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Intestinal Stem Cell Aging: Origins and Interventions

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Abstract

Regenerative processes that maintain the function of the gastrointestinal (GI) epithelium are critical for health and survival of multicellular organisms. In insects and vertebrates, intestinal stem cells (ISCs) regenerate the GI epithelium. ISC function is regulated by intrinsic, local, and systemic stimuli to adjust regeneration to tissue demands. These control mechanisms decline with age, resulting in significant perturbation of intestinal homeostasis. Processes that lead to this decline have been explored intensively in *Drosophila melanogaster* in recent years and are now starting to be characterized in mammalian models. This review presents a model for age-related regenerative decline in the fly intestine and discusses recent findings that start to establish molecular mechanisms of age-related decline of mammalian ISC function.

SOMATIC STEM CELLS: LOCAL, SYSTEMIC, AND CELL AUTONOMOUS REGULATION

Most animals harbor an intrinsic capacity to heal and regenerate tissues. Central to this capacity is the availability of a population of cells with sufficient potency to recreate all differentiated cell types needed for tissue function. Such somatic stem cells (SSCs) exist in many tissues in vertebrates, where they have to be carefully controlled to maintain their regenerative potential while preventing deregulation that could lead to dysplasias and neoplasias.

Depending on the needs of each tissue, resident SSC populations are controlled and activated in specific ways: SSCs in the muscle and the upper airways, for example, are often maintained in a quiescent state and only activated after tissue damage, while SSCs in the small intestinal epithelium are always active, providing a continuous stream of progenitor cells that go on to differentiate into the cell types lining the intestinal lumen (1–3). The precise context-dependent regulation of SSC activity and function is achieved through a combination of cell-intrinsic, local, and systemic regulators, which all impinge on stem cell function and thus on regenerative capacity and structure of the tissue (1–4). This renders stem cells susceptible to changes in their microenvironment as well as the broader physiological status of the animal, including to its metabolic and inflammatory condition. This susceptibility allows SSCs to appropriately respond to changes in the organism's condition, but it also poses a risk for the long-term health of regenerating tissues. As the animal ages, for example, systemic stress signals tend to be elevated, resulting in skewed SSC responses to damage (**Figure 1**). At the same time, the long-term maintenance of SSCs in a quiescent state means that SSCs are exposed to environmental and intrinsic stressors often for many years before they are needed for a regenerative response. The resulting accumulation of molecular damage in organelles, as well as of DNA, lipids, and proteins, has been described as a possible driver of SSC decline, especially in tissues such as the muscle (3, 5–9).

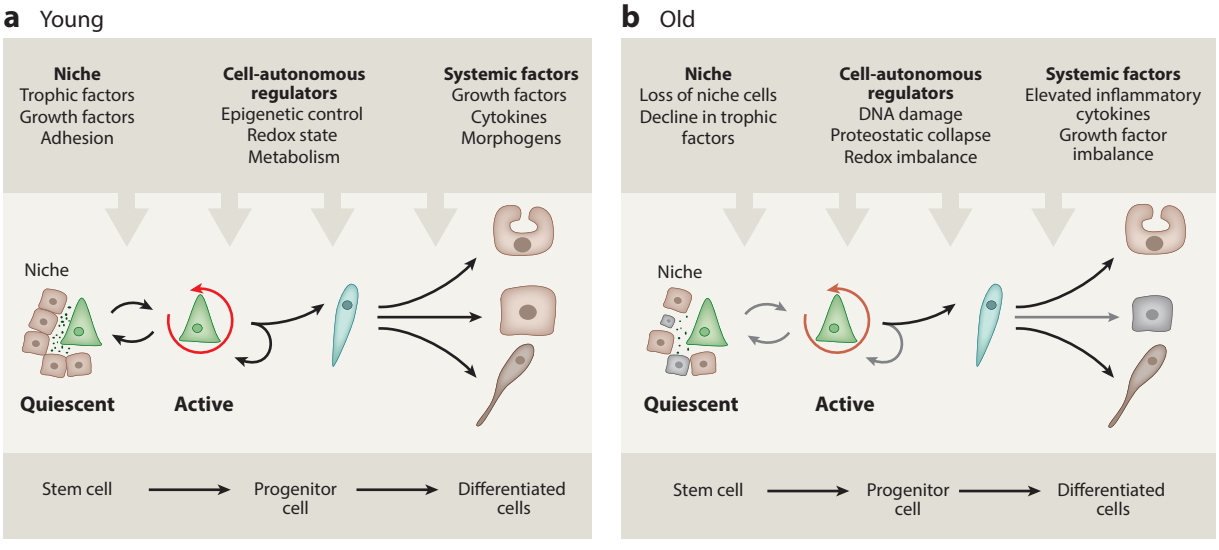


Figure 1

Regulation and aging of somatic stem cells. (*a*) Intrinsic (cell-autonomous), local (niche), and systemic factors regulate somatic stem cell maintenance, quiescence, activation, lineage specification, and differentiation. (*b*) In the aging animal, many of these factors decline or become deregulated, resulting in the loss of stem cells, deregulated stem cell activity, skewed differentiation potential, and misdifferentiation of daughter cells.

Strikingly, heterochronic parabiosis experiments have demonstrated that SSCs can be rejuvenated by changes in the systemic environment, indicating that systemic factors can over-ride the defects accumulated in these cells. Such rejuvenation can improve regenerative activity in many tissues and bears promise for the development of interventions that promote tissue repair in older patients (3, 5, 6).

SSCs in barrier epithelia are exposed to a unique combination of intrinsic and extrinsic stressors, yet they also exhibit remarkable regenerative potential and resilience. Understanding these qualities and gaining insight into age-related changes in such SSCs are crucial for the development of interventions into a range of chronic inflammatory diseases. The *Drosophila* gastrointestinal (GI) tract has served as a powerful and genetically accessible model system for the exploration of the biology and age-related decline of regenerative capacity in barrier epithelia (4, 8–12). In this system, a deep understanding of regulatory mechanisms of intestinal stem cell (ISC) activity and lineage specification is combined with the complexity of an epithelium that interacts with the microbiota, mounts innate immune responses, and serves as a semipermeable barrier to the exterior milieu. Accordingly, important insight into age-related changes in cell-autonomous, paracrine, and endocrine regulation of ISC activity and function has been obtained in this system in the last decade (4, 8–12).

In this review, I discuss our current understanding of the interplay of intrinsic, local, and systemic changes that cause regenerative decline in barrier epithelia of the GI tract, with a specific focus on lessons from the *Drosophila* model and how they apply to vertebrate stem cell biology. I specifically highlight new findings elucidating the causes and consequences of age-related changes in the function of ISCs in *Drosophila* and mice and focus on intervention strategies that may improve stem cell function, regenerative homeostasis, and longevity.

BARRIER EPITHELIA: REGENERATION, INFLAMMATION, METABOLISM, AND IMMUNITY

Epithelia lining the GI and urogenital tracts, as well as the airways, exhibit unique properties due to their exposure to the exterior milieu, their close interaction with the microbiota, and their function as a tight but semipermeable barrier for water, air, nutrients, immune factors, and digestive enzymes. It is therefore no surprise that these tissues exhibit a unique regenerative capacity and contain robust SSC populations. These tissues also exhibit unique plasticity and have evolved a variety of mechanisms to replace lost stem cells by dedifferentiation (13–16).

At the same time, the high functional diversity of such epithelia, which include epithelia that contain acid-producing cells in the gastric region, epithelia that are dominated by ciliated cells in the airway, and epithelia that form extensive crypt/villi structures in the small intestine, is reflected by the diversity of regenerative approaches. In the upper airways, for example, basal stem cells are mostly quiescent and only activated after tissue damage, while in the small intestine, stem cells in the crypt base are mostly active, generating a continuous supply of new cells that form the villus epithelium. This diversity of SSC behaviors is likely a consequence of the diversity of epithelial microenvironments. SSCs respond to growth factors, cytokines, and cell contact cues in their local environment and are dynamically regulated by signals emanating from neighboring cells. A detailed understanding of the homeostatic maintenance of SSC function in these epithelia thus requires deep insight into such local signals and the multitude of signals that can impinge on SSCs in conditions of nutrient deprivation, infection, inflammation, and mechanical damage (17–21).

Intestinal epithelia are particularly vulnerable to misregulation of homeostatic mechanisms, as mechanical and chemical stressors damage this epithelium regularly, whereas exposure to enteropathogens is common, the commensal microbiota has to be managed, the epithelial barrier

has to be maintained, and enzymes and nutrients have to be secreted and resorbed. Misregulation of homeostatic mechanisms is a likely cause of intestinal dysbiosis and chronic inflammation, pathologies that can in turn negatively influence epithelial regeneration and cause dysplasias and cancers (22–27). Understanding the various interactions between intestinal regeneration, epithelial functions, the commensal microbiota, and the immune system is thus critical to develop strategies for intervention and prevention of GI diseases.

Aging is among the main risk factors for a range of chronic and inflammatory diseases, and diseases of the GI tract are no exception. Elderly individuals are susceptible to infectious and inflammatory diseases (22), show increased incidence in colorectal cancer (28), exhibit metabolic imbalance (29), and are prone to GI infections (30). Age-related changes in the GI tract may not only cause a higher incidence of inflammatory diseases of the gut but also a decline in overall health and life span. Several age-related complications such as obesity (31), insulin resistance (32), and general frailty (33) have been associated with changes in the intestinal microbiota.

Conditions that often contribute to the development of such GI diseases are premalignant metaplasias and dysplasias. In the human GI tract, epithelial metaplastic lesions increase the risk of intestinal cancer (34). Such lesions are characterized by ectopic replacement of epithelial cell types. In Barrett's metaplasia, the esophageal squamous epithelium acquires properties of the gastric or intestinal columnar epithelium, resulting in a higher risk of esophageal adenocarcinomas (35, 36). Dysplasias, in turn, are characterized by aberrant cell proliferation and differentiation. Dysplastic lesions are believed to follow metaplasias in epithelial carcinogenesis and can contribute to the progression toward invasive carcinoma (37, 38).

The discovery and characterization of ISC populations in flies and mice in the past decade (39–41) and the study of their age-related dysfunction have led to the realization that these epithelial diseases are likely a consequence of misdirected epithelial regeneration caused by changes in the regulation of ISC activity and function. *Drosophila* has served as a particularly productive model in this respect, allowing detailed dissection of cell-autonomous, local, and systemic signaling mechanisms that are deregulated in aging animals and contribute to the loss of epithelial homeostasis in the GI tract. I describe this model in detail in the following section and propose a model for the age-related loss of tissue homeostasis in the fly gut that also shortens overall life span of the animal.

DROSOPHILA INTESTINAL STEM CELLS: A MODEL FOR COMPLEX REGULATION OF GASTROINTESTINAL REGENERATION

The *Drosophila* GI tract has served as a rich and productive model for the elucidation of homeostatic mechanisms that maintain integrity of barrier epithelia. The GI tract of the fly is lined by a pseudostratified monolayered epithelium that is regionally compartmentalized and surrounded by visceral muscle, innervated, and contacted by tracheal tubules (12). This epithelium is regenerated upon damage by Escargot (Esg)-expressing ISCs. ISCs are multipotent and regenerate cells with secretory/absorptive function like enterocytes and gastric copper cells, as well as cells with endocrine function like enteroendocrine cells (EEs). ISC potential is regionally diversified, with posterior midgut ISCs, for example, producing primarily enterocytes and EEs, and ISCs in the gastric region generating three different cell types: enterocyte-like cells, EEs, and copper cells (8, 12). It remains unclear whether this diversity is developmentally entrained and thus regulated by intrinsic determinants of ISC identity or whether it is a consequence of the microenvironment.

Regulation of Intestinal Stem Cell Proliferation

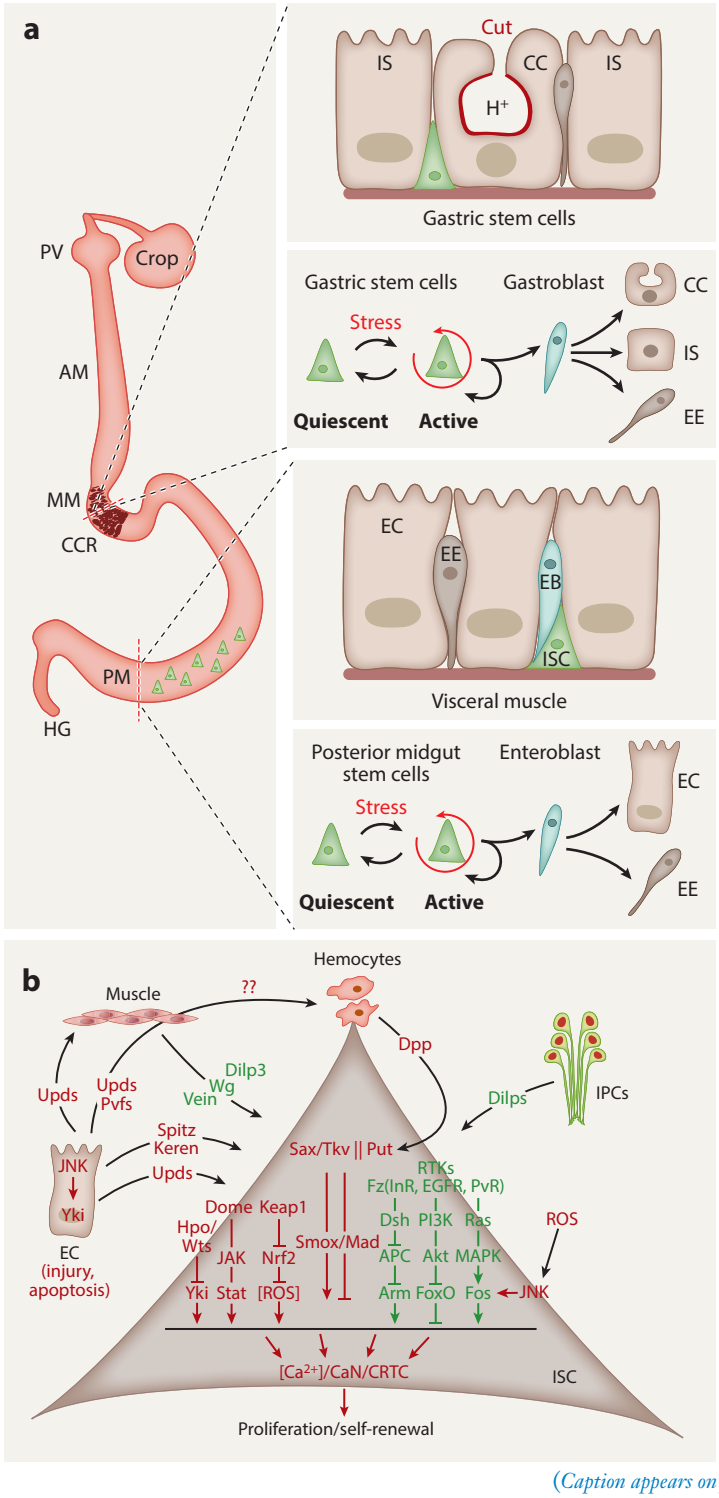
In contrast to that in the mammalian small intestine, ISC proliferation in flies is dynamic. Under homeostatic conditions, ISCs are mostly quiescent, resulting in relatively slow turnover

of the epithelium. In response to stressful insults, however, ISCs are activated within hours, allowing rapid and efficient restoration of the damaged epithelium. This response is observed upon infection with enteropathogens, as well as in response to DNA damage, oxidative stress, or surfactant challenge (4, 11, 12). In cases in which the challenge is temporary, such as during infection with the mild enteropathogen *Erwinia carotovora carotovora 15* (*Ecc15*), ISCs return to quiescence upon reestablishment of a functional epithelium, and the animal can thus survive multiple challenges [albeit, with a progressive loss of stem cells (42)]. The activation of ISCs results in an asymmetric division, which allows self-renewal of ISCs, as well as the generation of either EE- or enterocyte-committed postmitotic progenitor cells called enteroblasts (4, 12). The commitment into the EE or enterocyte lineage occurs at the level of the stem cell and can be modulated by Robo/Slit signaling through a negative feedback loop in which EEs secrete the ligand Slit to engage Robo2 receptors in ISCs, preventing their specification into the EE fate (43). Differentiation of enteroblasts into enterocytes is governed by Notch signaling, which is activated in enterocyte-committed enteroblasts by Delta ligands expressed on ISCs (4, 12). Interestingly, ISCs can also undergo symmetric divisions under certain conditions, such as during adaptive resizing (when the intestine is growing during a refeeding phase after starvation) (44).

The mechanisms governing activation of ISCs after a challenge have been intensively investigated since the first description and characterization of ISCs in 2006 (39, 40). These studies have led to a comprehensive model for the control of ISC proliferation during regenerative episodes (4, 12). Signals derived from damaged enterocytes, hemocytes, visceral muscle, and more distant tissues stimulate proliferative activity of ISCs. Major signaling pathways involved in this activation include the epidermal growth factor receptor (EGFR), Jun N-terminal kinase (JNK), Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT), bone morphogenetic protein (BMP), and WNT signaling pathways. These pathways respond to EGF-like ligands (such as Vein) expressed in the visceral muscle, to reactive oxygen species (ROS) and inflammatory cytokines [Unpaireds (Upds)] emanating from enterocytes, to BMP-like ligands (Dpp) secreted by hemocytes, and to Wnt-like ligands (Wg) derived from enteroblasts (**Figure 2**).

A critical question emerging from these studies is how individual ISCs integrate and decode the large number of mitogenic signals to engage in the appropriate context-specific proliferative response. One mechanism of integration is the need for simultaneous activation of various pathways to spur ISC proliferation. It has been shown, for example, that the EGFR pathway is required, but not sufficient for ISC proliferation under homeostatic conditions, as quiescent ISCs exhibit dual phosphorylation of the downstream kinase ERK. Combined activation of EGF-induced ERK and ROS-induced JNK signaling is in turn required and sufficient to promote ISC proliferation (45). This specific integration is achieved by dual phosphorylation of the downstream transcription factor Fos on unique ERK- and JNK-responsive sites. Ca^{2+} signaling has been reported as a second mechanism of signal integration in the control of ISC proliferation. Elevation of cytosolic [Ca^{2+}] can be observed in ISCs in response to activation of a range of different promitotic signaling pathways and is required and sufficient to trigger ISC proliferation by stimulating CaN and CREB-regulated transcriptional coactivator (CRTC) activity (46, 47).

These findings indicate that the transition from a quiescent to an activated state is defined in ISCs by unique molecular changes that are a consequence of the concerted action of the signaling pathways mentioned above. It will be interesting to test this hypothesis in the context of cellular metabolism, as recent studies indicate that changes in energy metabolism are critical to ensure or sustain appropriate proliferative responses. ISC proliferation is influenced by mitochondrial pyruvate intake, mitochondrial fission and fusion, and lipid metabolism (48–50). A central coordinator of nutrient sensing, translation, and autophagy, the TOR signaling pathway, is also required for efficient entry of ISCs into the active state (42).



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

The *Drosophila* gastrointestinal (GI) tract and intestinal stem cell (ISC) regulation. The *Drosophila* GI tract is compartmentalized and exhibits regionally differentiated regeneration of the GI epithelium. The main compartments include the proventriculus (PV), anterior midgut (AM), and middle midgut (MM) that include the copper cell region (CCR), posterior midgut (PM), and hindgut (HG). Schematics for lineage relationships of gastric stem cells (GSSCs) and posterior midgut stem cells (ISCs) are shown in panel *a*. GSSCs are activated by stress and generate gastroblasts (GBs), which can go on to differentiate into copper cells (CC), interstitial cells (IS), and enteroendocrine cells (EEs). ISCs generate enteroblasts (EBs) that differentiate into enterocytes (ECs) or EEs. Control mechanisms regulating ISC maintenance and proliferation are shown in panel *b*. Unpaireds (Upds) activate the Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway through the receptor domeless (dome) in response after stress-induced secretion from ECs is triggered by Jun N-terminal kinase (JNK) signaling, which activates the transcription factor Yorkie (Yki). Upds from ECs also activate vein expression in the muscle, which in turn activates epidermal growth factor receptor (EGFR) signaling in ISCs. Wingless (Wg) derived from the muscle has been proposed to support ISC survival by activating canonical Wnt signaling through Frizzled (Fz), Dishevelled (Dsh), adenomatous polyposis coli (APC), and armadillo (Arm). EGFR as well as other receptor tyrosine kinases (RTKs), such as the insulin receptor (InR) and the PDGF/VEGF receptor (PvR), engage the phosphatidyl inositol 3 kinase (PI3k) and Akt signaling pathway to inhibit forkhead box O (FoxO) activity, and the Ras/mitogen-activated protein kinase (MAPK) pathway to activate Fos. Hemocytes secrete decapentaplegic (Dpp) to engage the saxophone (Sax)/Smad on X (Smox) pathway during ISC activation, and Dpp will also engage the thickveins (Tkv)/mothers against Dpp (Mad) pathway to inhibit ISC proliferation in the later phase of the regenerative response. Both Tkv and Sax interact with the Type II receptor Punt (Put). Insulin-producing cells (IPCs) can influence ISC proliferation through the secretion of *Drosophila* insulin-like peptides (Dilps). ISCs also respond to reactive oxygen species (ROS) through the JNK signaling pathway. Mitogenic signals are integrated by ISCs through the calcium (Ca^{2+}), calcineurin (CaN), CREB-regulated transcription coactivator (CRTC) signaling pathway.

Epithelial Immunity in *Drosophila* and the Microbiota

As a barrier epithelium, the *Drosophila* intestinal epithelium interacts with and manages the microbiota and is exposed to enteropathogens. As a consequence, epithelial regeneration, epithelial immunity, and inflammation are closely linked, a likely evolutionary adaptation to a challenging environment. ISC function and activity are uniquely sensitive to infection with enteropathogens and can be influenced by the microbiota. Three main defense mechanisms manage the interactions of the epithelium and the bacterial flora (11, 51): the peritrophic matrix, the peptidoglycan recognition protein (PGRP)/immune deficiency (IMD)/relish pathway, and the p38/phospholipase C gamma (PLCg)/calcium/dual oxidase (Duox) signaling pathway. The peritrophic matrix consists of chitin and glycoproteins secreted by the proventriculus [with possible contributions by enterocytes (52)] that cover the intestinal epithelium. It prevents direct contact of microbes and other lumen contents with epithelial cells, and loss of peritrophic matrix components renders flies susceptible to infections (53). The PGRP/IMD/Relish pathway controls expression of antimicrobial peptides in response to recognition of bacterial peptidoglycans (54–67). It is kept inactive in homeostatic conditions by a variety of negative regulators, including Caudal (68), PGRPs of the SC, LB, and LF class (59, 69, 70), USP36 (71), and PIRK (72). Loss of these regulators can result in a shift in commensal populations, activation of stress signaling in the epithelium, and excessive stem cell proliferation, resulting in epithelial dysplasia (68, 73, 74). The p38/PLCg/Ca^{2+}/Duox signaling pathway controls the production of ROS by enterocytes. Duox is a member of the NADPH oxidase family and is transcriptionally induced in enterocytes in response to p38 MAPK activation (64, 75). It is activated in response to a bacterial challenge by a pathway involving activation of phospholipase Cβ (PLCβ) and triggers inositol-1,4,5-triphosphate (IP3)-induced Ca^{2+} release. Ca^{2+} binds and activates Duox (64, 76, 77). The epithelium produces an enzyme that protects against the cytotoxic effects of ROS: extracellular immune-related catalase (77). Yet during an infection,

ISCs respond to Duox-mediated ROS production by entering a proliferative state. A combination of this ROS signal, enterocyte-derived inflammatory cytokines (primarily Upds), and visceral muscle–derived Vein promote the proliferative response of ISCs to infection, ensuring rapid tissue regeneration upon tissue damage (4, 45, 78–80).

While these defense mechanisms control the response of the epithelium to enteropathogens, they also shape, and are influenced by, the natural microbiota. The *Drosophila* intestine contains a relatively simple microbiota comprising approximately 5–20 microbial species. Major constituents are beneficial microbes, such as *Acetobacter pomorum* and *Lactobacillus plantarum*, which promote growth and development in flies when reared on a restricted diet (68, 81–84). These microbes, in contrast to pathobionts, do not activate the intestinal immune system, allowing colonization of the gut. The epithelial immune system differentiates between pathobionts and commensals due to their differential secretion of uracil, which is secreted by pathobionts such as *Vibrio fluvialis*, *Klebsiella pneumonia*, *Erwinia carotovora carotovora*, *Shigella sonnei*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, but not by symbionts such as *A. pomorum*, *L. plantarum*, and *Commensalibacter intestini*. Uracil induces Hedgehog (Hh) signaling, which is required for intestinal expression of the calcium-dependent cell adhesion molecule cadherin 99C (Cad99C) and subsequent Cad99C-dependent formation of endosomes. These endosomes play essential roles in uracil-induced ROS production by acting as signaling platforms for PLCβ/PKC/Ca^{2+}-dependent Duox activation (66).

Interestingly, the species composition of the microbiota is not the only determinant of homeostasis in host/commensal interactions in the fly gut. Recent studies suggest that it is also the location of microbial colonization that shapes innate immune responses and inflammatory processes in the intestinal epithelium. Disrupting the luminal microenvironment, for example, in conditions in which the acidic gastric region is dysfunctional, allows ectopic colonization of specific gut regions by bacteria that normally colonize other regions. This also results in significant changes in microbiota composition and the induction of the above-referenced inflammatory response. Accordingly, loss of the gastric region has been shown to contribute to the age-related decline in intestinal homeostasis and to shorten life span (85).

The microbiota also produces metabolites that can influence ISC function and overall homeostasis. This function of the microbiota is only beginning to be understood, and more work is needed to obtain a comprehensive view of the influence of microbial metabolites on ISC function. A recent study has found that in conditions of elevated *L. plantarum* colonization, bacteria-derived lactic acid triggers the activation of the intestinal NADPH oxidase Nox, causing ROS production, intestinal damage, ISC overproliferation, and shortened life span. ROS production in this interaction requires lactate oxidation by the host intestinal lactate dehydrogenase, suggesting that changes in host gene expression can modulate the influence of the microbiota on tissue homeostasis (86). A second study reported that mono-association with *L. plantarum* disrupts intestinal homeostasis and shortens life span, confirming the deleterious effects of excessive or unbalanced colonization with normally beneficial bacteria (87).

Aging and the Fly Intestinal Epithelium

The need for coordination between immune and inflammatory responses and epithelial regeneration sensitizes the fly intestine to stress-induced loss of homeostasis. This is particularly evident in the intestine of aging flies. The intestinal epithelium in old *Drosophila* exhibits uncontrolled ISC proliferation and epithelial dysplasia that is associated with a loss of barrier function (58, 80, 88, 89). The causes and consequences of these phenotypes have been studied intensively in recent years (4, 65), and it is now possible to propose a comprehensive model for the loss of epithelial function in the aging *Drosophila* intestine (**Figure 3**). However, while the wide-ranging

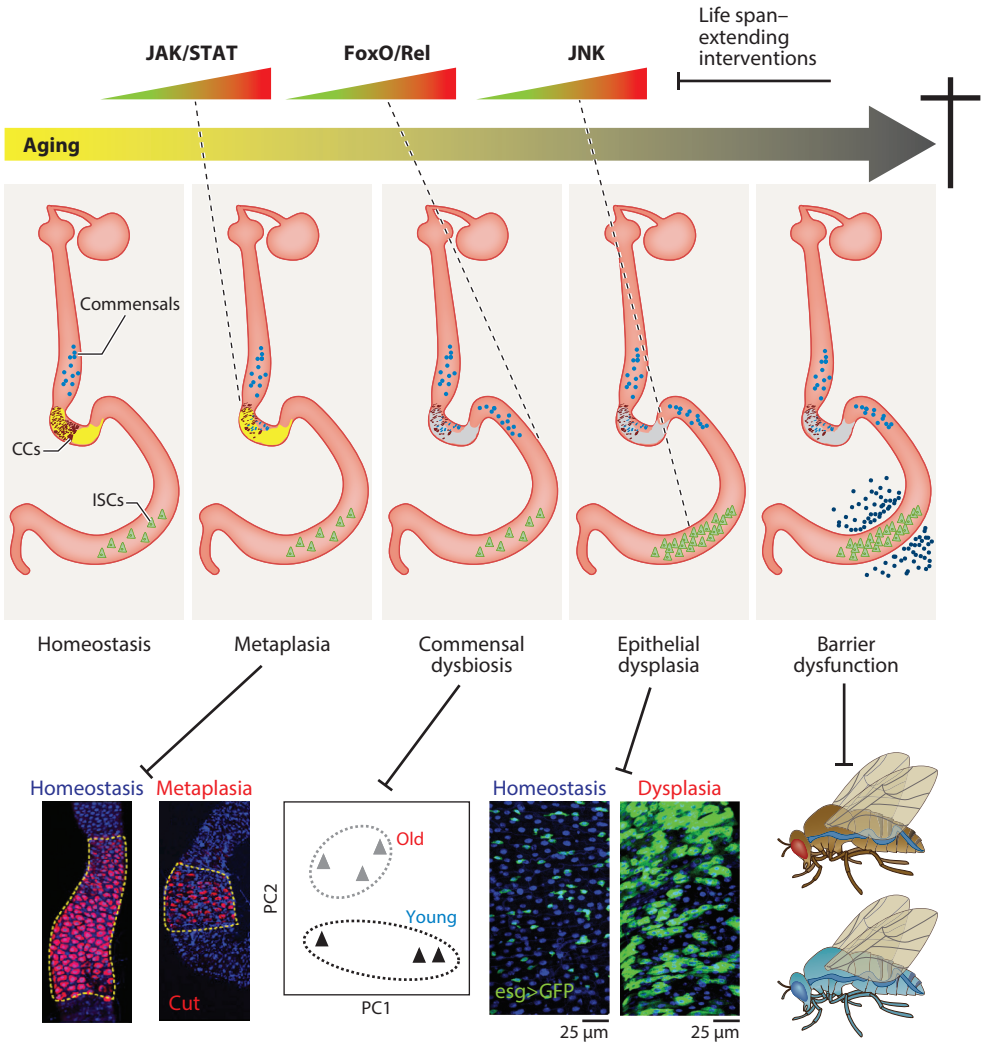


Figure 3

Aging of the *Drosophila* GI tract. A model for the progressive decline of epithelial homeostasis and the development of epithelial dysplasia and barrier disruption. Loss of barrier function precedes death in flies. It can be measured by exposing flies to a blue food dye that only penetrates the gut epithelium upon barrier dysfunction and then turns the fly blue. Pathways that cause distinct steps in the progression of tissue dysfunction are shown. JAK/STAT activation of copper cells (identified by Cut staining) results in their transdifferentiation and loss, which in turn results in loss of luminal acidity (shown in *yellow*) and bacterial dysbiosis. FoxO/Relish (Rel) activation in the enterocytes of the posterior midgut contributes to commensal dysbiosis, which in turn results in epithelial dysplasia by activating inflammatory signaling, including JNK signaling in ISCs. Regional or cell type–specific perturbation of these pathways can improve homeostasis and extend life span. Abbreviations: CC, copper cell; GI, gastrointestinal; ISC, intestinal stem cell; JAK, Janus-activated kinase; JNK, Jun N-terminal kinase; STAT, signal transducer and activator of transcription.

and combined age-related changes in cellular composition, morphology, signaling, and gene expression, as well as regenerative activity in the aging epithelium, have been extensively explored, our understanding of cell-intrinsic changes that may drive age-related stem cell dysfunction remains incomplete. In the following section, I present a possible model for age-related epithelial

degeneration and then discuss new studies that provide the first insight into the intrinsic molecular changes that impact ISC function.

The development of epithelial dysplasia in aging flies is closely linked to changes in the microbiota (18, 90–95). Recent studies suggest that these changes are a consequence of the age-related loss of acidity in the lumen of the gastric region due to the transdifferentiation of gastric epithelial copper cells into posterior midgut enterocytes (85). This metaplasia is induced by ectopic JAK/STAT signaling activity in copper cells of the gastric epithelium, which is in turn a consequence of chronically elevated Upd inflammatory cytokine expression, both from local sources in the intestine and from peripheral tissues such as the fatbody (85). This results in insufficient acidification of the gastric lumen and a colonization of the posterior midgut by commensals that usually colonize the anterior midgut.

Ectopic colonization of the posterior midgut is associated with an expansion and a shift in composition of the microbiota. This dysbiosis triggers chronic activation of the p38/Duox inflammatory response in the posterior midgut epithelium and thus chronic production of ROS. Accordingly, the aging intestinal epithelium exhibits high levels of ROS and ROS-induced signaling such as JNK signaling, platelet-derived growth factor (PDGF)/vascular endothelial growth factor (VEGF) signaling, and disruption of normal Nrf2 signaling (58, 80, 88, 89). The chronic exposure to such signals results in continuous proliferative activation of ISCs and the overproduction of ISC daughter cells. Epithelial dysplasia is a consequence of this overproduction of cells but is also associated with a misdifferentiation of daughter cells. These cells exhibit ectopic Notch signaling activity and Dl expression, continue expressing stem cell markers such as Esg, initiate but do not complete differentiation into enterocytes, and accumulate on the basal side of the epithelium, disrupting its structure as well as function (58, 80, 89).

Finally, epithelial dysplasia is associated with epithelial barrier dysfunction, which is predictive of fly death (96). Accordingly, multiple studies have demonstrated that the overall longevity of the animal correlates with the age of onset and the severity of the described intestinal changes (74). Based on the described discrete steps in the progression toward loss of epithelial homeostasis, multiple studies have tested cell type–specific and temporally controlled interventions to perturb specific steps and could show that such interventions extend life span (11, 74, 90–93, 95). These include suppressing JAK/STAT signaling in gastric epithelial cells, modulating the innate immune response in midgut enterocytes, preventing excessive ISC proliferation, and promoting maintenance of the epithelial barrier (9, 12, 74, 85, 96). Interventions that control nutrient availability for ISCs, such as dietary restriction, also result in reduced ISC overproliferation and extended life span (97, 98).

Although the proposed progression of age-related intestinal dysfunction is consistent with a large number of findings, several recent observations indicate that the chain of causality may be more complicated than anticipated or that additional ISC-intrinsic factors contribute to the loss of homeostasis in the aging intestinal epithelium:

1. The exact causal relationship between dysplasia and barrier dysfunction remains elusive, and recent studies suggest that epithelial barrier dysfunction can be a consequence of changes in enterocytes that are not mediated by ISC overproliferation. In fact, loss of barrier function can be observed in the absence of ISC overproliferation, and it is thus possible that barrier dysfunction develops independently in the aging intestine and contributes to or causes epithelial dysplasia. A recent study has found that altered expression of Snakeskin (Ssk), a septate junction-specific protein, can modulate intestinal homeostasis, microbial dynamics, immune activity, and life span in *Drosophila*. Strikingly, intestinal upregulation of Ssk improves intestinal barrier function during aging, limits dysbiosis, and extends life span,

indicating that strengthening the epithelial barrier is sufficient to rescue many of the other parameters of GI aging described above (99, 100). Further strengthening the notion that changes in bacteria, alterations in ISC proliferation, and loss of barrier integrity can be uncoupled, it was found that loss of the *Drosophila* tricellular junction protein gliotactin (Gli) in enterocytes results in activation of stress signaling in ISCs and an increase in ISC proliferation, even under axenic conditions, as well as a gradual disruption of the intestinal barrier (101). Further studies are needed to characterize the relationships between these phenotypes in detail.

2. Age-related dysfunction in a variety of intrinsic signaling pathways and processes in ISCs indicates that ISC dysfunction can develop independently of the described bacterial dysbiosis and inflammatory response. Recent studies have described a loss of proteostatic capacity in older ISCs due to a loss of Nrf2 activity (102), a decline of mitochondrial function in stem and progenitor cells (49), increased endoplasmic reticulum stress (93), activation of retrotransposon expression and ensuing DNA damage (103), changes in autophagy (104) and heterochromatin (105), and increased polymerase III transcriptional activity (106). All of these mechanisms seem to contribute to dysplasia in the aging intestine, yet it remains unclear how these processes interact at the level of the stem cell and with the wider inflammatory condition developing in the epithelium (90–95). Strikingly, ISCs of old flies also display frequent somatic mutations, resulting in neoplasias (94), and the fly intestine may thus also serve as a model for the age-related increase in cancer formation in vertebrates (49, 90–95, 104). These stem cell–intrinsic mechanisms of age-related decline also serve as targets for interventions, as improved homeostasis, and in some cases even life span extension, was observed when perturbations were targeted toward ISCs in the studies described above (93, 103–106).

Additional research is needed to understand the relative contributions of these processes to the age-related dysfunction of the epithelium and to identify the rate-limiting steps in the progression from a healthy regenerating epithelium to ISC dysfunction, epithelial dysplasia, and barrier dysfunction. Such insight would help to identify interventions that not only improve intestinal function but also benefit the health of the whole organism and thus increase health span and life span.

Critically, an area that is currently understudied in flies is the control and maintenance of ISC identity. It remains unclear whether ISC multipotency changes during aging, whether such changes may contribute to epithelial dysplasia, and whether mechanisms that maintain identity and lineage commitment are useful targets for intervention to improve tissue function. Such studies are ongoing in the community, and it can be expected that new insight will be obtained in the near future.

MAMMALIAN INTESTINAL STEM CELLS

The GI tract of mammals is significantly more complex than that of flies, but the general principles of stem cell regulation, the interaction of barrier epithelia with the microbiota, and the processes that control epithelial regeneration appear to be broadly conserved. As in flies, the GI tract of mammals is compartmentalized, resulting in significant epithelial diversity. Accordingly, epithelial regeneration and stem cell regulation differ in the various regions of the GI tract. Over the last decade, much has been learned about the stem cell compartment in the mouse GI tract, especially about leucine-rich repeat-containing G protein–coupled receptor 5 positive ($Lgr5^+$) cells in the crypts of the small intestine. The use of in vivo and ex vivo (organoid) mouse models for the study of stem cell identity and function has significantly improved our understanding of the regulation of epithelial homeostasis and of regeneration in the mammalian small intestine (107, 108).

The small intestine is lined by a monostratified epithelium that folds into numerous finger-like protrusions called villi that project into the intestinal lumen, maximizing surface area for digestion and absorption. Tubular invaginations between these villi, known as crypts, contain stem cells, Paneth cells, and transit amplifying (TA) cells, whereas the villi contain enterocytes, goblet cells, and EEs. Paneth cells and ISCs populate the base of the crypt and are closely associated with each other. While secreting antimicrobial substances and the hydrolytic enzyme lysozyme, Paneth cells also provide trophic factors for ISCs, promoting their survival and function. ISCs divide to self-renew and generate TA cells, which in turn undergo multiple rounds of cell division to amplify their numbers as they migrate along the crypt axis toward the base of the villus, where they differentiate (41, 109, 110).

Self-renewing and multipotent ISCs in crypts of the mouse small intestine had been predicted based on lineage tracing studies with mouse chimeras and mutagen-marked cells (110, 111) but were only identified and characterized in the last decade after ISC-specific markers had been found (107). These studies proposed two different stem cell populations in the crypt: LGR5-expressing crypt base columnar stem cells that intercalate with Paneth cells at the crypt base (41), and Bmi1-expressing +4 stem cells, which are located four cell diameters apical of the crypt base (112). Lineage tracing using *Lgr5*-driven inducible Cre recombinase results in epithelial cell clones that span from the crypt base to the tip of the villus and contain all major epithelial cell types (41, 108). $Bmi1^+$ cells can also self-renew, proliferate, and generate all the different cell lineages of the small intestine, supporting epithelial self-renewal and crypt maintenance (112). Both $LGR5^+$ and $Bmi1^+$ cells can generate intestinal organoids in culture (113). After ablation of $LGR5^+$ cells, $Bmi1^+$ cells can restore the crypt base cell population, providing evidence for significant plasticity of cell identities in the crypt (15), a concept that has been supported by findings that the crypt base can be repopulated by other cell types as well, including differentiated endocrine and Paneth cells (15, 16, 107, 114–117). A recent study has also proposed the presence of a distinct revival stem cell population that can repopulate the crypt in conditions of severe damage (118).

Human cells in the base of colonic crypts also behave as multipotent stem cells (119), and *Lgr5-GFP*-positive cells isolated from teratomas can generate long-lived intestinal organoids (120), indicating that ISC identity and function may be conserved between mice and humans.

In addition to the well-characterized crypt basal stem cells of the small intestine, stem cells in other areas of the GI tract have also been studied, albeit to a lesser extent. This includes stem cell populations in the esophagus and stomach, which regenerate fundamentally different epithelia. The esophagus is lined by a stratified squamous epithelium and lacks established niche structures, such as crypts in the small intestine. A layer of proliferating basal cells attached to the basement membrane produces several layers of differentiated cells, which are shed into the lumen and replaced by cells derived from the basal cells. While basal cells have stem cell properties, it remains unclear whether a unique self-renewing and quiescent stem cell population exists in the esophagus (121–125). The stomach, in turn, is lined by an epithelium composed of crypt-like structures called gastric units that produce gastric acid (in the corpus) or the hormone gastrin (in the Antrum) (126). A highly proliferative zone of the isthmus of these gastric units contains stem-like cells (126–128). Lineage tracing based on Cre recombinase driven by the *Villin* promoter, *Lgr5-Cre*, or *Sox2-Cre* has further identified stem cell populations along the gland in the pylorus (129), at the base of the gland in the pylorus (130), and in the pylorus and corpus near the isthmus region of the gland (131), respectively. $Troy^+$/$Mist^+$ chief cells at the bottom of the gastric unit in the corpus have further been proposed to act as reserve stem cells to regenerate all cell types over a longer period of time (132, 133).

A detailed understanding of the regulation and maintenance of most stem cell populations in the mammalian GI tract remains elusive. However, studies on $Lgr5^+$ crypt basal cells in the small

intestine have provided insight into ISC maintenance and regulation by factors from the stem cell niche (134). A multitude of signaling pathways control ISC activity and function, including Wnt, BMP, and Hh and Notch signaling (135). Most critically, a gradient of Wnt activity along the crypt axis maintains ISC regenerative capacity (136–142), and its loss leads to complete ablation of intestinal crypts. Wnt signaling further determines cell fates within and the spatial organization of the crypt (143).

High Wnt activity in nonproliferating Paneth cells is required for their terminal differentiation (144, 145), and this effect is mediated by the Wnt target gene *Sox9* (146–148). BMP signaling, in turn, negatively regulates ISC proliferation and self-renewal, potentially by inhibiting Wnt signaling (137, 149–152). Proliferation of stem and progenitor cells is also maintained by Notch signaling, in contrast to the fly intestine, where Notch activation promotes differentiation (149, 153–155). Inhibiting Notch signaling in the mouse converts $LGR5^+$ ISCs into secretory cells, likely through activation of the Wnt pathway (156), while ectopic activation of Notch in the crypt leads to the expansion of proliferating cells and inhibits the generation of secretory cell types (157).

Lgr5-expressing stem cells can be found throughout the small intestine and in the pyloric region of the stomach but are absent from the main body of the stomach (corpus) (130) and the esophageal epithelium (158). Differing levels of Wnt signaling activity may be driving this diversification: Although Wnt is required to maintain basal stem cells in the distal pyloric region (130), *Lgr5*-negative basal stem cells in the stomach corpus exhibit different levels of Wnt activity (132).

Intestinal Inflammation

Similar to the IMD/Relish pathway in flies, activation of the Relish homolog nuclear factor-κB (NF-κB) by the mammalian tumor necrosis factor receptor (TNFR) pathway is critical for epithelial immunity (159–162). NF-κB activation in epithelial cells modulates immune responses (163), and chronic activation of NF-κB and of the TNFR pathway in epithelial cells contributes to intestinal inflammation (160, 161, 164). The role of ISC deregulation in intestinal inflammation and inflammatory disorders is complex, but it is likely that interventions that target ISC function can serve to improve the trajectory of inflammatory bowel diseases. One example is the use of interleukin (IL)-22 as a possible therapy for inflammatory bowel diseases (165, 166).

ISCs may also mediate the beneficial effects of dietary perturbations on inflammatory bowel diseases. Recent work in a mouse model for colitis reports that a short fasting period followed by normal diet modulates the microbiota and has beneficial effects on inflammation, stem cell proliferation, and tissue maintenance in the small intestine (167). Accordingly, ISCs are responsive to changes in diet, including to dietary restriction, which has a beneficial effect on ISC activity in both young and old mice (168–170). Even a short-term (24-h) fast can have beneficial effects, as it augments ISC function by inducing fatty acid oxidation in ISCs (170).

Strikingly, there may be a link between diet and barrier dysfunction in mammals, as a recent study reported that hyperglycemia promotes intestinal barrier permeability in mouse models of obesity and diabetes. This effect is mediated by GLUT2-dependent transcriptional regulation in intestinal epithelial cells and alteration of tight and adherence junction integrity. Treating hyperglycemia, deleting GLUT2 in intestinal epithelial cells, or inhibiting glucose metabolism restored barrier function and bacterial containment. The study further showed that systemic influx of intestinal microbiome products correlates with individualized glycemic control in humans (171).

How these different physiological parameters, inflammatory signals, and controls of epithelial regeneration are influenced by aging and how they interact to influence age-related changes in the mammalian intestine remain unclear. Recent studies have started to provide insight into these questions by focusing on the age-related dysfunction of ISCs.

Aging in the Mammalian Intestine

Compared to those in the fly intestine, age-related changes in the intestinal epithelium of mammals remain understudied. Studies in the 1990s reported an age-related decline in repair capacity of the mouse small intestine, showing that 28- to 30-month-old mice exhibit a significant decline in size and number of intestinal crypts after high doses of irradiation (172). Later, it was shown that the regenerative capacity of the intestinal epithelium declines upon DNA damage induced by short telomeres and ROS (173, 174). Recent studies in mice suggest that the age-related loss of homeostasis described in flies, which includes microbial dysbiosis, barrier dysfunction, and systemic inflammation, is mirrored by similar age-related changes in the GI tract of mammals (175). Maintaining mice under germ-free conditions was found to prevent the age-related increase in circulating proinflammatory cytokines and resulted in longer life spans. Cohousing experiments further demonstrated that old but not young conventionally raised animals can transfer proinflammatory conditions to germ-free mice.

However, these studies did not examine ISC function directly and did not explore the interaction between age-related local, luminal, or systemic changes to gain insight into the causes of the age-related decline in repair capacity. A recent flurry of studies has now taken advantage of the more sophisticated understanding of mouse ISC biology that has developed in the last decade and has started to shed light on the effects of aging on ISCs.

A specific focus has been on age-related changes in the function of $Lgr5^+$ ISCs of the small intestine. Nalapareddy and colleagues (176) have reported changes in architecture, cell numbers, and cell composition in the intestinal epithelium of old compared to young mice and found that the regenerative capacity of ISCs from old mice is diminished. Morphological changes include a decrease in crypt numbers, an increase in crypt length and width, an increase in villi length, and elevated numbers of cells per crypt (**Figure 4**). It was further shown that fewer proliferating cells can be observed at the base of the crypt, and while the numbers of ISCs do not change in old mice compared to young animals, their function declines, resulting in fewer lineage-traced $Lgr5^+$ cell-derived clones in old animals (176, 177). This is consistent with a reduction in stem cell–specific gene expression, particularly in Wnt pathway genes, in the crypt of old mice, as well as with a decline in tissue repair after injury (176). Strikingly, this ISC dysfunction is still observed in ex vivo crypt-derived organoids, which show a decline in viability and a decline in crypt-like buds after three passages in culture compared to organoids derived from young mice. A recent study has found a similar age-related decline in the organoid-forming capacity of colonic crypts in humans (178). This decline in organoid viability is likely a consequence of stem cell autonomous changes and/or of changes in paracrine niche factors. Accordingly, Pentinmikko et al. (178) report that coculture with Paneth cells from young mice partially rescues the organoid forming deficiency of old $Lgr5^+$ ISCs, while Nalapareddy and colleagues (176) find that exposure of mouse organoids to elevated levels of Wnt3a is sufficient to rescue age-related phenotypes. Although Wnt signaling in the intestinal epithelium is critical for tissue homeostasis in young mice, regulation of Wnt ligand expression and of Wnt pathway activity in the crypt is complex, and further work is needed to establish the causes and consequences of Wnt signaling decline in the aging crypt (140, 149). Pentinmikko and colleagues (178) propose that one mechanism causing Wnt signaling decline is the increased expression of the secreted Wnt deacylase Notum in aged Paneth cells.

Other studies have reported sometimes similar, sometimes conflicting phenotypes of aging ISCs: Moorefield et al. (179) report increased villus height and Paneth cells in 18- to 22-month-old mice, increased ISC proliferation, and expansion of the ISC population (identified in this study by moderate expression of Sox9), while also demonstrating that crypt organoid cultures from old mice yield fewer and less-complex organoids. The authors also find increased apoptosis

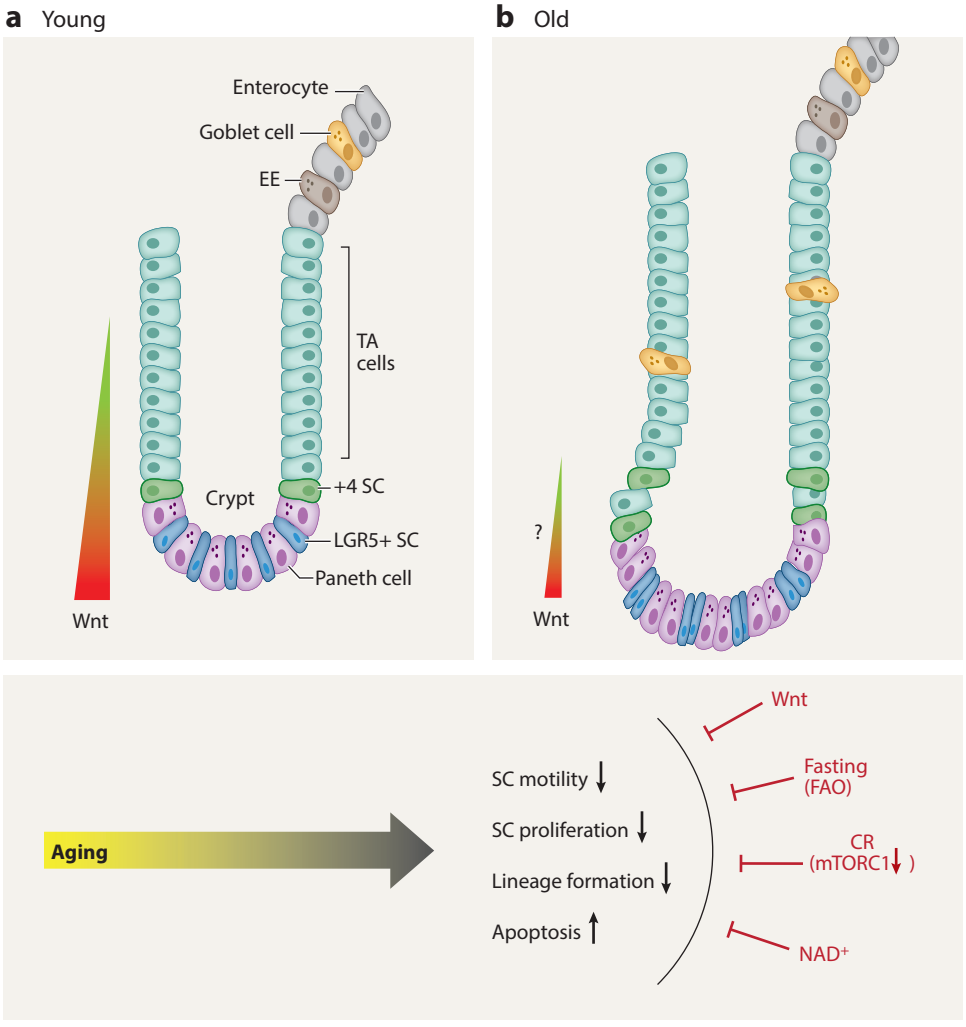


Figure 4

Aging of the stem cell compartment of the small intestine of mice. (*a*) Current studies have described changes in crypts of the small intestine that can be reversed through specific perturbations, such as Wnt or NAD^+ supplementation, CR, and fasting, which induces FAO in ISCs. (*b*) As described, age-related changes are variable, and additional insight is needed to establish the relationship between molecular changes, disruption of tissue morphology, and functional changes. Abbreviations: CR, calorie restriction; EE, enteroendocrine cell; FAO, fatty acid oxidation; ISC, intestinal stem cell; NAD^+, nicotinamide adenine dinucleotide; TA, transit amplifying.

in the intestinal epithelium of old mice. Cui and colleagues (180), in turn, find that ISCs from 24-month-old mice form cyst-like organoids devoid of differentiated cells. Such organoids are characteristic of conditions with elevated canonical Wnt signaling. Accordingly, the authors report higher expression of Wnt target genes in crypts of old mice and find that reducing R-spondin-1 in the culture can suppress the cyst phenotype.

The discrepancy in findings related to Wnt signaling activity in aging intestinal crypts reflects the difficulty of establishing the origin of dysfunctional regenerative capacity in aging intestines

of mice. Critically, increased or sustained Wnt pathway activity contributes to colorectal cancers, complicating the path to restoration of regenerative capacity if Wnt signaling activity is in fact reduced in old crypts (181). Accordingly, a connection between age-related molecular changes and the increased propensity for colorectal cancer has recently been described in the colon: In colon-derived organoids from old mice, spontaneous epigenetic silencing by promoter hypermethylation (which mimics human aging-like phenotypes) leads to activation of the Wnt pathway, causing a stem-like state and differentiation defects and resulting in higher sensitivity to transformation by *Braf*^{V600E} compared to organoids from young mice (182).

Although the exact phenotypes of ISCs of aging mice have yet to be robustly characterized, various studies have reported potential intervention strategies to restore function. Mihaylova et al. (170) have found that fasting can promote ISC function by inducing fatty acid oxidation, whereas Yousefi et al. (183) report that calorie restriction improves the regenerative capacity of the intestinal epithelium by mTORC1 inhibition in injury-resistant reserve ISCs. mTORC1 activity is critical for the transition of many SSC types to an active state but can significantly impair the function of these cells by promoting differentiation. Accordingly, exposure to rapamycin can significantly improve the maintenance of airway basal stem cells in aging mice (42, 184). ISC function in old mice can further be restored by treatment with the Notum inhibitor ABC99 (178).

Another study reported that ISCs but not Paneth cells decline in number and proliferative activity in aging mice, and that levels of SIRT1 and mTORC1 activity decline in these cells. Treatment with the NAD^+ precursor nicotinamide riboside reverses this phenotype and can promote repair of gut damage. Strikingly, this effect is found to be blocked by rapamycin or the SIRT1 inhibitor EX527 (185). These findings obviously contrast with some of the others discussed above, and more work is needed to resolve these controversies. It seems likely that two main confounding elements come together to cause diverse observations: (*a*) a lack of attention to regional variability even within the small intestine and (*b*) the use of single time points to report old mouse phenotypes. It is likely that careful characterization and comparison of age-related changes in ISCs isolated from different specific regions of the GI tract will provide insight that can resolve the first concerns, while more comprehensive characterization of developing age-related dysfunctions at various time points along the life span of mice will resolve the second concern.

Additional techniques that provide more detailed in vivo insight into stem cell function are also required to obtain a more comprehensive and robust picture of ISC dysfunction in the aging animal. Of particular interest is the use of live imaging approaches, which in mice require significant technical investment, but can lead to important insight into ISC function in homeostasis and in aging animals. Choi et al. (186), for example, have recently reported a coordinated motion of ISCs after focal damage in intestinal crypts, which allows reestablishment of the intercalation of Paneth cells and ISCs. This process is impaired in crypts of old mice, indicating that the decline in regenerative capacity in the intestine of aging animals is a consequence not only of reduced ISC proliferation or differentiation capacity, but also of cellular motility. The mechanisms of this age-related decline remain unclear.

CONCLUDING OBSERVATIONS

The detailed characterization of age-related changes in the GI tract of flies has led to a molecular model for the establishment of ISC dysfunction. This model allows positing hypotheses for the development of similar dysfunction in vertebrates and can inform the development of interventions that aim to restore tissue function in the elderly and to treat diseases that exhibit inflammatory and regenerative disturbances of the GI epithelium. Further in-depth characterization of mammalian

ISC aging is needed to expand this body of work, to rigorously test hypotheses emerging from the fly model, and to develop therapeutic approaches based on an understanding of ISC aging.

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LITERATURE CITED

1. Simons BD, Clevers H. 2011. Stem cell self-renewal in intestinal crypt. *Exp. Cell Res.* 317:2719–24
2. Rock JR, Hogan BL. 2011. Epithelial progenitor cells in lung development, maintenance, repair, and disease. *Annu. Rev. Cell Dev. Biol.* 27:493–512
3. Rando TA. 2006. Stem cells, ageing and the quest for immortality. *Nature* 441:1080–86
4. Biteau B, Hochmuth CE, Jasper H. 2011. Maintaining tissue homeostasis: dynamic control of somatic stem cell activity. *Cell Stem Cell* 9:402–11
5. Neves J, Sousa-Victor P, Jasper H. 2017. Rejuvenating strategies for stem cell-based therapies in aging. *Cell Stem Cell* 20:161–75
6. Jones DL, Rando TA. 2011. Emerging models and paradigms for stem cell ageing. *Nat. Cell Biol.* 13:506–12
7. Chandel NS, Jasper H, Ho TT, Passegue E. 2016. Metabolic regulation of stem cell function in tissue homeostasis and organismal ageing. *Nat. Cell Biol.* 18:823–32
8. Li H, Jasper H. 2016. Gastrointestinal stem cells in health and disease: from flies to humans. *Dis. Model. Mech.* 9:487–99
9. Wang L, Karpac J, Jasper H. 2014. Promoting longevity by maintaining metabolic and proliferative homeostasis. *J. Exp. Biol.* 217:109–18
10. Jasper H, Kennedy BK. 2012. Niche science: the aging stem cell. *Cell Cycle* 11:2959–60
11. Ayyaz A, Jasper H. 2013. Intestinal inflammation and stem cell homeostasis in aging *Drosophila melanogaster*. *Front. Cell. Infect. Microbiol.* 3:98
12. Miguel-Aliaga I, Jasper H, Lemaitre B. 2018. Anatomy and physiology of the digestive tract of *Drosophila melanogaster*. *Genetics* 210:357–96
13. Tata PR, Mou H, Pardo-Saganta A, Zhao R, Prabhu M, et al. 2013. Dedifferentiation of committed epithelial cells into stem cells *in vivo*. *Nature* 503:218–23
14. Lucchetta EM, Ohlstein B. 2017. Amitosis of polyploid cells regenerates functional stem cells in the *Drosophila* intestine. *Cell Stem Cell* 20:609–20.e6
15. Tian H, Biehs B, Warming S, Leong KG, Rangell L, et al. 2011. A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable. *Nature* 478:255–59
16. Tetteh PW, Basak O, Farin HF, Wiebrands K, Kretzschmar K, et al. 2016. Replacement of lost *Lgr5*-positive stem cells through plasticity of their enterocyte-lineage daughters. *Cell Stem Cell* 18:203–13
17. Barker N, Bartfeld S, Clevers H. 2010. Tissue-resident adult stem cell populations of rapidly self-renewing organs. *Cell Stem Cell* 7:656–70
18. Buchon N, Osman D, David FP, Fang HY, Boquete JP, et al. 2013. Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. *Cell Rep.* 3:1725–38
19. Marianes A, Spradling AC. 2013. Physiological and stem cell compartmentalization within the Drosophila midgut. *eLife* 2:e00886
20. Li H, Qi Y, Jasper H. 2013. Dpp signaling determines regional stem cell identity in the regenerating adult *Drosophila* gastrointestinal tract. *Cell Rep.* 4:10–18
21. Strand M, Micchelli CA. 2011. Quiescent gastric stem cells maintain the adult *Drosophila* stomach. *PNAS* 108:17696–701
22. Clemente JC, Ursell LK, Parfrey LW, Knight R. 2012. The impact of the gut microbiota on human health: an integrative view. *Cell* 148:1258–70

23. Gonda TA, Tu S, Wang TC. 2009. Chronic inflammation, the tumor microenvironment and carcinogenesis. *Cell Cycle* 8:2005–13
24. Kaser A, Zeissig S, Blumberg RS. 2010. Inflammatory bowel disease. *Annu. Rev. Immunol.* 28:573–621
25. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, et al. 2012. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res.* 22:292–98
26. Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, et al. 2010. Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res.* 70:1430–40
27. Uronis JM, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C. 2009. Modulation of the intestinal microbiota alters colitis-associated colorectal cancer susceptibility. *PLOS ONE* 4:e6026
28. Patel BB, Yu Y, Du J, Levi E, Phillip PA, Majumdar APN. 2009. Age-related increase in colorectal cancer stem cells in macroscopically normal mucosa of patients with adenomas: a risk factor for colon cancer. *Biochem. Biophys. Res. Commun.* 378:344–47
29. Roberts SB, Rosenberg I. 2006. Nutrition and aging: changes in the regulation of energy metabolism with aging. *Physiol. Rev.* 86:651–67
30. Duncan SH, Flint HJ. 2013. Probiotics and prebiotics and health in ageing populations. *Maturitas* 75:44–50
31. Kallus SJ, Brandt LJ. 2012. The intestinal microbiota and obesity. *J. Clin. Gastroenterol.* 46:16–24
32. De Bandt J-P, Waligora-Dupriet A-J, Butel M-J. 2011. Intestinal microbiota in inflammation and insulin resistance: relevance to humans. *Curr. Opin. Clin. Nutr. Metab. Care* 14:334–40
33. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, et al. 2012. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488:178–84
34. Slack JM. 2007. Metaplasia and transdifferentiation: from pure biology to the clinic. *Nat. Rev. Mol. Cell Biol.* 8:369–78
35. Falk GW. 2002. Barrett's esophagus. *Gastroenterology* 122:1569–91
36. Hvid-Jensen F, Pedersen L, Drewes AM, Sorensen HT, Funch-Jensen P. 2011. Incidence of adenocarcinoma among patients with Barrett's esophagus. *N. Engl. J. Med.* 365:1375–83
37. Correa P, Houghton J. 2007. Carcinogenesis of *Helicobacter pylori*. *Gastroenterology* 133:659–72
38. Ullman T, Odze R, Farraye FA. 2009. Diagnosis and management of dysplasia in patients with ulcerative colitis and Crohn's disease of the colon. *Inflamm. Bowel. Dis.* 15:630–38
39. Micchelli CA, Perrimon N. 2006. Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature* 439:475–79
40. Ohlstein B, Spradling A. 2006. The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature* 439:470–74
41. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, et al. 2007. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449:1003–7
42. Haller S, Kapuria S, Riley RR, O'Leary MN, Schreiber KH, et al. 2017. mTORC1 activation during repeated regeneration impairs somatic stem cell maintenance. *Cell Stem Cell* 21:806–18.e5
43. Biteau B, Jasper H. 2014. Slit/Robo signaling regulates cell fate decisions in the intestinal stem cell lineage of *Drosophila*. *Cell Rep.* 7:1867–75
44. O'Brien LE, Soliman SS, Li X, Bilder D. 2011. Altered modes of stem cell division drive adaptive intestinal growth. *Cell* 147:603–14
45. Biteau B, Jasper H. 2011. EGF signaling regulates the proliferation of intestinal stem cells in *Drosophila*. *Development* 138:1045–55
46. Deng H, Gerencser AA, Jasper H. 2015. Signal integration by Ca regulates intestinal stem-cell activity. *Nature* 528:212–17
47. Xu C, Luo J, He L, Montell C, Perrimon N. 2017. Oxidative stress induces stem cell proliferation via TRPA1/RyR-mediated Ca^{2+} signaling in the *Drosophila* midgut. *eLife* 6:e22441
48. Singh SR, Zeng X, Zhao J, Liu Y, Hou G, et al. 2016. The lipolysis pathway sustains normal and transformed stem cells in adult *Drosophila*. *Nature* 538:109–13
49. Koehler CL, Perkins GA, Ellisman MH, Jones DL. 2017. Pink1 and Parkin regulate *Drosophila* intestinal stem cell proliferation during stress and aging. *J. Cell Biol.* 216:2315–27

50. Schell JC, Wisidagama DR, Bensard C, Zhao H, Wei P, et al. 2017. Control of intestinal stem cell function and proliferation by mitochondrial pyruvate metabolism. *Nat. Cell Biol.* 19:1027–36
51. Ferrandon D. 2013. The complementary facets of epithelial host defenses in the genetic model organism *Drosophila melanogaster*: from resistance to resilience. *Curr. Opin. Immunol.* 25:59–70
52. Hegedus D, Erlandson M, Gillott C, Toprak U. 2009. New insights into peritrophic matrix synthesis, architecture, and function. *Annu. Rev. Entomol.* 54:285–302
53. Kuraishi T, Binggeli O, Opota O, Buchon N, Lemaitre B. 2011. Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *PNAS* 108:15966–71
54. Nehme NT, Liégeois S, Kele B, Giammarinaro P, Pradel E, et al. 2007. A model of bacterial intestinal infections in *Drosophila melanogaster*. *PLOS Pathog*. 3:e173
55. Liehl P, Blight M, Vodovar N, Boccard F, Lemaitre B. 2006. Prevalence of local immune response against oral infection in a *Drosophila/Pseudomonas* infection model. *PLOS Pathog*. 2:e56
56. Ryu JH, Ha EM, Oh CT, Seol JH, Brey PT, et al. 2006. An essential complementary role of NF-κB pathway to microbicidal oxidants in *Drosophila* gut immunity. *EMBO J.* 25:3693–701
57. Tzou P, Ohresser S, Ferrandon D, Capovilla M, Reichhart JM, et al. 2000. Tissue-specific inducible expression of antimicrobial peptide genes in *Drosophila* surface epithelia. *Immunity* 13:737–48
58. Buchon N, Broderick NA, Chakrabarti S, Lee W-J, Lemaitre B. 2009. Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev*. 23:2333–44
59. Zaidman-Rémy A, Hervé M, Poidevin M, Pili-Floury S, Kim MS, et al. 2006. The *Drosophila* amidase PGRP-LB modulates the immune response to bacterial infection. *Immunity* 24:463–73
60. Bosco-Drayon V, Poidevin M, Boneca IG, Narbonne-Reveau K, Royet J, Charroux B. 2012. Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota. *Cell Host Microbe* 12:153–65
61. Neyen C, Poidevin M, Roussel A, Lemaitre B. 2012. Tissue- and ligand-specific sensing of gram-negative infection in *Drosophila* by PGRP-LC isoforms and PGRP-LE. *J. Immunol.* 189:1886–97
62. Ferrandon D, Imler J-L, Hetru C, Hoffmann JA. 2007. The *Drosophila* systemic immune response: sensing and signalling during bacterial and fungal infections. *Nat. Rev. Immunol.* 7:862–74
63. Lemaitre B, Hoffmann J. 2007. The host defense of *Drosophila melanogaster*. *Annu. Rev. Immunol*. 25:697–743
64. Ha EM, Lee KA, Seo YY, Kim SH, Lim JH, et al. 2009. Coordination of multiple dual oxidase-regulatory pathways in responses to commensal and infectious microbes in *Drosophila* gut. *Nat. Immunol.* 10:949–57
65. Buchon N, Broderick NA, Lemaitre B. 2013. Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat. Rev. Microbiol*. 11:615–26
66. Lee K-A, Kim S-H, Kim E-K, Ha E-M, You H, et al. 2013. Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. *Cell* 153:797–811
67. Davis MM, Engström Y. 2012. Immune response in the barrier epithelia: lessons from the fruit fly *Drosophila melanogaster*. *J. Innate Immun*. 4:273–83
68. Ryu JH, Kim SH, Lee HY, Bai JY, Nam YD, et al. 2008. Innate immune homeostasis by the homeobox gene *Caudal* and commensal-gut mutualism in *Drosophila*. *Science* 319:777–82
69. Maillet F, Bischoff V, Vignal C, Hoffmann J, Royet J. 2008. The *Drosophila* peptidoglycan recognition protein PGRP-LF blocks PGRP-LC and IMD/JNK pathway activation. *Cell Host Microbe* 3:293–303
70. Paredes JC, Welchman DP, Poidevin M, Lemaitre B. 2011. Negative regulation by amidase PGRPs shapes the *Drosophila* antibacterial response and protects the fly from innocuous infection. *Immunity* 35:770–79
71. Thevenon D, Engel E, Avet-Rochex A, Gottar M, Bergeret E, et al. 2009. The *Drosophila* ubiquitin-specific protease dUSP36/Scny targets IMD to prevent constitutive immune signaling. *Cell Host Microbe* 6:309–20
72. Lhocine N, Ribeiro PS, Buchon N, Wepf A, Wilson R, et al. 2008. PIMS modulates immune tolerance by negatively regulating *Drosophila* innate immune signaling. *Cell Host Microbe* 4:147–58

73. Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. 2009. *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe* 5:200–11
74. Biteau B, Karpac J, Supoyo S, Degennaro M, Lehmann R, Jasper H. 2010. Lifespan extension by preserving proliferative homeostasis in *Drosophila*. *PLOS Genet*. 6:e1001159
75. Ha EM, Lee KA, Park SH, Kim SH, Nam HJ, et al. 2009. Regulation of DUOX by the Gαq-phospholipase Cβ-Ca^{2+} pathway in *Drosophila* gut immunity. *Dev. Cell* 16:386–97
76. Ryu JH, Ha EM, Lee WJ. 2009. Innate immunity and gut-microbe mutualism in *Drosophila*. *Dev. Comp. Immunol*. 34:369–76
77. Ha EM, Oh CT, Bae YS, Lee WJ. 2005. A direct role for dual oxidase in *Drosophila* gut immunity. *Science* 310:847–50
78. Jiang H, Edgar BA. 2009. EGFR signaling regulates the proliferation of *Drosophila* adult midgut progenitors. *Development* 136:483–93
79. Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA. 2009. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* 137:1343–55
80. Biteau B, Hochmuth CE, Jasper H. 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell* 3:442–55
81. Shin SC, Kim SH, You H, Kim B, Kim AC, et al. 2011. *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science* 334:670–74
82. Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F. 2011. *Lactobacillus plantarum* promotes drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing. *Cell Metab*. 14:403–14
83. Wong CN, Ng P, Douglas AE. 2011. Low-diversity bacterial community in the gut of the fruitfly *Drosophila melanogaster*. *Environ. Microbiol*. 13:1889–900
84. Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A. 2011. Bacterial communities of diverse *Drosophila* species: ecological context of a host-microbe model system. *PLOS Genet*. 7:e1002272
85. Li H, Qi Y, Jasper H. 2016. Preventing age-related decline of gut compartmentalization limits microbiota dysbiosis and extends lifespan. *Cell Host Microbe* 19:240–53
86. Iatsenko I, Boquete JP, Lemaitre B. 2018. Microbiota-derived lactate activates production of reactive oxygen species by the intestinal NADPH oxidase Nox and shortens *Drosophila* lifespan. *Immunity* 49:929–42.e5
87. Fast D, Duggal A, Foley E. 2018. Monoassociation with *Lactobacillus plantarum* disrupts intestinal homeostasis in adult *Drosophila melanogaster*. *mBio* 9:e01114-18
88. Choi NH, Kim JG, Yang DJ, Kim YS, Yoo MA. 2008. Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell* 7:318–34
89. Hochmuth CE, Biteau B, Bohmann D, Jasper H. 2011. Redox regulation by Keap1 and Nrf2 controls intestinal stem cell proliferation in *Drosophila*. *Cell Stem Cell* 8:188–99
90. Rera M, Bahadorani S, Cho J, Koehler CL, Ulgherait M, et al. 2011. Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog. *Cell Metab*. 14:623–34
91. Guo L, Karpac J, Tran SL, Jasper H. 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell* 156:109–22
92. Chen H, Zheng X, Zheng Y. 2014. Age-associated loss of lamin-B leads to systemic inflammation and gut hyperplasia. *Cell* 159:829–43
93. Wang L, Ryoo HD, Qi Y, Jasper H. 2015. PERK limits *Drosophila* lifespan by promoting intestinal stem cell proliferation in response to ER stress. *PLOS Genet*. 11:e1005220
94. Siudeja K, Nassari S, Gervais L, Skorski P, Lameiras S, et al. 2015. Frequent somatic mutation in adult intestinal stem cells drives neoplasia and genetic mosaicism during aging. *Cell Stem Cell* 17:663–74
95. Clark RI, Salazar A, Yamada R, Fitz-Gibbon S, Morselli M, et al. 2015. Distinct shifts in microbiota composition during *Drosophila* aging impair intestinal function and drive mortality. *Cell Rep*. 12:1656–67
96. Rera M, Clark R, Walker D. 2012. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *PNAS* 109:21528–33

97. Akagi K, Wilson KA, Katewa SD, Ortega M, Simons J, et al. 2018. Dietary restriction improves intestinal cellular fitness to enhance gut barrier function and lifespan in *D. melanogaster*. *PLOS Genet*. 14:e1007777
98. Regan JC, Khericha M, Dobson AJ, Bolukbasi E, Rattanavirotkul N, Partridge L. 2016. Sex difference in pathology of the ageing gut mediates the greater response of female lifespan to dietary restriction. *eLife* 5:e10956
99. Salazar AM, Resnik-Docampo M, Ulgherait M, Clark RI, Shirasu-Hiza M, et al. 2018. Intestinal snakeskin limits microbial dysbiosis during aging and promotes longevity. *Science* 9:229–43
100. Resnik-Docampo M, Sauer V, Schinaman JM, Clark RI, Walker DW, Jones DL. 2018. Keeping it tight: the relationship between bacterial dysbiosis, septate junctions, and the intestinal barrier in *Drosophila*. *Fly* 12:34–40
101. Resnik-Docampo M, Koehler CL, Clark RI, Schinaman JM, Sauer V, et al. 2017. Tricellular junctions regulate intestinal stem cell behaviour to maintain homeostasis. *Nat. Cell Biol.* 19:52–59
102. Rodriguez-Fernandez IA, Qi Y, Jasper H. 2019. Loss of a proteostatic checkpoint in intestinal stem cells contributes to age-related epithelial dysfunction. *Nat. Commun.* 10:1050
103. Sousa-Victor P, Ayyaz A, Hayashi R, Qi Y, Madden DT, et al. 2017. Piwi is required to limit exhaustion of aging somatic stem cells. *Cell Rep*. 20:2527–37
104. Nagy P, Sandor GO, Juhasz G. 2018. Autophagy maintains stem cells and intestinal homeostasis in *Drosophila*. *Sci. Rep*. 8:4644
105. Jeon HJ, Kim YS, Kim JG, Heo K, Pyo JH, et al. 2018. Effect of heterochromatin stability on intestinal stem cell aging in *Drosophila*. *Mech. Ageing Dev*. 173:50–60
106. Filer D, Thompson MA, Takhaveev V, Dobson AJ, Kotronaki I, et al. 2017. RNA polymerase III limits longevity downstream of TORC1. *Nature* 552:263–67
107. Barker N. 2014. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. *Nat. Rev. Mol. Cell Biol.* 15:19–33
108. Clevers H. 2013. The intestinal crypt, a prototype stem cell compartment. *Cell* 154:274–84
109. Hermiston ML, Gordon JI. 1995. Organization of the crypt-villus axis and evolution of its stem cell hierarchy during intestinal development. *Am. J. Physiol. Gastrointest. Liver Physiol.* 268:G813–22
110. Bjerknes M, Cheng H. 1999. Clonal analysis of mouse intestinal epithelial progenitors. *Gastroenterology* 116:7–14
111. Ponder BA, Schmidt GH, Wilkinson MM, Wood MJ, Monk M, Reid A. 1985. Derivation of mouse intestinal crypts from single progenitor cells. *Nature* 313:689–91
112. Sangiorgi E, Capecchi MR. 2008. *Bmi1* is expressed *in vivo* in intestinal stem cells. *Nat. Genet.* 40:915–20
113. Yan KS, Chia LA, Li X, Ootani A, Su J, et al. 2012. The intestinal stem cell markers Bmi1 and Lgr5 identify two functionally distinct populations. *PNAS* 109:466–71
114. Li N, Yousefi M, Nakauka-Ddamba A, Jain R, Tobias J, et al. 2014. Single-cell analysis of proxy reporter allele-marked epithelial cells establishes intestinal stem cell hierarchy. *Stem Cell Rep*. 3:876–91
115. Jones JC, Brindley CD, Elder NH, Myers MG Jr., Rajala MW, et al. Cellular plasticity of Defa4^{Cre}-expressing Paneth cells in response to Notch activation and intestinal injury. *Cell. Mol. Gastroenterol. Hepatol.* 7:533–54
116. Yu S, Tong K, Zhao Y, Balasubramanian I, Yap GS, et al. 2018. Paneth cell multipotency induced by Notch activation following injury. *Cell Stem Cell* 23:46–59.e5
117. Sei Y, Feng J, Samsel L, White A, Zhao X, et al. Mature enteroendocrine cells contribute to basal and pathological stem cell dynamics in the small intestine. *Am. J. Physiol. Gastrointest. Liver Physiol.* 315:G495–510
118. Ayyaz A, Kumar S, Sangiorgi B, Ghoshal B, Gosio J, et al. 2019. Single-cell transcriptomes of the regenerating intestine reveal a revival stem cell. *Nature* 569:121–25
119. Jung P, Sato T, Merlos-Suarez A, Barriga FM, Iglesias M, et al. Isolation and *in vitro* expansion of human colonic stem cells. *Nat. Med.* 17:1225–27
120. Forster R, Chiba K, Schaeffer L, Regalado SG, Lai CS, et al. 2014. Human intestinal tissue with adult stem cell properties derived from pluripotent stem cells. *Stem Cell Rep*. 2:838–52

121. Jeong Y, Rhee H, Martin S, Klass D, Lin Y, et al. 2016. Identification and genetic manipulation of human and mouse oesophageal stem cells. *Gut* 65:1077–86
122. Kalabis J, Oyama K, Okawa T, Nakagawa H, Michaylira CZ, et al. 2008. A subpopulation of mouse esophageal basal cells has properties of stem cells with the capacity for self-renewal and lineage specification. *J. Clin. Investig.* 118:3860–69
123. Pan Q, Nicholson AM, Barr H, Harrison LA, Wilson GD, et al. 2013. Identification of lineage-uncommitted, long-lived, label-retaining cells in healthy human esophagus and stomach, and in metaplastic esophagus. *Gastroenterology* 144:761–70
124. DeWard AD, Cramer J, Lagasse E. 2014. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell Rep*. 9:701–11
125. Doupe DP, Alcolea MP, Roshan A, Zhang G, Klein AM, et al. 2012. A single progenitor population switches behavior to maintain and repair esophageal epithelium. *Science* 337:1091–93
126. Mills JC, Shivdasani RA. 2011. Gastric epithelial stem cells. *Gastroenterology* 140:412–24
127. Bjerknes M, Cheng H. 2002. Multipotential stem cells in adult mouse gastric epithelium. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283:G767–77
128. Lee ER, Leblond CP. 1985. Dynamic histology of the antral epithelium in the mouse stomach: II. Ultrastructure and renewal of isthmal cells. *Am. J. Anat.* 172:205–24
129. Qiao XT, Ziel JW, McKimpson W, Madison BB, Todisco A, et al. 2007. Prospective identification of a multilineage progenitor in murine stomach epithelium. *Gastroenterology* 133:1989–98
130. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, et al. 2010. Lgr5^{+ve} stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* 6:25–36
131. Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, et al. 2011. Sox2^{+} adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell* 9:317–29
132. Stange DE, Koo BK, Huch M, Sibbel G, Basak O, et al. 2013. Differentiated *Troy*$^{+}$ chief cells act as reserve stem cells to generate all lineages of the stomach epithelium. *Cell* 155:357–68
133. Nam KT, Lee HJ, Sousa JF, Weis VG, O'Neal RL, et al. 2010. Mature chief cells are cryptic progenitors for metaplasia in the stomach. *Gastroenterology* 139:2028–37.e9
134. Walker MR, Patel KK, Stappenbeck TS. 2009. The stem cell niche. *J. Pathol.* 217:169–80
135. Santos AJM, Lo YH, Mah AT, Kuo CJ. 2018. The intestinal stem cell niche: homeostasis and adaptations. *Trends Cell Biol*. 28:1062–78
136. van de Wetering M, Sancho E, Verweij C, de Lau W, Oving I, et al. 2002. The β-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* 111:241–50
137. Kosinski C, Li VS, Chan AS, Zhang J, Ho C, et al. 2007. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. *PNAS* 104:15418–23
138. Korinek V, Barker N, Moerer P, van Donselaar E, Huls G, et al. 1998. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* 19:379–83
139. Fevr T, Robine S, Louvard D, Huelsken J. 2007. Wnt/β-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells. *Mol. Cell Biol.* 27:7551–59
140. Pinto D, Gregorieff A, Begthel H, Clevers H. 2003. Canonical Wnt signals are essential for homeostasis of the intestinal epithelium. *Genes Dev*. 17:1709–13
141. Kuhnert F, Davis CR, Wang HT, Chu P, Lee M, et al. 2004. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *PNAS* 101:266–71
142. Muncan V, Sansom OJ, Tertoolen L, Phesse TJ, Begthel H, et al. 2006. Rapid loss of intestinal crypts upon conditional deletion of the Wnt/Tcf-4 target gene *c-Myc*. *Mol. Cell. Biol.* 26:8418–26
143. Schepers A, Clevers H. 2012. Wnt signaling, stem cells, and cancer of the gastrointestinal tract. *Cold Spring Harb. Perspect. Biol.* 4:a007989
144. Andreu P, Colnot S, Godard C, Gad S, Chafey P, et al. 2005. Crypt-restricted proliferation and commitment to the Paneth cell lineage following *Apc* loss in the mouse intestine. *Development* 132:1443–51
145. van Es JH, Jay P, Gregorieff A, van Gijn ME, Jonkheer S, et al. 2005. Wnt signalling induces maturation of Paneth cells in intestinal crypts. *Nat. Cell Biol.* 7:381–86
146. Bastide P, Darido C, Pannequin J, Kist R, Robine S, et al. 2007. Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. *J. Cell Biol.* 178:635–48

147. Mori-Akiyama Y, van den Born M, van Es JH, Hamilton SR, Adams HP, et al. 2007. SOX9 is required for the differentiation of Paneth cells in the intestinal epithelium. *Gastroenterology* 133:539–46
148. Batlle E, Henderson JT, Beghtel H, van den Born MM, Sancho E, et al. 2002. β-Catenin and TCF mediate cell positioning in the intestinal epithelium by controlling the expression of EphB/ephrinB. *Cell* 111:251–63
149. van der Flier LG, Clevers H. 2009. Stem cells, self-renewal, and differentiation in the intestinal epithelium. *Annu. Rev. Physiol.* 71:241–60
150. Hardwick JC, Van Den Brink GR, Bleuming SA, Ballester I, Van Den Brande JM, et al. 2004. Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology* 126:111–21
151. Haramis AP, Begthel H, van den Born M, van Es J, Jonkheer S, et al. 2004. De novo crypt formation and juvenile polyposis on BMP inhibition in mouse intestine. *Science* 303:1684–86
152. He XC, Zhang J, Tong WG, Tawfik O, Ross J, et al. 2004. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-β-catenin signaling. *Nat. Genet.* 36:1117–21
153. Milano J, McKay J, Dagenais C, Foster-Brown L, Pognan F, et al. 2004. Modulation of notch processing by γ-secretase inhibitors causes intestinal goblet cell metaplasia and induction of genes known to specify gut secretory lineage differentiation. *Toxicol. Sci.* 82:341–58
154. Wong GT, Manfra D, Poulet FM, Zhang Q, Josien H, et al. 2004. Chronic treatment with the γ-secretase inhibitor LY-411,575 inhibits β-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J. Biol. Chem.* 279:12876–82
155. van Es JH, van Gijn ME, Riccio O, van den Born M, Vooijs M, et al. 2005. Notch/γ-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 435:959–63
156. Tian H, Biehs B, Chiu C, Siebel CW, Wu Y, et al. 2015. Opposing activities of Notch and Wnt signaling regulate intestinal stem cells and gut homeostasis. *Cell Rep.* 11:33–42
157. Fre S, Huyghe M, Mourikis P, Robine S, Louvard D, Artavanis-Tsakonas S. 2005. Notch signals control the fate of immature progenitor cells in the intestine. *Nature* 435:964–68
158. von Rahden BH, Kircher S, Lazariotou M, Reiber C, Stuermer L, et al. 2011. LgR5 expression and cancer stem cell hypothesis: clue to define the true origin of esophageal adenocarcinomas with and without Barrett's Esophagus? *J. Exp. Clin. Cancer Res.* 30:23
159. Hoffmann JA. 2003. The immune response of *Drosophila*. *Nature* 426:33–38
160. de Jong HK, Parry CM, van der Poll T, Wiersinga WJ. 2012. Host-pathogen interaction in invasive Salmonellosis. *PLOS Pathog.* 8:e1002933
161. Meylan F, Richard AC, Siegel RM. 2011. TL1A and DR3, a TNF family ligand-receptor pair that promotes lymphocyte costimulation, mucosal hyperplasia, and autoimmune inflammation. *Immunol. Rev.* 244:188–96
162. Xavier RJ, Podolsky DK. 2007. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 448:427–34
163. Pasparakis M. 2012. Role of NF-κB in epithelial biology. *Immunol. Rev.* 246:346–58
164. Wullaert A, Bonnet MC, Pasparakis M. 2011. NF-κB in the regulation of epithelial homeostasis and inflammation. *Cell Res.* 21:146–58
165. Lindemans CA, Calafiore M, Mertelsmann AM, O'Connor MH, Dudakov JA, et al. 2015. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 528:560–64
166. Mizoguchi A, Yano A, Himuro H, Ezaki Y, Sadanaga T, Mizoguchi E. 2018. Clinical importance of IL-22 cascade in IBD. *J. Gastroenterol.* 53:465–74
167. Rangan P, Choi I, Wei M, Navarrete G, Guen E, et al. 2019. Fasting-mimicking diet modulates microbiota and promotes intestinal regeneration to reduce inflammatory bowel disease pathology. *Cell Rep.* 26:2704–19.e6
168. Yilmaz ÖH, Katajisto P, Lamming DW, Gültekin Y, Bauer-Rowe KE, et al. 2012. mTORC1 in the Paneth cell niche couples intestinal stem-cell function to calorie intake. *Nature* 486:490–95
169. Beyaz S, Mana MD, Roper J, Kedrin D, Saadatpour A, et al. 2016. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* 531:53–58

170. Mihaylova MM, Cheng CW, Cao AQ, Tripathi S, Mana MD, et al. 2018. Fasting activates fatty acid oxidation to enhance intestinal stem cell function during homeostasis and aging. *Cell Stem Cell* 22:769–78.e4
171. Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, et al. 2018. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. *Science* 359:1376–83
172. Martin K, Potten CS, Roberts SA, Kirkwood TB. 1998. Altered stem cell regeneration in irradiated intestinal crypts of senescent mice. *J. Cell Sci.* 111(Part 16):2297–303
173. Nalapareddy K, Choudhury AR, Gompf A, Ju Z, Ravipati S, et al. CHK2-independent induction of telomere dysfunction checkpoints in stem and progenitor cells. *EMBO Rep*. 11:619–25
174. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, et al. 2014. Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. *Nat. Commun.* 2:4172
175. Thevaranjan N, Puchta A, Schulz C, Naidoo A, Szamosi JC, et al. 2017. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host Microbe* 21:455–66.e4
176. Nalapareddy K, Nattamai KJ, Kumar RS, Karns R, Wikenheiser-Brokamp KA, et al. 2017. Canonical Wnt signaling ameliorates aging of intestinal stem cells. *Cell Rep*. 18:2608–21
177. Kozar S, Morrissey E, Nicholson AM, van der Heijden M, Zecchini HI, et al. 2013. Continuous clonal labeling reveals small numbers of functional stem cells in intestinal crypts and adenomas. *Cell Stem Cell* 13:626–33
178. Pentinmikko N, Iqbal S, Mana M, Andersson S, Cognetta AB 3rd, et al. 2019. Notum produced by Paneth cells attenuates regeneration of aged intestinal epithelium. *Nature* 571:398–402
179. Moorefield EC, Andres SF, Blue RE, Van Landeghem L, Mah AT, et al. 2017. Aging effects on intestinal homeostasis associated with expansion and dysfunction of intestinal epithelial stem cells. *Aging* 9:1898–915
180. Cui H, Tang D, Garside GB, Zeng T, Wang Y, et al. 2019. Wnt signaling mediates the aging-induced differentiation impairment of intestinal stem cells. *Stem Cell Rev*. 15:448–55
181. Radtke F, Clevers H. 2005. Self-renewal and cancer of the gut: two sides of a coin. *Science* 307:1904–9
182. Tao Y, Kang B, Petkovich DA, Bhandari YR, In J, et al. 2019. Aging-like spontaneous epigenetic silencing facilitates Wnt activation, stemness, and $Braf^{V600E}$-induced tumorigenesis. *Cancer Cell* 35:315–28.e6
183. Yousefi M, Nakauka-Ddamba A, Berry CT, Li N, Schoenberger J, et al. 2018. Calorie restriction governs intestinal epithelial regeneration through cell-autonomous regulation of mTORC1 in reserve stem cells. *Stem Cell Rep*. 10:703–11
184. Rodgers JT, King KY, Brett JO, Cromie MJ, Charville GW, et al. 2014. mTORC1 controls the adaptive transition of quiescent stem cells from G_0 to G_{Alert}. *Nature* 510:393–96
185. Igarashi M, Miura M, Williams E, Jaksch F, Kadowaki T, et al. 2019. NAD^+ supplementation rejuvenates aged gut adult stem cells. *Aging Cell* 18:e12935
186. Choi J, Rakhilin N, Gadamsetty P, Joe DJ, Tabrizian T, et al. 2018. Intestinal crypts recover rapidly from focal damage with coordinated motion of stem cells that is impaired by aging. *Sci. Rep.* 8:10989