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All for one and one for all: Regionalization of the *Drosophila* intestine

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ABSTRACT

Physiological responses are the ultimate outcomes of the functional interactions and proper organization of the different cell types that make up an organ. The digestive tract represents a good example where such structure/function correlation is manifested. To date, the molecular mechanisms that establish and/or maintain gut segmentation and functional specialization remain poorly understood. Recently, the use of model systems such as *Drosophila* has enriched our knowledge about the gut organization and physiology. Here, we review recent studies deciphering the morphological and functional properties of the *Drosophila* adult midgut compartments. Intestinal compartments are established through the differentiation of regionalized stem cell populations in concert with the joint activity of patterned transcription factors and locally produced morphogens. The maintenance of a compartmentalized gut structure is vital to the organism, allowing sequentially the ingestion and digestion of food, absorption of nutrients, and excretion of waste products in addition to the compartmentalization of immune and homeostatic functions. Further characterization of the gene regulatory networks underlying gut compartmentalization will pave the way for a better understanding of gastrointestinal function in insects and mammals, in both health and disease conditions.

1. Introduction

In metazoans, organs are usually divided into discrete structures enabling highly specialized functions. As a good example, the alimentary canal displays an extreme case of functional segmentation, in which successive compartments along the antero–posterior axis comprise various cell types that are characterized by distinct architectures and functions (Karasov et al., 2011; Stainier, 2005). This spatial variation allows for the sequential processing of food, from ingestion, through digestion and absorption to excretion (Karasov et al., 2011). Hence, the proper function of the gastrointestinal tract is not only bound to its overall integrity but also to its ability to maintain a proper functional and segmented organization.

Moreover, the digestive tract faces multiple stresses due to its role as a barrier with the external milieu and the permanent interaction with microbes, as well as simple mechanical food abrasion. In response to this challenging environment, the gut epithelium is renewed constantly in most animals; being one of the most highly mitotic organs (Radtke and Clevers, 2005). This feature is achieved via the activity of intestinal stem cells (ISCs) scattered along the epithelium forming the inner lining of the digestive tract. ISCs divide and differentiate to compensate the loss of cells due to daily environmental insults (Radtke and Clevers, 2005). Thus the gut is capable of maintaining a stereotypic organization while it is constantly renewing. However, to date, understanding the cellular and molecular mechanisms that are involved in the organization and maintenance of the adult digestive system remains a major challenge.

2. *Drosophila melanogaster*: an emerging model to study intestinal and stem cell biology

The alimentary canal of adult *D. melanogaster* is established during metamorphosis and fully matures within the first 48 h post-eclosion (Buchon et al., 2013b; Demerec, 1994; Takashima et al., 2011). It consists of a simple epithelial tube divided into foregut, midgut, and hindgut. The foregut is of ectodermal origin and includes the mouth, the pharynx, the esophagus, and the crop (Hakim et al., 2010). The crop is an impermeable bag-like structure that allows food mixing, detoxification, and storage (Stoffolano and Haselton, 2013). A specialized sphincter, the cardia, connects the

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foregut to the midgut and regulates food passage toward the midgut. The midgut, which is the only adult tissue originating from the endoderm, occupies a large part of the abdomen with an average length of 6 mm (Buchon et al., 2013b), and is the principal site of food digestion and nutrient absorption. Historically, scientists have divided the adult midgut into three segments: anterior, middle and posterior. This organization has been defined following the identification of an acidic region in the middle of the midgut that comprises specialized cells, the copper cells, which ensure a local low pH compartment (Dubreuil, 2004). Several studies have shown that genes encoding antimicrobial peptides and digestive enzymes are expressed in a patterned manner along the midgut (Abraham and Doane, 1978; Buchon et al., 2009b; Shanbhag and Tripathi, 2009; Terra and Ferreira, 1994; Wang et al., 2009). This suggests that despite its small size, the midgut of *Drosophila* is divided into physiologically specialized regions. The midgut precedes the hindgut, a tissue of ectodermal origin composed of the pylorus, the ileum and the rectum (Demerec, 1994). The pylorus parallels the cardia and functions like a valve that controls the transit of the luminal content by constricting surrounding muscles. Malpighian tubules, which are specialized excretory structures analogous to the mammalian kidney, branch at the midgut-hindgut junction and discharge waste product into the hindgut (Beyenbach et al., 2010). The ileum mediates the absorption of water and ions and participates in excretion of waste.

Similarly to its mammalian counterpart, the midgut epithelium of *Drosophila* is renewed by the activity of ISCs (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). ISCs divide mostly asymmetrically, generating both a new ISC to maintain the pool of stem cells in the gut and a progenitor called enteroblast (EB) (de Navascués et al., 2012; Goulas et al., 2012; Ohlstein and Spradling, 2007). Enteroblasts are transient and post-mitotic cells that gradually differentiate into either the absorptive and digestive enterocytes (ECs) or the secretory entero-endocrine cells (EEs) (Ohlstein and Spradling, 2007). Alternatively, it has been proposed that commitment to the endocrine lineage could be established in a distinct progenitor, the pre-EE rather than enteroblasts (Biteau and Jasper, 2014; Zeng and Hou, 2015). Over the last ten years, the *Drosophila* midgut has become a useful system to gain insights into the molecular mechanisms that control ISC behavior and gut physiology (Buchon et al., 2013a). JAK/STAT, EGFR, Wnt, Notch, Hippo, Hedgehog, and BMP pathways are among the key conserved signaling pathways regulating ISC proliferation and differentiation (Biteau and Jasper, 2011; Buchon et al., 2009a, 2010; Cordero et al., 2012; Guo et al., 2013; H. Jiang et al., 2011, 2009; Karpowicz et al., 2010; H. Li et al., 2013; Lin et al., 2008; Ohlstein and Spradling, 2007; Osman et al., 2012; Ren et al., 2010; Shaw et al., 2010; Singh et al., 2011; Staley and Irvine, 2010; J. Zhou et al., 2014). The *Drosophila* intestine is capable of rapid regeneration in response to acute chemical or biotic insults, such as infection (Amcheslavsky et al., 2009; Buchon et al., 2009b; H. Jiang et al., 2009). Upon damage, the gut epithelium initiates a homeostatic feedback loop that couples enterocyte loss to ISC proliferation. The visceral muscles originating from the mesoderm have a critical role during intestinal regeneration. Indeed, two layers of visceral muscles, comprising an external layer of longitudinal muscles, as well as an internal layer of circular muscles, surround the gut epithelium. The latter appears to be involved in the regulation of ISC activity by responding to signals from the epithelium, such as the JAK-STAT cytokine Upd3, by releasing key growth factors such as the EGF vein or wingless ligands to control ISCs behavior (Biteau and Jasper, 2011; Buchon et al., 2010; H. Jiang et al., 2011; Lin et al., 2008; Lin and Xi, 2008). The enteroblasts also control ISC activity through the expression of different JAK-STAT, EGFR and Wnt ligands (Buchon et al., 2010; Cordero et al., 2012; H. Jiang et al., 2011; Liu et al., 2010; F. Zhou et al., 2013). Despite this emerging picture of a non-autonomous control of ISC homeostasis, it remains unclear how different pathways are integrated within the ISC to control their maintenance and lineage differentiation. Apart from a few analyses, most conclusions related to ISC homeostasis have been formulated based on studies performed on the posterior adult midgut, without considering that the digestive tube presents structural and functional differences along its length.

3. The *Drosophila* midgut consists of five major compartments

In order to understand the underlying complexity of the *Drosophila* adult midgut, we and others used morphometric, histochemical and transcriptomic approaches to delineate areas of functional compartmentalization (Buchon et al., 2013b; Marianes et al., 2013). We first identified six major constrictions that compartmentalized the intestinal lumen into five main chambers through a morphometric analysis. Using a fluorescent brush border marker that stains the apical side of enterocytes and classical histological analyses, we further divided those five compartments into 8 distinct subregions (Fig. 1) and described their respective biochemical properties that appear highly specific. Furthermore, an extensive mapping of gene expression profile revealed the existence of 14 distinct subregions (Buchon et al., 2013b). Interestingly, the morphometric, histological, and the genetic data converged, suggesting the presence of sharp boundaries delineating the gut regions. Independently, Marianes and Spradling have used both light and electron microscopy to investigate structural differences along the whole midgut. Coupled to the characterization of the expression patterns of hundreds of reporter lines, they identified the presence of 10 discrete regions along the *Drosophila* midgut (Marianes et al., 2013). Strikingly, these 10 regions are a part of the 14 subregions, demonstrating a good consensus between both studies (Fig. 1) (O'Brien, 2013).

The two main sphincters of the gut, the cardia and the pylorus, are innervated by a subset of neurons located in the central nervous system (Cognigni et al., 2011). These neurons also innervate exclusively the most anterior (R1 and R2a) and most posterior regions (R5) of the midgut (Buchon et al., 2013b). This suggests that the local innervation of midgut extremities could regulate food transit in and out of the midgut. However, peristalsis appears so far to be the only mechanism regulating transit inside the midgut, which could be regulated by entero-endocrine cells enriched in the midgut domains devoid of neurons (Buchon et al., 2013b; Cognigni et al., 2011; LaJeunesse et al., 2009). EEs secrete numerous neuropeptides that are highly patterned along the gut length but their physiological functions and mode of action remain largely unknown (Beehler-Evans and Micchelli, 2015; Song et al., 2014; Veenstra, 2009). However, recent reports have provided evidence for a role of EEs in modulating ISC function (Amcheslavsky et al., 2014; Scopelliti et al., 2014), gut homeostasis and gut metabolism (Song et al., 2014). Altogether, these findings show that the *Drosophila* digestive tract is not a simple tube, but a highly compartmentalized organ with a diverse array of functions.

4. Midgut compartments display functional specialization for digestion and immune defense

To gain general insights into the functions of the five main midgut compartments, regional transcriptomes have been generated using microarrays (Buchon et al., 2013b) and RNAseq (Marianes et al., 2013). Both studies demonstrate that each midgut compartment performs unique physiological functions and acts as an integral metabolic, digestive and immune unit, corroborating

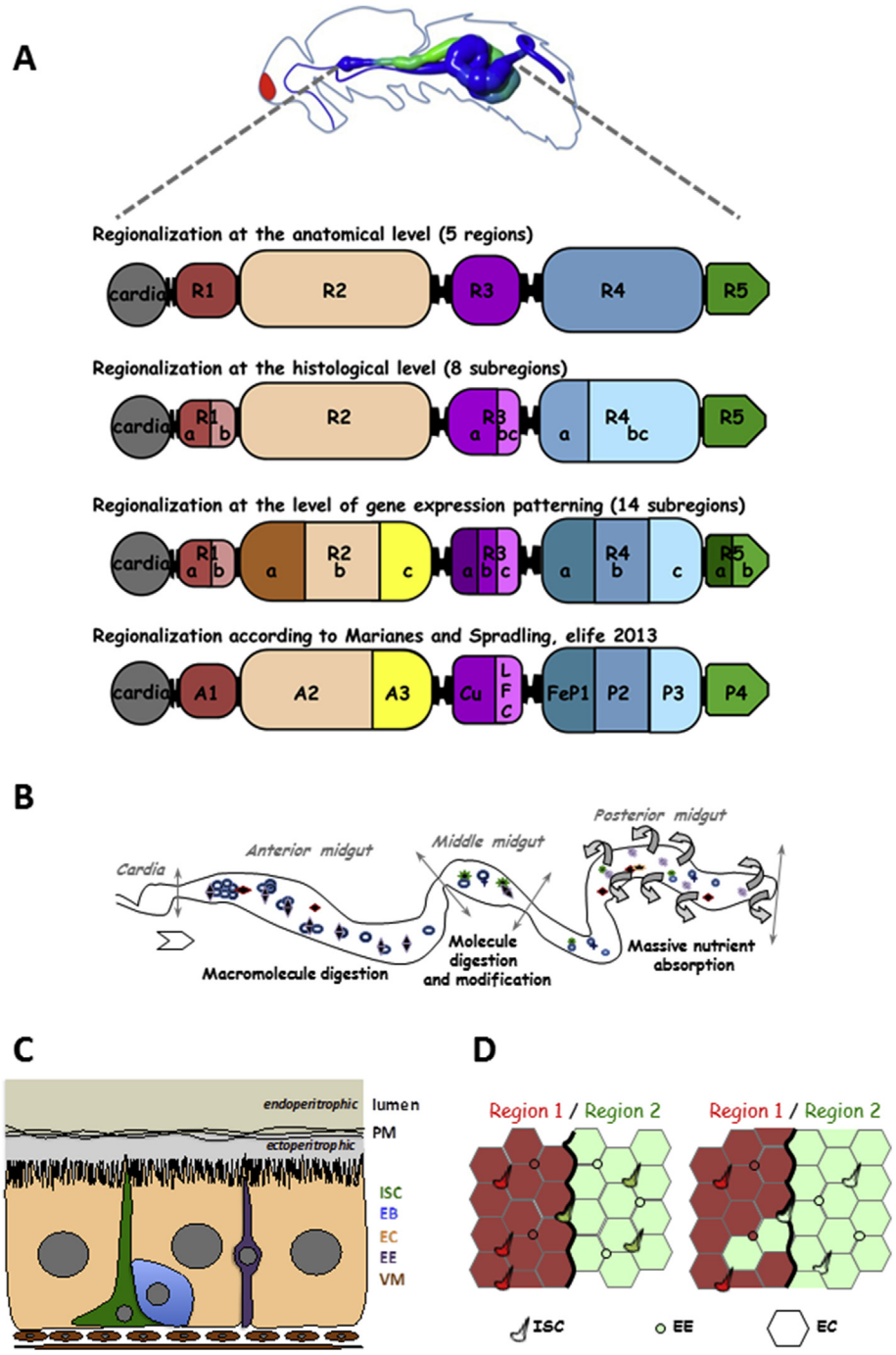

Fig. 1. A The *Drosophila* midgut consists of 5 major regions (R1-R5) which are further subdivided into 8 histological and 14 genetic subregions. B Schematic illustration of the sequential processing of food and nutrient absorption along the gut compartments. C The intestinal epithelium is constantly renewed through the activity of Intestinal Stem Cells (ISCs) which divide and generate new stem cells and transient committed enteroblasts (EBs). Enteroblasts differentiate into either absorptive/digestive epithelial (enterocytes, ECs) or secretory (entero-endocrine, EEs) cells. The gut epithelium is basally supported by visceral muscles (VM) and apically separated from the lumen by a chitinous structure, the peritrophic membrane (PM). D Intestinal compartments are established and maintained due in part to the activity of regional stem cell populations. The daughter cells of ISCs located at region boundaries do not mix except in rare cases, in which the daughter cells retain the fate of their region of origin.

previous observations showing that the expression of some digestive enzymes and transporters are restricted to discrete territories of the gut (Abraham and Doane, 1978; Terra and Ferreira, 1994; Wang et al., 2009). For instance, the anterior midgut compartments express enzymes involved in the digestion of macromolecules such as starch, lipids, and large proteins (Fig. 1). The middle midgut ensures an acidic environment that is required in the processing of some molecules into simpler sugars, amino acids and fatty acids. The low pH also helps in the reduction of metallic ions, which are subsequently absorbed at the proximal regions of the posterior midgut. Finally, the posterior midgut is dedicated to the massive absorption of small nutrients that are destined to rapid use or storage (Fig. 1). The posterior midgut, coherently with its absorptive function, expresses genes encoding lipid transporters at a high level. Accordingly, numerous lipid droplets are detected in the anterior and posterior regions. Although some functions are

confined to a region of the midgut, most are just enriched in some regions, while being lowly expressed in others.

In addition to digestion, nutrient transport, and absorption, other midgut functions show some regional specificity. The *Drosophila* gut immune response is mostly manifested through the generation of reactive oxygen species by the NADPH oxidase Duox, and the synthesis of antimicrobial peptides (AMPs) regulated by the Imd pathway (Buchon et al., 2013a). Interestingly, most AMPs are strongly expressed in the anterior midgut in comparison to the posterior compartments, suggesting that the anterior midgut acts as a first defense barrier (Buchon et al., 2013b). Moreover, some AMPs are selectively expressed in specific regions (*e.g. Def*, *DptB*, *Att-A*, *Dro3*), indicating that regional identity modulates the immune machinery in a precise manner (Buchon et al., 2013b). After immune challenge, the expression levels of *Dro3 and DptB* reporters are highly induced in both the foregut and anterior midgut (R1 and cardia predominantly) (Bosco-Drayon et al., 2012; Buchon et al., 2013b, 2009b; Osman et al., 2012), but also expand spatially (for *DptB*) to the posterior regions where no basal activity is normally detected (Buchon et al., 2009b). The Imd pathway is activated upon the detection of bacterial cell wall components, *i.e.* DAP-type peptidoglycan, by peptidoglycan recognition proteins (PGRPs). PGRP-LC encodes a membrane bound receptor, while PGRP-LE encodes an intracellular receptor. Both gene expression data (Buchon et al., 2013b) and mutant phenotypes (Bosco-Drayon et al., 2012; Neyen et al., 2012) indicated that bacterial recognition is mediated by PGRP-LC in the foregut, anterior midgut and the hindgut, while PGRP-LE is acting predominantly in the middle and posterior midgut. It is tempting to speculate that regional differences in the mechanisms underlying bacterial recognition allow maintaining a basal level of activation specific to individual gut regions according to local epithelium structure and permeability. As the midgut is more permeable than the foregut and the hindgut that are lined by a cuticular exoskeleton, the detection of bacteria by the intracellular PGRP-LE receptor could allow decreasing immune reactivity to peptidoglycans derived from commensals or ingested within the food. Finally, immune regulators are also patterned along the midgut. For instance, the transcription factor caudal is expressed only in the posterior midgut, where it downregulates the expression of AMPs (Ryu et al., 2008). This specific decrease in immune reactivity in the posterior midgut could also be a mechanism to tolerate the intestinal flora that accumulates densely in the distal midgut compartments along with the luminal content (Broderick et al., 2014). Altogether, these results suggest that the immune response is patterned and differentially modulated in terms of immune recognition, regulation and activity. Of note, many genes encoding detoxification enzymes (such as cytochrome P450s, genes involved in the response to oxidative stress, serpins, alcohol and acylglycerol catabolisms) are strongly enriched in the crop, suggesting that the Crop could act as a detoxification chamber neutralizing toxic aliments prior to their passage in the cardia and the midgut (Buchon et al., 2013b).

5. Establishment and stability of intestinal regionalization

D. melanogaster displays two different feeding styles during its life. As larvae, the insect feeds continuously on solid or semi liquefied food using its mouth hooks, and as an adult, it ingests liquid food via its proboscis. As the gut is completely reconstructed during metamorphosis, one could ask whether the compartmentalization of the adult midgut parallels that of the larval stage, or whether there is a complete reshaping of gut structure-function to ensure the adaptation to adult lifestyle (Takashima et al., 2011). On a large scale, the ultrastructure of the larval and adult midgut is similar. Like the adult, the larval midgut is composed of three major segments, the anterior, middle and posterior midgut. The middle midgut undoubtedly corresponds to the adult counterpart, hosting copper cells that generate a local acidic pH (Dubreuil, 2004). In addition, based on ultrastructural features, it has been proposed that larval anterior enterocytes are absorptive, the middle enterocytes comprise a mixed population of absorptive and secretory cells, and the posterior enterocytes are mostly absorptive; an organization reminiscent of the adult midgut (Shanbhag and Tripathi, 2009, 2005). However, a number of differences could be observed. First, the acidity level varied along the successive midgut domains of the larvae in comparison to the adult stage (Shanbhag and Tripathi, 2009). In larvae, the luminal content of the anterior segment and the anterior part of the posterior segment is between neutral to mild alkalinity (pH > 7 and <8), the middle midgut is highly acidic (pH < 3) and the distal part of the posterior midgut is highly alkaline (pH > 10) (Shanbhag and Tripathi, 2009). In the adult, the luminal contents of anterior and posterior midgut regions are mildly alkaline (pH 7–9), while the middle midgut segment is acidic (pH < 4.0) as in larvae (Shanbhag and Tripathi, 2009). Second, reporter transgenes showing a patterned expression in the adult also show regionalized expression in the larval midgut, albeit both expressions are not collinear (Buchon et al., 2013b). Finally, both larval and adult guts display specific anatomical structures serving the need of each specific life stage. The larval gut is equipped with the gastric caeca, which are composed of four outgrown compartments attached to the anterior midgut at the level of the cardia. Conserved in some other insects, these anatomical structures may serve to take nutrients out of the bulk flow enabling therefore longer and distinct digestion or as reservoirs for symbiotic microbes. In adults, the crop is a bi-lobed structure annexed only to the adult gut and may be of particular importance for food storage to be used in case of nutrient scarcity (Stoffolano and Haselton, 2013). To what extent these stage-specific structures could impact the function of the different midgut regions have not been yet addressed. Thus, an integrative analysis is required to define how many regions compose the larval midgut and what their respective functions are.

A careful observation of guts derived from newly emerged flies revealed that a full regional subdivision of the adult midgut is not acquired until a few hours after eclosion. This suggests that the basic molecular programs responsible for regional identity are turned on late during development (Buchon et al., 2013b). Additionally, the adult midgut is populated after the proliferation in late larval stages of adult midgut progenitors (AMPs), which are grouped in nests along the larval midgut and spread to colonize the transient pupal and future adult midgut (H. Jiang and Edgar, 2009; Mathur et al., 2010; Micchelli et al., 2011; Takashima et al., 2011). At the pupal stage, these adult midgut progenitors can still be exchanged between the hindgut and midgut, indicating that precursor's identity is not predetermined during larval or early pupal stages (Takashima et al., 2011, 2013). However, in the case of the adult middle midgut, the specification of the copper cell region occurs during a defined window of metamorphosis. At this stage, progenitor cells expressing the transcription factor escargot are receptive for the Bone Morphogenetic Protein (BMP) ligand secreted from the visceral muscles, which induces locally the regional fate of those cells (Driver and Ohlstein, 2014). Further induction of the BMP pathway in the adult midgut can generate an expansion of cells with some copper cell features (expression of the *Cut* marker), but is not capable of generating fully differentiated copper cells (Driver and Ohlstein, 2014; H. Li et al., 2013). This suggests that the BMP pathway alone in adults is insufficient to alter cell fate and transform any cell type (ISC, EB, or ECs) into mature copper cells. Moreover, BMP has a general role in controlling the regenerative response upon infection and damage (Guo

et al., 2013; Z. Li et al., 2013; J. Zhou et al., 2014), and could be involved in the maintenance of ISC fate throughout the midgut (Tian and J. Jiang, 2014). Thus, regional cues for the copper cell compartment (middle midgut or region 3, see Fig. 1) are provided at a specific time window, during metamorphosis (Driver and Ohlstein, 2014), and the BMP pathway is further used in the adult midgut to control epithelium homeostasis and ISC proliferation, but cannot define regional fate anymore (Guo et al., 2013; H. Li et al., 2013). These data strongly argue that regional properties are already embedded into middle midgut specific stem cells late during metamorphosis and maintained throughout adult life. It remains to be determined whether such a model is applicable outside of this peculiar region and to what extent visceral muscles regulate regional identity.

Importantly, once established, intestinal compartmentalization remains stable throughout life, regardless of nutritional changes or acute damage (Buchon et al., 2013b). However, during aging, intestinal regionalization is irreversibly altered, and associated with an abnormal proliferation and differentiation of stem cells (Biteau et al., 2008; Choi et al., 2008). This leads to the hypothesis that deterioration of intestinal regionalization may be a causal factor for the loss of intestinal homeostasis during aging (Buchon et al., 2013b).

6. Maintenance of intestinal regionalization involves a complex gene regulatory network

Sixty percent of the transcription factors encoded in the fly genome are expressed in the adult midgut. Interestingly, some of them are known to regulate normal embryonic gut development. For instance, GATAe, a key determinant of embryonic midgut endoderm (Okumura et al., 2005), is also required at the adult stage in enterocytes along the midgut's entire length for regional gene expression (Buchon et al., 2013b). The midgut-specific transcription factor, Labial, coordinates both copper cell morphology and function similarly to its role in the embryo and larvae (Buchon et al., 2013b; Dubreuil et al., 2001). Therefore, genetic programs shaping gut architecture and functions in the embryo are recycled throughout the adult life to maintain gut homeostasis. How these developmental factors cooperate with other transcriptional regulators such as the pan-gut transcription factor *bigmax* and the regionally acting *Ptx1* gene needs to be elucidated (Buchon et al., 2013b).

Morphogens are also known as major regulators of tissue patterning and organization. These diffusible factors are expressed in a concentration dependent manner to provide positional information to the neighboring cells leading to their specification in different cell types. Interestingly, the Wnt/Wg signaling is expressed in a gradient manner, with the highest levels of Wnt around the major boundaries separating the five compartments (Buchon et al., 2013b). This observation suggests that these constrictions could act as boundaries between compartments but also as organizing centers for these regions. In addition, many genes encoding digestive enzymes are expressed in gradients in the vicinity of intestinal boundaries and their expressions are correlated to the patterns of Wnt/Wg activity. Deciphering how Wnt/Wg regulates graded gene expression remains to be addressed. Of note, GATA3 and Wnt are also detected at the junction linking the stomach to the intestine in mammals, suggesting a functional conservation of the genetic determinants involved in the segmentation of the digestive system.

Alternatively, as is exemplified above, ISCs in the middle midgut can be regionally specified during metamorphosis and are able to keep their positional information throughout life (Driver and Ohlstein, 2014). Those two models are not antagonistic, and it is possible that a combination of epigenetic modifications inside ISCs act in concert with regional secreted factors to shape enterocyte differentiation and patterned gene expression. One major challenge facing future studies is to identify the relative contributions of adult signals and developmentally programmed cues in the maintenance and plasticity of those gut regions.

7. Intestinal stem cells display compartmentalized properties

To what extent do regional ISCs contribute to functional gut compartmentalization? Performing genetic clonal analyses in *Drosophila* showed for the first time, that most ISCs residing in the vicinity of region boundaries differentiate into daughter cells of the same regional fate (Marianes et al., 2013). Even in exceptional situations where daughter cells move to the neighboring compartment, they retained their regional identity (Fig. 1). Along the same line, tumors induced by the down-regulation of Notch pathway in progenitor cells did not cross region boundaries (Marianes et al., 2013). This indicates that gut compartmentalization is in part mediated by intrinsic ISC genetic programs. Future work should investigate potential regional epigenetic mechanisms responsible of this stem cell lineage "memory". Altogether, these results suggest that, in addition to ISC region-specific fate, cell adhesion cues are likely involved in maintaining intact region boundaries, preventing the mixing of localized cell types.

Differences in ISC behavior were also revealed across various ISC populations along the digestive system. In the cardia, type I Gastric Stem Cells (type I GaSCs), which are a subpopulation of ISCs, reside in the epithelium and control the formation and the renewal of the whole cardia, the esophagus, the crop, and probably a part of the region 1 of the midgut (Singh et al., 2011). Contrary to other midgut stem cells, GaSCs are grouped in a cluster throughout life within the cardia at the foregut-midgut junction and do not spread throughout the epithelium within the different compartments. Similar ISC behavior is observed at the midgut-hindgut junction (Fox and Spradling, 2009; Takashima et al., 2013, 2008). Differences in the ISC division rate are also observed. ISCs of the anterior (R2) and posterior (R4) midgut are more proliferative than other ISC populations, with ISCs in the posterior R4 compartment dividing at least once a day (Marianes et al., 2013). In strong contrast, ISCs of the middle midgut (Gastric Stem Cells or GSSCs) proliferate at a very low rate, dividing once every 4–5 days. It remains controversial whether those quiescent stem cells express classical markers of ISCs, such as the Notch ligand Delta (Marianes et al., 2013; Strand and Micchelli, 2011). It is possible that the detected level of Delta depends on basal renewal rates, and thus rearing conditions. Delta would therefore represent a marker of active stem cells, rather than a stemness marker. Finally, different ISC subpopulations show distinct levels of susceptibility to oncogenic transformation. In the anterior midgut R1, ISCs are more susceptible to transformation by a double mutation in the oncogenes Ras and Apc, while ISCs of R4 are more susceptible to develop tumors in response to Notch down regulation (Marianes et al., 2013; Martorell et al., 2014). Altogether these studies demonstrate that ISC activity varies strongly between regions. Future studies should determine how much of those regional properties are secondary consequences of different regional physiologies, and how much are cell autonomous properties of regional ISCs.

8. Future directions

Recent analyses of the *Drosophila* midgut using morphological, histological and genetic techniques have greatly advanced our understanding of the regulatory networks underlying adult intestinal compartmentalization. More efforts are needed to uncover the

mechanisms involved in the early establishment of gut regionalization, as well as the regional characteristics of ISCs and their daughter cells. These studies will also help identify some key determinants of tumor growth in the gut, whether they are region specific or aging related. Furthermore, how gut regionalization is maintained upon epithelium renewal induced by acute gut damage is still an open field to explore. Another line of research could be to analyze how the overall gut structure and its underlying molecular mechanisms are conserved in other *Drosophila* species and even other insect groups. Since adaptation to new sources of food participates in the speciation processes, it would be interesting to compare the gut organization in other *Drosophila* species that feed on different substrates or even other dipterans that are carnivore or detritivores. The use of *D. melanogaster* as a model system will improve our understanding of insect gut physiology, and pave the way for future control strategy of agricultural pests and disease vectors. Furthermore, the high similarity in intestinal structure and function between *Drosophila* and mammals confirms the great utility of this invertebrate genetic model to address fundamental biological questions related to humans in both health and disease.

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References

Abraham, I., Doane, W.W., 1978. Genetic regulation of tissue-specific expression of amylase structural genes in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. 75, 4446–4450.

Amcheslavsky, A., Jiang, J., Ip, Y.T., 2009. Tissue damage-induced intestinal stem cell division in Drosophila. Cell Stem Cell 4, 49–61. http://dx.doi.org/10.1016/j.stem.2008.10.016.

Amcheslavsky, A., Song, W., Li, Q., Nie, Y., Bragatto, I., Ferrandon, D., Perrimon, N., Ip, Y.T., 2014. Enteroendocrine cells support intestinal stem-cell-mediated homeostasis in Drosophila. Cell Rep. 9, 32–39. http://dx.doi.org/10.1016/j.celrep.2014.08.052.

Beehler-Evans, R., Micchelli, C.A., 2015. Generation of enteroendocrine cell diversity in midgut stem cell lineages. Development 142, 654–664. http://dx.doi.org/10.1242/dev.114959.

Beyenbach, K.W., Skaer, H., Dow, J.A.T., 2010. The developmental, molecular, and transport biology of Malpighian tubules. Annu. Rev. Entomol. 55, 351–374. http://dx.doi.org/10.1146/annurev-ento-112408-085512.

Biteau, B., Hochmuth, C.E., Jasper, H., 2008. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging Drosophila gut. Cell Stem Cell 3, 442–455. http://dx.doi.org/10.1016/j.stem.2008.07.024.

Biteau, B., Jasper, H., 2011. EGF signaling regulates the proliferation of intestinal stem cells in Drosophila. Development 138, 1045–1055. http://dx.doi.org/10.1242/dev.056671.

Biteau, B., Jasper, H., 2014. Slit/Robo signaling regulates cell fate decisions in the intestinal stem cell lineage of Drosophila. Cell Rep. 7, 1867–1875. http://dx.doi.org/10.1016/j.celrep.2014.05.024.

Bosco-Drayon, V., Poidevin, M., Boneca, I.G., 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, 153–165. http://dx.doi.org/10.1016/j.chom.2012.06.002.

Broderick, N.A., Buchon, N., Lemaitre, B., 2014. Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. MBio 5, e01117–14. http://dx.doi.org/10.1128/mBio.01117-14.

Buchon, N., Broderick, N.A., Chakrabarti, S., Lemaitre, B., 2009a. Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in Drosophila. Genes. Dev. 23, 2333–2344. http://dx.doi.org/10.1101/gad.1827009.

Buchon, N., Broderick, N.A., Kuraishi, T., Lemaitre, B., 2010. Drosophila EGFR pathway coordinates stem cell proliferation and gut remodeling following infection. BMC Biol. 8, 152. http://dx.doi.org/10.1186/1741-7007-8-152.

Buchon, N., Broderick, N.A., Lemaitre, B., 2013a. Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. Nat. Rev. Micro 11, 615–626. http://dx.doi.org/10.1038/nrmicro3074.

Buchon, N., Broderick, N.A., Poidevin, M., Pradervand, S., Lemaitre, B., 2009b. Drosophila intestinal response to bacterial infection: activation of host defense and stem cell proliferation. Cell Host Microbe 5, 200–211. http://dx.doi.org/10.1016/j.chom.2009.01.003.

Buchon, N., Osman, D., David, F.P.A., Yu Fang, H., Boquete, J.-P., Deplancke, B., Lemaitre, B., 2013b. Morphological and molecular characterization of adult midgut compartmentalization in Drosophila. Cell Rep. 3, 1725–1738. http://dx.doi.org/10.1016/j.celrep.2013.04.001.

Choi, N.-H., Kim, J.-G., Yang, D.-J., Kim, Y.-S., Yoo, M.-A., 2008. Age-related changes in Drosophila midgut are associated with PVF2, a PDGF/VEGF-like growth factor. Aging Cell 7, 318–334. http://dx.doi.org/10.1111/j.1474-9726.2008.00380.x.

Cognigni, P., Bailey, A.P., Miguel-Aliaga, I., 2011. Enteric neurons and systemic signals couple nutritional and reproductive status with intestinal homeostasis. Cell Metab. 13, 92–104. http://dx.doi.org/10.1016/j.cmet.2010.12.010.

Cordero, J.B., Stefanatos, R.K., Scopelliti, A., Vidal, M., Sansom, O.J., 2012. Inducible progenitor-derived wingless regulates adult midgut regeneration in Drosophila. EMBO J. http://dx.doi.org/10.1038/emboj.2012.248.

de Navascués, J., Perdigoto, C.N., Bian, Y., Schneider, M.H., Bardin, A.J., Martinez Arias, A., Simons, B.D., 2012. Drosophila midgut homeostasis involves neutral competition between symmetrically dividing intestinal stem cells, 31, 2473–2485. http://dx.doi.org/10.1038/emboj.2012.106.

Demerec, M., 1994. Biology of Drosophila. Cold Spring Harbor Laboratory Pr.

Driver, I., Ohlstein, B., 2014. Specification of regional intestinal stem cell identity during Drosophila metamorphosis. Development 141, 1848–1856. http://dx.doi.org/10.1242/dev.104018.

Dubreuil, R.R., 2004. Copper cells and stomach acid secretion in the Drosophila midgut. Int. J. Biochem. Cell Biol. 36, 745–752.

Dubreuil, R.R., Grushko, T., Baumann, O., 2001. Differential effects of a labial mutation on the development, structure, and function of stomach acid-secreting cells in *Drosophila melanogaster* larvae and adults. Cell Tissue Res. 306, 167–178. http://dx.doi.org/10.1007/s004410100422.

Fox, D.T., Spradling, A.C., 2009. The Drosophila hindgut lacks constitutively active adult stem cells but proliferates in response to tissue damage. Cell Stem Cell 5, 290–297. http://dx.doi.org/10.1016/j.stem.2009.06.003.

Goulas, S., Conder, R., Knoblich, J.A., 2012. The par complex and integrins direct asymmetric cell division in adult intestinal stem cells. Cell Stem Cell 11, 529–540. http://dx.doi.org/10.1016/j.stem.2012.06.017.

Guo, Z., Driver, I., Ohlstein, B., 2013. Injury-induced BMP signaling negatively regulates Drosophila midgut homeostasis. J. Cell Biol. 201, 945–961. http://dx.doi.org/10.1083/jcb.201302049.

Hakim, R.S., Baldwin, K., Smagghe, G., 2010. Regulation of midgut growth, development, and metamorphosis. Annu. Rev. Entomol. 55, 593–608. http://dx.doi.org/10.1146/annurev-ento-112408-085450.

Jiang, H., Edgar, B.A., 2009. EGFR signaling regulates the proliferation of Drosophila adult midgut progenitors. Development 136, 483–493. http://dx.doi.org/10.1242/dev.026955.

Jiang, H., Grenley, M.O., Bravo, M.-J., Blumhagen, R.Z., Edgar, B.A., 2011. EGFR/Ras/MAPK signaling mediates adult midgut epithelial homeostasis and regeneration in Drosophila. Cell Stem Cell 8, 84–95. http://dx.doi.org/10.1016/j.stem.2010.11.026.

Jiang, H., Patel, P.H., Kohlmaier, A., Grenley, M.O., Mcewen, D.G., Edgar, B.A., 2009. Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the Drosophila midgut. Cell 137, 1343–1355. http://dx.doi.org/10.1016/j.cell.2009.05.014.

Karasov, W.H., Martínez del Rio, C., Caviedes-Vidal, E., 2011. Ecological physiology of diet and digestive systems. Annu. Rev. Physiol. 73, 69–93. http://dx.doi.org/10.1146/annurev-physiol-012110-142152.

Karpowicz, P., Perez, J., Perrimon, N., 2010. The Hippo tumor suppressor pathway regulates intestinal stem cell regeneration. Development 137, 4135–4145. http://dx.doi.org/10.1242/dev.060483.

LaJeunesse, D., Johnson, B., Presnell, J., Catignas, K., Zapotoczny, G., 2009. Regulated Peristalsis into the Acidic Region of the Drosophila Larval Midgut is Controlled by a Novel Component of the Autonomic Nervous System.

Li, H., Qi, Y., Jasper, H., 2013a. Dpp signaling determines regional stem cell identity in the regenerating adult Drosophila gastrointestinal tract. Cell Rep. 4, 10–18. http://dx.doi.org/10.1016/j.celrep.2013.05.040.

Li, Z., Zhang, Y., Han, L., Shi, L., Lin, X., 2013b. Trachea-derived dpp controls adult midgut homeostasis in Drosophila. Dev. Cell 24, 133–143. http://dx.doi.org/10.1016/j.devcel.2012.12.010.

Lin, G., Xi, R., 2008. Intestinal stem cell, muscular niche and wingless signaling. Fly 2, 310–312.

Lin, G., Xu, N., Xi, R., 2008. Paracrine wingless signalling controls self-renewal of Drosophila intestinal stem cells. Nature 455, 1119–1123. http://dx.doi.org/10.1038/nature07329.

Liu, W., Singh, S.R., Hou, S.X., 2010. JAK-STAT is restrained by Notch to control cell proliferation of the Drosophila intestinal stem cells. J. Cell Biochem. 109, 992–999. http://dx.doi.org/10.1002/jcb.22482.

Marianes, A., Spradling, A.C., Brand, A., 2013. Physiological and stem cell compartmentalization within the Drosophila midgut. elife 2. http://dx.doi.org/10.7554/eLife.00886.

Martorell, O., Merlos-Suárez, A., Campbell, K., Barriga, F.M., Christov, C.P., Miguel-Aliaga, I., Batlle, E., Casanova, J., Casali, A., 2014. Conserved mechanisms of tumorigenesis in the Drosophila adult midgut. PLoS ONE 9, e88413. http://dx.doi.org/10.1371/journal.pone.0088413.

Mathur, D., Bost, A., Driver, I., Ohlstein, B., 2010. A transient niche regulates the specification of Drosophila intestinal stem cells. Science 327, 210–213. http://dx.doi.org/10.1126/science.1181958.

Micchelli, C.A., Perrimon, N., 2006. Evidence that stem cells reside in the adult Drosophila midgut epithelium. Nature 439, 475–479. http://dx.doi.org/10.1038/nature04371.

Micchelli, C.A., Sudmeier, L., Perrimon, N., Tang, S., Beehler-Evans, R., 2011. Identification of adult midgut precursors in Drosophila. Gene Expr. Patterns 11, 12–21. http://dx.doi.org/10.1016/j.gep.2010.08.005.

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. http://dx.doi.org/10.4049/jimmunol.1201022.

O'Brien, L.E., 2013. Regional specificity in the Drosophila midgut: setting boundaries with stem cells. Cell Stem Cell 13, 375–376. http://dx.doi.org/10.1016/j.stem.2013.09.008.

Ohlstein, B., Spradling, A.C., 2006. The adult Drosophila posterior midgut is maintained by pluripotent stem cells. Nature 439, 470–474. http://dx.doi.org/10.1038/nature04333.

Ohlstein, B., Spradling, A.C., 2007. Multipotent Drosophila intestinal stem cells specify daughter cell fates by differential notch signaling. Science 315, 988–992. http://dx.doi.org/10.1126/science.1136606.

Okumura, T., Matsumoto, A., Tanimura, T., Murakami, R., 2005. An endoderm-specific GATA factor gene, dGATAe, is required for the terminal differentiation of the Drosophila endoderm. Dev. Biol. 278, 576–586. http://dx.doi.org/10.1016/j.ydbio.2004.11.021.

Osman, D., Buchon, N., Chakrabarti, S., Huang, Y.-T., Su, W.-C., Poidevin, M., Tsai, Y.-C., Lemaitre, B., 2012. Autocrine and paracrine unpaired signaling regulate intestinal stem cell maintenance and division. J. Cell Sci. 125, 5944–5949. http://dx.doi.org/10.1242/jcs.113100.

Radtke, F., Clevers, H., 2005. Self-renewal and cancer of the gut: two sides of a coin. Science 307, 1904–1909. http://dx.doi.org/10.1126/science.1104815.

Ren, F., Wang, B., Yue, T., Yun, E.-Y., Ip, Y.T., Jiang, J., 2010. Hippo signaling regulates Drosophila intestine stem cell proliferation through multiple pathways. Proc. Natl. Acad. Sci. 107, 21064–21069. http://dx.doi.org/10.1073/pnas.1012759107.

Ryu, J.-H., Kim, S.-H., Lee, H.-Y., Bai, J.Y., Nam, Y.-D., Bae, J.-W., Lee, D.G., Shin, S.C., Ha, E.-M., Lee, W.-J., 2008. Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. Science 319, 777–782. http://dx.doi.org/10.1126/science.1149357.

Scopelliti, A., Cordero, J.B., Diao, F., Strathdee, K., White, B.H., Sansom, O.J., Vidal, M., 2014. Local control of intestinal stem cell homeostasis by enteroendocrine cells in the adult Drosophila midgut. - PubMed - NCBI. Curr. Biol. 24, 1199–1211.

Shanbhag, S., Tripathi, S., 2005. Electrogenic H+ transport and pH gradients generated by a V-H+ -ATPase in the isolated perfused larval Drosophila midgut. J. Membr. Biol. 206, 61–72. http://dx.doi.org/10.1007/s00232-005-0774-1.

Shanbhag, S., Tripathi, S., 2009. Epithelial ultrastructure and cellular mechanisms of acid and base transport in the Drosophila midgut. J. Exp. Biol. 212, 1731–1744. http://dx.doi.org/10.1242/jeb.029306.

Shaw, R.L., Kohlmaier, A., Polesello, C., Veelken, C., Edgar, B.A., Tapon, N., 2010. The Hippo pathway regulates intestinal stem cell proliferation during Drosophila adult midgut regeneration. Development 137, 4147–4158. http://dx.doi.org/10.1242/dev.052506.

Singh, S.R., Zeng, X., Zheng, Z., Hou, S.X., 2011. The adult Drosophila gastric and stomach organs are maintained by a multipotent stem cell pool at the foregut/midgut junction in the cardia (proventriculus). Cell Cycle 10, 1109–1120.

Song, W., Veenstra, J.A., Perrimon, N., 2014. Control of lipid metabolism by tachykinin in Drosophila. Cell Rep. 9, 40–47.

Stainier, D.Y.R., 2005. No organ left behind: tales of gut development and evolution. Science 307, 1902–1904. http://dx.doi.org/10.1126/science.1108709.

Staley, B.K., Irvine, K.D., 2010. Warts and Yorkie mediate intestinal regeneration by influencing stem cell proliferation. Curr. Biol. 20, 1580–1587. http://dx.doi.org/10.1016/j.cub.2010.07.041.

Stoffolano, J.G., Haselton, A.T., 2013. The adult Dipteran crop: a unique and overlooked organ. Annu. Rev. Entomol. 58, 205–225. http://dx.doi.org/10.1146/annurev-ento-120811-153653.

Strand, M., Micchelli, C.A., 2011. Quiescent gastric stem cells maintain the adult Drosophila stomach. Proc. Natl. Acad. Sci. 108, 17696–17701. http://dx.doi.org/10.1073/pnas.1109794108.

Takashima, S., Mkrtchyan, M., Younossi-Hartenstein, A., Merriam, J.R., Hartenstein, V., 2008. The behaviour of Drosophila adult hindgut stem cells is controlled by Wnt and Hh signalling. Nature 454, 651–655. http://dx.doi.org/10.1038/nature07156.

Takashima, S., Paul, M., Aghajanian, P., Younossi-Hartenstein, A., Hartenstein, V., 2013. Migration of Drosophila intestinal stem cells across organ boundaries. Development 140, 1903–1911. http://dx.doi.org/10.1242/dev.082933.

Takashima, S., Younossi-Hartenstein, A., Ortiz, P.A., Hartenstein, V., 2011. A novel tissue in an established model system: the Drosophila pupal midgut. Dev. Genes Evol. 221, 69–81. http://dx.doi.org/10.1007/s00427-011-0360-x.

Terra, W.R., Ferreira, C., 1994. Insect digestive enzymes: properties, compartmentalization and function. Comp. Biochem. Physiol. 109, 1–62.

Tian, A., Jiang, J., 2014. Intestinal epithelium-derived BMP controls stem cell self-renewal in Drosophila adult midgut, 3, e01857. http://dx.doi.org/10.7554/eLife.01857.

Veenstra, J.A., 2009. Peptidergic paracrine and endocrine cells in the midgut of the fruit fly maggot. Cell Tissue Res. 336, 309–323. http://dx.doi.org/10.1007/s00441-009-0769-y.

Wang, X., Wu, Y., Zhou, B., 2009. Dietary zinc absorption is mediated by ZnT1 in *Drosophila melanogaster*. FASEB J. 23, 2650–2661. http://dx.doi.org/10.1096/fj.08-126649.

Zeng, X., Hou, S.X., 2015. Enteroendocrine cells are generated from stem cells through a distinct progenitor in the adult Drosophila posterior midgut. Development 142, 644–653. http://dx.doi.org/10.1242/dev.113357.

Zhou, F., Rasmussen, A., Lee, S., Agaisse, H., 2013. The UPD3 cytokine couples environmental challenge and intestinal stem cell division through modulation of JAK/STAT signaling in the stem cell microenvironment. Dev. Biol. 373, 383–393. http://dx.doi.org/10.1016/j.ydbio.2012.10.023.

Zhou, J., Florescu, S., Boettcher, A.-L., Luo, L., Dutta, D., Kerr, G., Cai, Y., Edgar, B.A., Boutros, M., 2014. Dpp/Gbb signaling is required for normal intestinal regeneration during infection. Dev. Biol. http://dx.doi.org/10.1016/j.ydbio.2014.12.017.