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THEME | *The Engineered Gut: Use of Stem Cells and Tissue Engineering to Study Physiological Mechanisms and Disease Processes*

Intestinal renewal across the animal kingdom: comparing stem cell activity in mouse and *Drosophila*

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Zwick RK, Ohlstein B, Klein OD. Intestinal renewal across the animal kingdom: comparing stem cell activity in mouse and *Drosophila*. *Am J Physiol Gastrointest Liver Physiol* 316: G313–G322, 2019. First published December 13, 2018; doi:10.1152/ajpgi.00353.2018.—The gastrointestinal (GI) tract renews frequently to sustain nutrient digestion and absorption in the face of consistent tissue stress. In many species, proliferative intestinal stem cells (ISCs) are responsible for the repair of the damage arising from chemical and mechanical aspects of food breakdown and exposure to pathogens. As the cellular source of all mature cell types of the intestinal epithelium throughout adulthood, ISCs hold tremendous therapeutic potential for understanding and treating GI disease in humans. This review focuses on recent advances in our understanding of ISC identity, behavior, and regulation during homeostasis and injury-induced repair, as revealed by two major animal models used to study regeneration of the small intestine: *Drosophila melanogaster* and *Mus musculus*. We emphasize recent findings from *Drosophila* that are likely to translate to the mammalian GI system, as well as challenging topics in mouse ISC biology that may be ideally suited for investigation in flies. For context, we begin by reviewing major physiological similarities and distinctions between the *Drosophila* midgut and mouse small intestine.

animal models; intestinal stem cells; midgut; regeneration; small intestine

INTESTINAL PHYSIOLOGY IN *DROSOPHILA* AND MAMMALS

An epithelial monolayer that serves as the primary site of food digestion runs through the *Drosophila* foregut, midgut, and hindgut, as well as the similar regions in the mammalian gut: the esophagus, small intestine, and large intestine (6, 38, 55) (Fig. 1). The mammalian small intestine, in turn, is divided into three regions from proximal to distal: the duodenum, jejunum, and ileum (Fig. 1). These three regions within the small intestine display gradual changes in structure and cell-type composition and a limited number of anatomical differences, such as the confinement of mucus-secreting Brunner's glands to the duodenum (18, 83). By contrast, evaluation of the *Drosophila* midgut at a high spatial resolution recently revealed 10–14 subdivisions with precise boundaries and structural and functional distinctions, including major differences in cellular morphology and physiology, gene expression, susceptibility to tumor formation, and intestinal stem cell (ISC) behavior (22, 63). It is possible that the *Drosophila* midgut contains more distinct compartmentalization than the similar region in mice; however, these findings also raise the intriguing possibility that the mammalian small intestine may exhibit more finely grained spatial differences than has currently been appreciated.

Unlike the straight epithelial monolayer in flies, the intestine in mice (and humans) folds into depressions and protrusions, called crypts and villi (18) (Fig. 1). Despite this prominent structural difference, the intestine of both species houses epithelial cells of the same basic lineages: absorptive enterocytes (ECs) and secretory enteroendocrine (ee) cells that execute the major functions of the gut. Within these lineages, mammals also possess several specialized cell types not found in *Drosophila*: antimicrobial-secreting Paneth cells, mucus-secreting goblet cells, and mechanosensing tuft cells (45) (Figs. 1 and 2).

ISC populations have been defined in both mice and flies. *Drosophila* midgut ISCs were identified via clonal analysis and evaluation of various cell markers (67, 75) and are positioned on top of the basement membrane along the length of the intestinal epithelium, next to specialized epithelial cell types (Fig. 1). In mice, ISCs were first reported in 1974 (26) and formally defined more than three decades later as fast-cycling leucine-rich repeat-containing G-protein-coupled receptor 5

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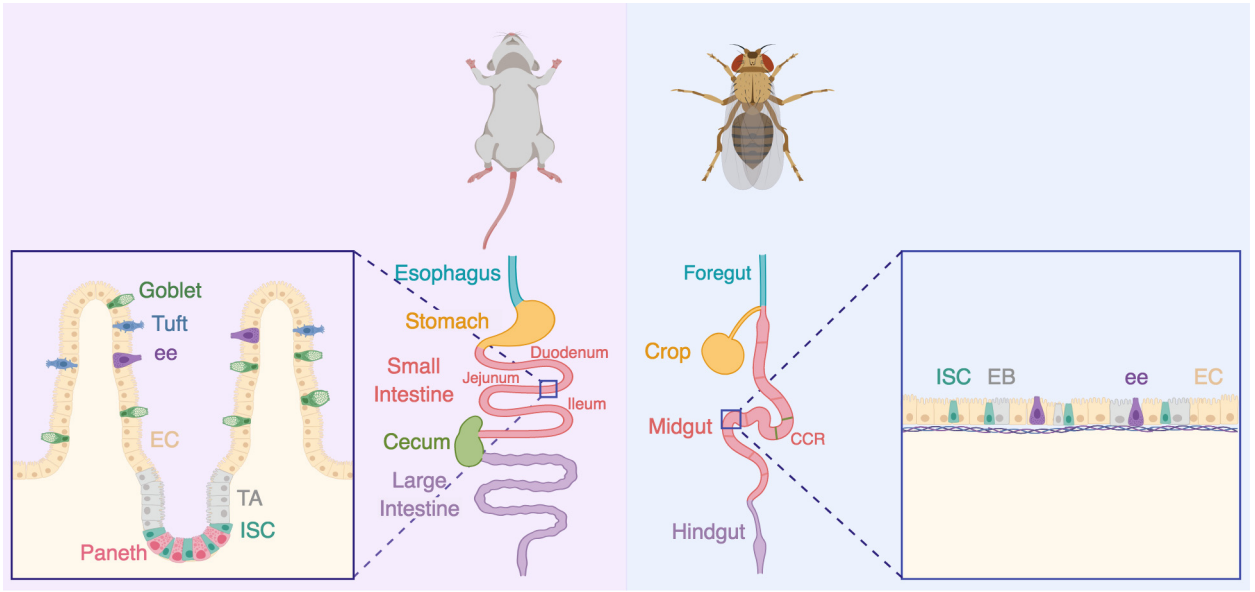

Fig. 1. Anatomy and physiology of the gastrointestinal (GI) tract in mice and *Drosophila*. Schematic model of the GI tract in mice (left), including the esophagus; stomach; duodenum, jejunum, and ileum within the small intestine; cecum; and large intestine, and in *Drosophila* (right), including the foregut; crop; subsections of the midgut, including the copper cell region (CCR); and hindgut. *Insets*: intestinal structure and cellular composition of the small intestine/midgut in each species, containing intestinal stem cells (ISCs) and epithelial cells of the absorptive and secretory lineages as labeled. EB, enteroblast; EC, enterocyte; ee, enteroendocrine; TA, transit amplifying.

(LGR5)-expressing cells (8) with the ability to generate organoids in vitro (85). These cells are interspersed between Paneth cells in the lower-most region of intestinal crypts (Fig. 1), leading to their commonly used name "crypt base columnar" (CBC) cells. The alternating pattern of Paneth cells and CBCs in mammalian crypts results from a cell division-coupled rearrangement (25, 65) in which Paneth cells wedge between dividing CBC daughter cells during cytokinesis (65). In contrast, the factors that dictate the spacing of ISCs within subsections of the *Drosophila* midgut are not well understood.

LINEAGE HIERARCHIES WITHIN THE INTESTINAL EPITHELIUM

Our current concept of the epithelial lineage hierarchy in the intestine of mice and flies is summarized in Fig. 2. In mice, the

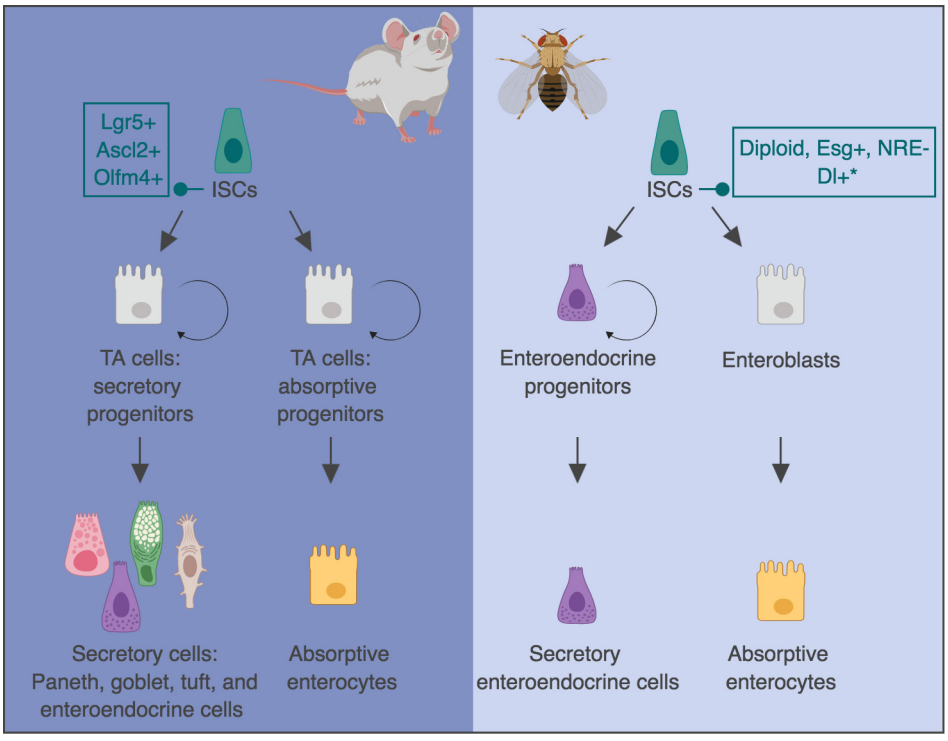

Fig. 2. Intestinal epithelial lineage hierarchies. In mice (left), crypt base columnar intestinal stem cells (ISCs) give rise to transit amplifying (TA) cells that serve as progenitors to mature cells of the secretory lineage [Paneth cells, goblet cells, tuft cells, and enteroendocrine (ee) cell subtypes] or the absorptive lineage [enterocytes (ECs)]. In *Drosophila* (right), ISCs give rise to either secretory ee cells or enteroblast progenitors that differentiate into ECs. Green boxes (*top*, left and right) contain commonly used ISC markers in each species. *Expression in actively cycling states.

traditional paradigm for ISC differentiation under homeostatic conditions (29) involves ISC progeny first committing to either the secretory or absorptive lineages (Fig. 2). These progenitors occupy a region within the crypt, termed the transit amplifying (TA) compartment, and undergo four to five divisions before shuffling from the crypt toward the villi to differentiate into mature cells of their respective lineages. In *Drosophila*, ISCs were previously proposed to generate a bipotent enteroblast (EB) progenitor in response to cell loss. EBs were then thought to commit rapidly to either an EC or ee cell fate in response to high or low Delta (Dl)-driven Notch signaling levels, respectively (74). More recent studies, however, showed that EBs are committed to differentiate into absorptive lineages, whereas secretory lineages do not transition through an EB intermediate (11, 17, 39, 108, 109). For differentiation in the absorptive lineage, ISCs produce membrane-bound Dl that activates the Notch receptor in newly produced EBs, promoting their differentiation into ECs (39). In a significant break from the former concept of homeostatic regulation of the secretory lineage, ee differentiation was found to be Notch independent, instead requiring asymmetric localization of the ee cell fate marker Prospero during ISC division (39) under control of transcription factors Escargot (Esg) and Scute (58). Furthermore, ee cells in *Drosophila* are produced via a mitotic progenitor cell (39), analogous to secretory TA cells in mammals (Fig. 2).

Several signaling pathways play highly conserved roles in the control and maintenance of the intestinal epithelial hierarchy. As in flies, Notch is one of the major niche signals critical for ISC maintenance and EC differentiation in mice (13, 35, 39, 90a, 99, 100). Egf signaling, which has long been known to regulate ISC proliferation and quiescence in *Drosophila* (16, 20, 47, 91), was recently shown also to regulate the quiescence of mouse-derived primary ISCs in vitro: the blocking of EGF receptor induces ISC quiescence and an ee cell-biased gene-expression signature (10). In addition to these examples, Wnt signaling is crucial to the regulation of ISC maintenance, proliferation, and differentiation. As previously reviewed (38), several lines of evidence have suggested that Wnt/Wingless signaling regulates invertebrate ISC behavior in some contexts, although this is only partially understood in *Drosophila* and has been a source of some debate. Collectively, these studies demonstrate that several pathways involved in control of ISC maintenance and differentiation are conserved between flies and mice, with practical implications for the comparison of *Drosophila* and mammalian lineage hierarchies.

A question of major interest in both vertebrates and invertebrates is how the intestinal epithelium maintains the appropriate balance of the absorptive and secretory lineages under homeostasis. A growing body of literature describes mechanisms that couple signaling and behavior of mature epithelial cells to ISC division and differentiation in the *Drosophila* midgut. Interestingly, the Dl ligand from newly formed ee daughter cells induces low Notch activity in ISCs that limits their production of ECs (39). Notch signaling is thus bidirectional: Dl expression by ISCs promotes EC differentiation, as described above, whereas ee cell-derived Dl represses ISC differentiation into ECs, maintaining ISC identity (39). The death of differentiated epithelial cells also impacts ISC behavior in *Drosophila*. EC apoptosis, including that which results from homeostatic cell loss, promotes compensatory ISC division (3, 38, 48, 59, 93). A population of differentiation-delayed EBs produced by ISCs under homeostatic conditions can also sense loss of differentiated cells via cell-to-cell contact and responds by rapidly undergoing terminal differentiation (4), providing an additional means by which ISCs and their progeny responds to local cellular demand in *Drosophila*. The mechanisms that regulate a steady number of absorptive and secretory cells under homeostasis are not well understood in mammals; these studies conducted in *Drosophila* suggest that differentiated epithelial cell types may represent a major source of signals controlling this balance.

ISC IDENTITY AND HETEROGENEITY

Markers that identify canonical stem cells are well established in the mammalian intestine, but unique stem cell markers are currently lacking in *Drosophila*. In mammals, actively cycling CBCs, which are regulated in large part by Wnt/β-catenin signaling, are most commonly defined by their selective expression of the Wnt pathway member *Lgr5* in the crypt (8). Hundreds of additional genes make up the transcriptional signature of CBCs, such as commonly used markers *Olfm4* and *Ascl2* (71) (Fig. 2), but some are also expressed in other progenitor cell types in the intestinal epithelium (90). In *Drosophila*, ISCs and their daughter EBs express *esg*, which is turned off as these cells become polyploid and differentiate into ECs (52, 60), as well as *headcase* (79) (Fig. 2). ISCs can also be defined as Esg^+, Notch response element (NRE)-negative, diploid cells that express *Dl* only while actively cycling (67). In apparent contradiction to these characterizations, Esg^+/Dl^+ cells accumulate in aged flies (15, 27) and injured intestines; however, these cells are strongly NRE positive and therefore, may be suspended in an EB-to-EC transition state due to differentiation defects (101a). Polyploid cells also express *esg* and Dl in response to tissue stress (61), but this may represent an early stage of EC reversion into a progenitor-like state. Whereas expression of genes enriched in EBs but not ISCs can distinguish the two esg^+ progenitor cell types, discovery of a single gene that is selectively expressed by *Drosophila* ISCs but not their progeny would be of significant value to the field.

Whereas it is emerging that a single, distinct ISC population exists in both mice and *Drosophila*, recent work also shows that individual cells that meet the criteria of these populations may display important functional differences. For example, superficially similar ISCs in female and male *Drosophila* display different proliferation kinetics, with ISCs in female flies dividing more frequently during normal turnover and in response to injury (78). Under homeostatic conditions, ISC-specific knockdown of the sex-determination pathway in female animals or conversely, feminization of ISCs in males reverses sex-specific differences in proliferation rates, demonstrating that sexual-determination genes regulate this aspect of ISC behavior (41). Enhanced ISC proliferation capacity is hypothesized to provide female flies with greater adaptability to metabolic demand during egg production, and in line with this, masculinized ISCs in females have reduced fecundity (41). Although many aspects of sex determination differ between insects and mammals, recent evidence suggests that sex specification in each species converges on common effector genes (30, 64, 77). Thus the possibility that mammals also

display sexual divergence in ISC behavior—perhaps during reproductive stages when metabolic need and the demand for host protection are high—would be an interesting area for future research.

Another major source of heterogeneity among *Drosophila* ISCs relates to their spatial position across the intestine. ISCs, residing in different subregions of the midgut, display distinct cycling rates and cell-fate decisions. The tracking of single, fluorescently labeled stem cells established that in certain subregions, ISCs generate progeny only within their own starting regions (63), raising the possibility that intrinsically different ISCs maintain different regions of the midgut. It was subsequently identified that exposure to bone morphogenetic protein (BMP) signals during a confined window of metamorphosis specializes some ISCs for the "copper cell region" (CCR) of the midgut (32). After this developmental time frame, microenvironment-derived BMP signals are no longer sufficient to induce a CCR-specific identity in ISCs, although they play important roles in maintaining CCR identity in previously specialized CCR ISCs (32, 37). Therefore, in at least one region of midgut and likely others, intrinsic differences in ISCs are established in early development, whereas signals from the microenvironment participate in the maintenance of tissue diversity across the adult midgut. In mammals, region-specific gene-expression profiles are also maintained in long-term culture of organoids derived from crypts of different regions of the small intestine in the absence of ongoing stimulus from the microenvironment, suggesting the presence of unappreciated intrinsic differences in crypt-derived epithelial cells from different regions (68). Further exploration of this possibility is needed in mammals, which may be guided by further investigation into how ISCs specify and maintain additional regions of the *Drosophila* midgut. ISC heterogeneity may have major clinical implications. If mammalian ISCs contain distinct regional subsets, as have been identified in *Drosophila*, then the pinpointing of these populations would be instrumental for the use of ISCs in regenerative medicine. Future studies in *Drosophila* and/or mice are also needed to explore whether ISC subsets could have differences in, for example, their propensity to drive gastrointestinal (GI) disease, potency to repair injury, or drug/radioresistance.

REGENERATION FOLLOWING INTESTINAL INJURY AND STRESS

The intestine can be repaired after tissue stress and injury by a variety of potential mechanisms (13, 45, 49, 102), including production of new, differentiated cells from CBCs and/or other putative ISC populations to replace those that were lost (Fig. 3*A*), reversion of differentiated cells into functional stem cells (Fig. 3*B*), and the reprogramming of ISCs into a proliferative fetal-like state (Fig. 3*C*).

In flies, various types of insults to the intestinal epithelium, including cell ablation with genetic models, bacterial infection, or feeding with tissue-damaging agents, trigger an ISC-driven repair response of division and differentiation to replace lost mature cells (2, 19, 21, 44, 49) (Fig. 3*A*). In mice, the site of intestinal injury seems to impact the repair response that will ensue. Two recent studies (72, 110), in which injury was localized to different points in the crypt-villus axis, illustrate this point. In one, villus damage, caused by an enteric rotavirus that specifically infects differentiated cell types, was repaired when ISCs were activated to divide and migrate up villi to replace lost cells (110), according to an ISC-driven mechanism of cellular replacement similar to that which occurs after numerous *Drosophila* injuries described above (Fig. 3*A*). The ISC response in this case was dependent on epithelial-derived Wnt signals, although it is unknown whether these signals act on ISCs directly or in a nonautonomous manner involving a feedback mechanism with additional cell types in the microenvironment. In a second scenario, crypt damage was induced by parasitic helminth larvae, which penetrate the epithelium and localize to the duodenal stroma within a multicellular granuloma (72). In this case, crypt cells immediately adjacent to granulomas undergo an IFN-γ-mediated reversion to a fetal gene-expression program. In vivo, *Lgr5* expression was shut off in the base of these crypts, and proliferation and expression of the IFN target gene *Sca-1* were induced. In vitro, these *Sca-1*$^+$ cells generate fetal-like spheroids and express a fetal-associated transcriptional program. Interestingly, other forms of crypt-localized injury in the small intestine, including irradiation and ablation of *Lgr5*$^+$ CBCs (72), as well as dextran sulfate sodium-induced colitis in the large intestine (107), produce a similar upregulation of *Sca1* expression. Thus, fetal reprogramming represents another general mode of regeneration that follows crypt injury in multiple parts of the GI tract (Fig. 3*C*). Whereas it is known that fetal reversion in the small intestine following helminth infection is at least partially mediated by IFN-γ-producing immune cells (72), the exact nature of ISC–immune cell interactions in controlling regeneration is an important area for future work.

In mice, several populations other than CBCs have been proposed to display stem cell-like behavior, especially in response to injury, which has led to the hypothesis that additional stem cell populations could maintain the intestinal epithelium in a context-specific manner (13). Most notably, a population positioned four cells above the base of the crypt (called "+4 cells") has been proposed to represent a reserve, radioresistant ISC population activated by tissue injury (13), hypothesized to replace CBCs lost by radiation or genetic ablation (56, 66, 92, 97, 105) (Fig. 3*A*). Although originally thought to be quiescent and label retaining, the population that is commonly referred to as +4 cells may actually represent a heterogenous cell population with different cycling, radioresistant, and regenerative properties (56). Recently, several studies have demonstrated that putative genetic markers of +4 cells, such as *Bmi1*, which is expressed by radioresistant and injury-inducible cells (104), are more broadly expressed throughout the intestinal epithelium than had been appreciated. RNA sequencing revealed that Bmi1$^+$ cells express a transcriptomic signature aligned with ee secretory cells (105). In response to irradiation (105) or CBC ablation (43), progeny of *Bmi1*$^+$ cells de-differentiates into CBCs in a process that involves chromatin rearrangement to a conformation that more closely resembles that of ISCs (43). It is possible that other populations may represent a reserve stem cell population. However, data advance our understanding of mammalian ISC hierarchies and stem/progenitor population inter-relatedness and add to a growing body of literature that reveals specific injury conditions that promote high levels of plasticity in progenitor and differentiated epithelial cell populations (23, 43, 95, 98, 105) (Fig. 3*B*). In *Drosophila*, evaluation of the regenerative re-

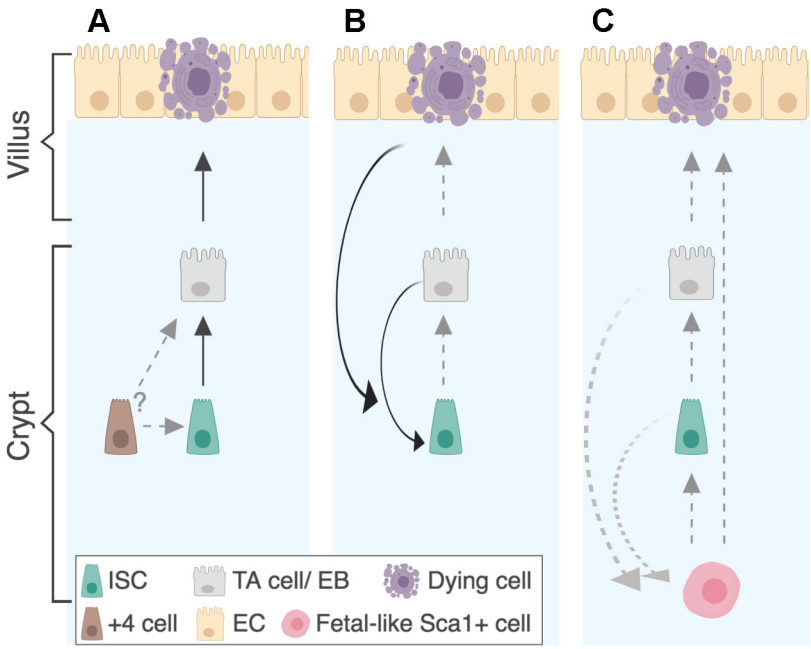

Fig. 3. Models of intestinal regeneration in response to injury. Potential cellular mechanisms of intestinal repair after injury include the following. *A*: replacement of progenitor and differentiated intestinal epithelial cells by intestinal stem cells (ISCs). The contribution of a second population of reserve ISCs, +4 cells, has also been proposed. *B*: de-differentiation of progenitor or mature cell types into a functional ISC population capable of replacing lost cells, potentially via standard differentiation pathways. *C*: reprogramming of ISCs and/or other epithelial cell types into a fetal-like cell type marked by a Sca-1$^+$ transcriptional signature. Mechanisms and cell types that require further confirmation are designated with dotted gray arrows or a question mark, respectively. Crypt and villus designations refer to cell position within mammalian small intestine. EB, enteroblast; EC, enterocyte; TA, transit amplifying.

sponse that occurs during refeeding, after fasting-induced ISC loss from large regions of the midgut, revealed that symmetrical ISC divisions do not replenish the population (61), as might be expected given the ISC-driven regeneration methods described above (Fig. 3*A*). Instead, polyploid ECs, which normally possess 4–16 genome copies, undergo ploidy reduction to reconstitute the population of *2n* ISCs (61). In this case, de-differentiation occurs via "amitosis": cell division in which genetic material is separated by nuclear invagination without a mitotic spindle, resulting in a binucleated cell that ultimately splits into two daughter cells (61).

Collectively, these studies reveal striking similarities in the cellular mechanisms of regeneration in *Drosophila* and mammals. Depending on the context of injury, both species demonstrate ISC-driven repair mechanisms (Fig. 3*A*), as well as plasticity of lineage-committed cells that allows them to reassume roles as functional stem cells (Fig. 3, *B* and *C*). Depolyploidization has been reported in other physiological scenarios in numerous organisms, including in cultured mouse embryos and human adrenal glands (53, 62). Whether this mechanism could also account for de-differentiation in other regenerating mammalian tissues, including the intestine, is an exciting avenue for future investigation. Conversely, future studies to identify which mechanistic aspects of mammalian de-differentiation are recapitulated during invertebrate intestinal repair, as well as the possibility that *Drosophila* ISCs could also undergo reprogramming (Fig. 3*C*), will drive further development in the use of flies to model intestinal regeneration.

MICROENVIRONMENTAL CONTROL OF ISCs

ISCs are exposed to a rich milieu of cellular and noncellular cues from the surrounding microenvironment, including other epithelial and immune cells, capillaries (or trachea, in *Drosophila*), muscle, nutrients, mechanical forces, and extracellular matrix (6, 45, 94). Although many of these sources of extracellular signals are shared between *Drosophila* and mice, the mammalian microenvironment contains a higher number of epithelial and immune subtypes than flies, as well as mesenchymal cells not present in *Drosophila*.

Debate over the cell type(s) that provide the Wnt and Notch signals, key to the regulation of ISC behavior in mice, has led to recent breakthroughs in our concept of the mammalian ISC niche (81). Paneth cells were an early candidate source of signals, given their proximity to CBCs and the demonstration that they produce Wnt, Notch, and EGF ligands integral to ISC maintenance and proliferation (13, 84). An important role for Paneth cells in metabolic regulation of ISCs has also been defined in several scenarios, including ISC response to calorie restriction (42, 106) and mitochondrial oxidative phosphorylation (80). Although it is clear that Paneth cells play a key role in the regulation of many aspects of ISC behavior, the proposal of this cell type as a true ISC "niche"—a localized environment that houses stem cells and is required for the imposition of stemness (70)—resulted from studies showing the requirement of Paneth cells for intestinal organoid establishment in vitro and CBC maintenance in vivo (84). Subsequently, however, it has been recognized that Paneth cells support intestinal organoids with Wnt signals that are produced redundantly by other cell types in the ISC microenvironment, and additional models of Paneth cell loss have not recapitulated the requirement of Paneth cells for CBC maintenance in vivo (33, 51). Whereas global genetic loss of *Wntless* (*Wls*), which is required for Wnt ligand secretion, depletes the ISC population, this phenotype is not observed after selective deletion of *Wls* in *Villin-Cre*$^+$ mature intestinal epithelial cells (97), in line with prior studies showing the continuity of intestinal homeostasis following genetic deletion of other Wnt pathway members from the same mature epithelial cells (34, 50, 82). These studies point to Wnt contribution from an extra-epithelial source in vivo.

The mesenchyme surrounding mammalian CBCs has long been recognized as a source of Wnt ligands, as well as BMP antagonists (94). Single molecule RNA fluorescence in situ hybridization was recently used to identify expression of Wnt

ligands, such as Wnt2b and Wnt5a, by numerous mesenchymal cell types in the ISC microenvironment (97). *Foxl1*-expressing mesenchymal cells, residing in close proximity to crypts, were specifically found to express high levels of growth factors that can induce Wnt signaling (5), as well as other positive and negative regulators of Wnt, sonic hedgehog, Bmp, and transforming growth factor β signaling (89); the expression of these ligands is compartmentalized depending on *Foxl1*$^+$ cell position relative to the epithelial crypt-villi axis (89). Depletion of this putative niche cell population using two diphtheria toxin-mediated cell-ablation approaches resulted in smaller crypts and villi, loss of ISCs, and depressed Wnt activity (5). Furthermore, although selective deletion of the Wnt functional maturation gene *Porcupine* in epithelial cells, does not impair intestinal function (50, 82), selective loss of *Porcupine* in *Foxl1*$^+$ cells leads to reduced Wnt signaling, loss of ISC and TA cell proliferation, and impaired epithelial renewal, ultimately resulting in massive crypt loss (89). In support of this finding, deletion of *Wls* from an overlapping *Gli1*-expressing stromal cell population also resulted in modest ISC loss and crypt collapse (31). Intriguingly, *Gli1*$^+$ cell numbers increase after colon damage, suggesting the possibility that these cells could sense tissue damage or interact bidirectionally with CBCs (31).

Whereas these studies demonstrate that mesenchymal cells provide niche support for mammalian ISCs, the identity of a true ISC niche in *Drosophila*, which lack this same stromal population, remains unknown. Intriguingly, however, following depletion, ISCs rebound to the same cell number as was present pre-depletion (61), suggesting the presence of a so-far unknown mechanism to regulate ISC number precisely in *Drosophila*. Future work to determine whether this aspect of stem cell behavior is controlled by signals from the microenvironment or intrinsic-sensing mechanisms is of major interest and may reveal novel means by which ISCs in both species are able to restore normal population sizes after loss (66, 92, 96, 104).

The plethora of molecules derived from the microenvironment that regulates ISC behavior in *Drosophila* and mammals—several of which overlap—has been detailed in numerous reviews (9, 13, 46). Recently, several additional microenvironmental factors have come into focus as important regulators of stem cell behavior. For one, the impact of mechanical forces on epithelial cell dynamics was investigated in a recent study by He et al. (40), who showed that a fraction of *Dl*$^+$ cells with ee cell potential expresses Piezo, a cation channel that senses mechanical forces. Piezo controls cell proliferation and ee cell numbers through Ca^{2+} signaling under homeostatic conditions and in response to transient mechanical stimuli, such as that produced by the swelling of the intestine after overfeeding (40). Furthermore, research from the laboratory of Ip and colleagues (57) identified that the Misshapen kinase serves as a mechanical sensor that responds to mechanical stimuli, including intestinal distention, after yeast ingestion in vivo and substrate stiffness in vitro. In response to GI stretching, the cellular localization and phosphorylation of Misshapen change, relieving inhibition of ISC-dependent growth by the Yorkie pathway and ultimately allowing intestinal growth (57). Work with primary mouse organoids also supports a role for mechanical forces in the control of ISC behavior, showing that extracellular matrix stiffness regulates ISC proliferation and differentiation (36). Specifically, soft laminin-based matrices promote organoid formation/differentiation, whereas stiffer fibrogen-based matrices enhance ISC expansion via yes-associated protein 1 signaling (36). Information gained from further investigation into mechanical control of ISC behavior will be important for applications in biomedical engineering and regenerative medicine.

In addition to the mechanical impact of food ingestion on the intestine, several recent studies have revealed the impact of nutritional cues on ISC behavior (1, 46, 88). Long-term calorie restriction in mice is known to both shorten villi and reduce the number of differentiated ECs and to increase ISC numbers nonautonomously via inhibition of mammalian target of rapamycin complex 1 in Paneth cells (42, 106). ISC population expansion in response to long-term calorie restriction in mice is in apparent contrast to the reduced number of ISC divisions in *Drosophila* in response to decreased nutritional intake, although the change in flies is also sensed nonautonomously via insulin signaling from EBs (28). More recently, it was established in mice that short-term fasts also impact ISC behavior, in this case acting directly on ISCs to augment fatty acid oxidation via a peroxisome proliferator-activated receptor γ-mediated mechanism, which results in improved ISC function (69). Interestingly, ISC numbers and activity decline with age, but a short-term (24-h) fasting regime was shown to boost the clonogenic potential of ISCs in aged mice in vitro and in vivo, raising the possibility that fasting can mitigate age-associated declines in the regenerative potential of the intestine (69). Similar to fasting, high-fat diets activate a peroxisome proliferator-activated receptor γ program that enhances ISC number and function in mice (14). The surprisingly similar response of ISCs to essentially opposite diets may be due to heightened exposure of ISCs to free fatty acids, which are increased in the plasma in response to both fasting and high-fat diet (albeit from different sources). Dietary cholesterol has also recently been shown to increase ISC numbers in mice (101) and differentiation into ee cells in flies (73). Collectively, these findings speak to the complexity of the ISC response to specific types of lipids and nutrient levels. Research to understand this response better is of high priority, given that high-fat diets can increase the risk for several types of human intestinal cancers, including colon cancer, via mechanisms that are not fully understood (24).

Stem cell regulation by neighboring organs is another understudied source of microenvironmental signals recently shown to regulate ISC behavior in *Drosophila*. Specifically, midgut ISCs in direct proximity (<30 μm) to the midgut-hindgut boundary were found to be less proliferative and tumor-initiation prone than ISCs that are further removed from the organ boundary. Midgut ISCs near the boundary also mounted a more robust repair response to induced cell death in the midgut-hindgut boundary than more distant ISCs (86), suggesting that microenvironmental signals from neighboring organs may play a role in informing aspects of regional ISC heterogeneity discussed above.

CONCLUSIONS AND OUTLOOK

Research in *Drosophila* and mice in the past 5 years has revealed essential information about the regulation of homeostatic turnover and injury repair by ISCs that can be exploited

therapeutically for GI conditions specifically and for regenerative medicine more broadly. As work to identify specific markers of ISCs has progressed in each species, important sources of heterogeneity within the ISC population, including spatial and sex-specific differences, have been discovered in *Drosophila* that warrant further exploration in vertebrates. By building on prior understanding of ISC-driven repair of the intestinal epithelium, an increasingly complex picture of injury response that varies, in part, based on the type and site of injury, is emerging. In particular, genetic and epigenetic plasticity of numerous epithelial cell types has recently been uncovered as an immediate response to injury. Future studies to clarify molecular and cellular pathways by which this epithelial reversion contributes to intestinal repair are needed. Further exploration into other emerging and lesser known aspects of the ISC microenvironment, including those discussed above, as well as inflammatory signals and immune regulation (7, 13), mesenteric adipocytes (103, 111), and the enteric nervous system (76, 87), also holds promise for better understanding the cues that regulate ISC behavior.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.K.Z. prepared figures; R.K.Z. drafted manuscript; R.K.Z., B.O., and O.D.K. edited and revised manuscript; R.K.Z., B.O., and O.D.K. approved final version of manuscript.

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