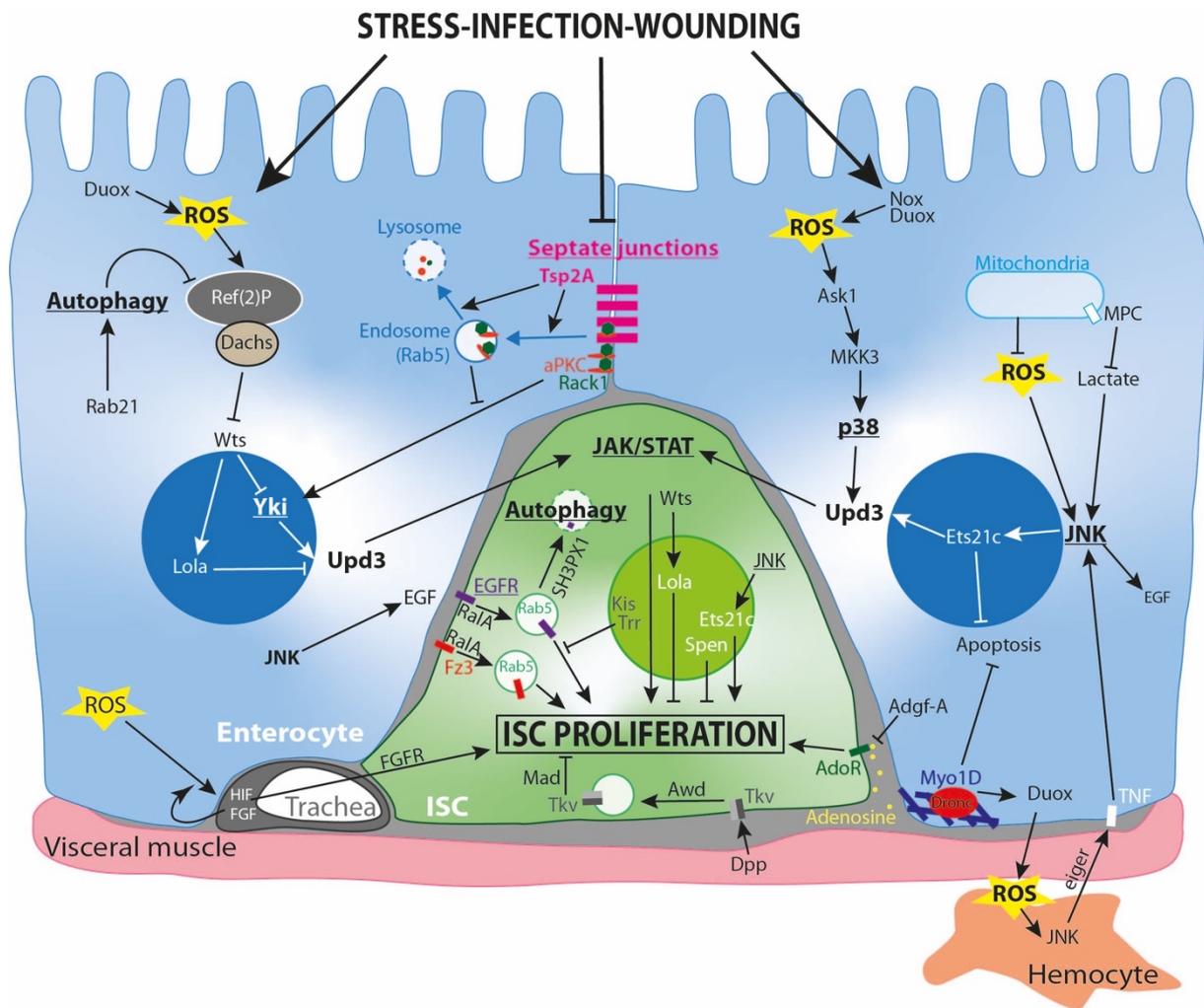


Figure 2. Cell autonomous and Non-cell autonomous control of proliferation in the midgut.

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

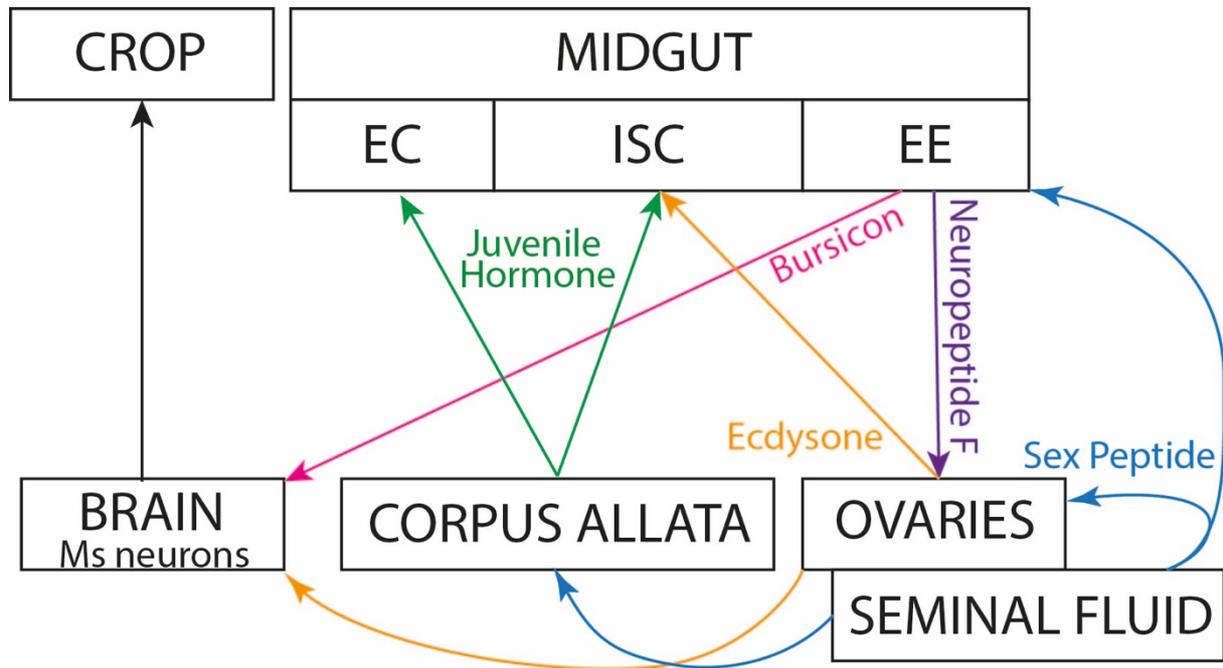


Figure 3: Steroid and peptide hormone regulation of gut physiology and stem cell proliferation

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].