Regional Specificity in the *Drosophila* Midgut: Setting Boundaries with Stem Cells

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Many organs consist of distinct subregions with specialized physiological roles, but how regional boundaries are upheld during cellular renewal is largely unknown. Recently, Buchon et al. (2013) and Marianes and Spradling (2013) showed that subregions of the *Drosophila* midgut are maintained by patterned transcription factors and compartmentalized stem cell progeny.

The complex physiology of solid organs necessitates that different parts of an organ specialize in different functional roles. A canonical example is food digestion in the gastrointestinal (GI) tract, where sequential compartments along the length of the gut tube perform successive steps of nutrient breakdown and absorption. Organ subregions have characteristic cell types and tissue structures that reflect their distinct roles. To work efficiently, an organ must both maintain the integrity of its subregions and amalgamate their functional outputs.

At the same time, most organs undergo continuous cellular turnover. The fact that compartments are maintained over a lifetime—despite constant replacement of their constituent cells—implies that active mechanisms enforce compartment boundaries. Although little understood, these regional identity mechanisms appear exceedingly robust; for instance, subregions are reestablished following massive injury to organs such as lung, small intestine, and midgut in *Drosophila* (O'Brien and Bilder, 2013). Each subregion typically has its own cohort of stem cells, raising the intriguing—but relatively unexamined—possibility that stem cells help uphold regional identities. Now, two recent studies (Buchon et al., 2013; Marianes and Spradling, 2013) use the *Drosophila* midgut to tackle this fundamental issue.

The *Drosophila* midgut emerged as a new genetic model for self-renewal only recently with the demonstration that stem cells replenish the midgut's epithelial lining in adult animals (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006). Physiologically equivalent to the mammalian stomach and small bowel, the fly midgut was rapidly found to share core features with mammalian intestine in terms of stem cell function, lineage, and molecular control (Biteau et al., 2011). Most studies of midgut stem cells have focused on the organ's dynamic posterior half. However, classical anatomists have long recognized that the entire length of the midgut tube contains histologically distinct zones (Lemaitre and Miguel-Aliaga, 2013). Restricted expression of digestive enzymes and abrupt transitions in luminal pH suggested that these midgut zones are functional units, performing successive steps of digestion as nutrients transit through the gut tube. Such a division of labor would be akin to the well-understood functional segmentation that characterizes vertebrate digestive tracts. In both mouse and fly, stem cells in different GI regions can show characteristic variations in cycling rates and expression of the established markers Lgr5 (mouse) and Delta (fly) (Barker et al., 2010; Strand and Micchelli, 2011), correlating stem cell variation with regional physiology. However, fundamental questions remain. What mechanisms maintain compartment boundaries during organ renewal and repair? And do stem cell differences direct—or merely reflect—compartment differences?

Now, Buchon et al. and Marianes and Spradling open the door to whole-organ understanding of the interrelationship between stem cells and organ compartmentalization. Through complementary genetic and morphometric approaches, the two groups independently arrived at similar nose-to-tail atlases of the midgut's major regions (Figure 1). Subsequent transcriptome analyses uncovered striking diversity in gene expression from region to region. Buchon et al., using microarray, identified a total of ~1,500 genes that show compartment-specific expression; Marianes and Spradling, using RNAseq, found that each compartment expresses a suite of 50–150 genes at least ten times higher than all other compartments. Each group also probed the mechanisms that specify and reinforce regional diversity, focusing on either genetic regulatory networks (Buchon et al., 2013) or compartment-specific stem cell differences (Marianes and Spradling, 2013).

Transcriptional profiles from both groups revealed a colinear organization of digestion and immunity along the midgut tube. Anterior compartments break down complex starches, fats, and proteins; posterior compartments finish degradation and transport nutrients. Families of digestive genes, such as trypsins, mannosidases, and lipases, form genomic clusters in which each gene has a distinct pattern of compartmentalized expression. Gut immunity is also regionalized. The midgut's anterior entry is a preeminent zone of microbial defense, with strongly enriched expression of Imd and JAK-STAT peptides. Individual regions throughout the gut express characteristic subsets of bacteria-sensing peptidoglycan recognition proteins (PGRPs). Together, the fine-grained analyses from Buchon and Marianes illuminate the richness of functional specialization within even a relatively simple organ.

The studies next proceeded to investigate when and how compartment identities arise. Buchon et al. found that region-specific gene profiles appear within the first few days of adult life

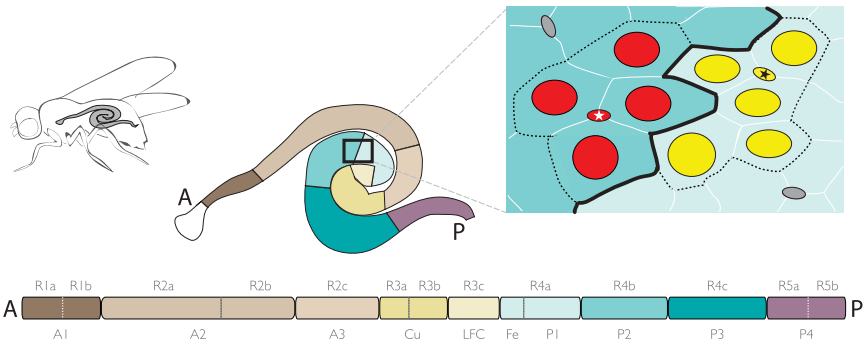

Figure 1. Distinct Stem Cell Populations, Transcriptomes, Histological Structures, and Physiological Functions Define Compartments of the Adult *Drosophila* Midgut

Midgut compartments defined by Buchon and by Marianes are schematized as colored segments in anatomic and linear views. Labels for region names indicate the consensus alignment from Buchon (top) and Marianes (bottom) (N. Buchon, D. Osman, B. Lemaitre, A.C. Spradling, and A. Marianes, personal communication). A, anterior; P, posterior. Right inset: lineage restriction of stem cell clones. Daughter cells occupy the same compartment as their mother stem cell, even at compartment boundaries. In the cartoon, stem cells are depicted as small ovals. Large red nuclei mark daughters of the red stem cell (white star), and large yellow nuclei mark daughters of the yellow stem cell (black star); each of the two stem cell clones is outlined with a dotted black line. The compartment boundary (thick black line) coincides with the boundary between red and yellow clones.

and are robust to dietary change and pathogenic infection before deteriorating with age. Although midgut regionalization evokes segmental patterning of the *Drosophila* embryo, the underlying regulatory networks appear largely distinct (Marianes and Spradling, 2013). Nonetheless, certain embryonic regulators do return to help pattern the adult midgut (Buchon et al., 2013). Notably, the GATAe transcription factor, a master regulator of midgut development, controls compartment-specific gene expression throughout the adult midgut. Localized transcription factors such as Labial and Ptx-1 work with GATAe and other pan-midgut regulators to further define individual regions. Wnt activity exhibits gradients around multiple compartment boundaries and may refine boundary placement. Interestingly, Gata4 and Wnts have been shown to demarcate regional boundaries in the mammalian bowel (San Roman and Shivdasani, 2011).

How are compartment identities maintained at the cellular level? Marianes and Spradling uncover an important clue: regional autonomy of stem cells. Daughter cells strictly occupy the same compartment as their mother stem cell, at least for five of six boundaries tested (Figure 1). Between regions, lineage restriction creates serpentine boundaries that match the borders of individual stem cell clones. Even stem-cell-derived tumors do not cross boundaries, possibly indicating that partitioning involves localized mechanisms such as differential cell adhesion or mechanical force. Intriguingly, several compartment boundaries coincide with sphincter-like constrictions in the gut tube. Significantly, midgut lineage restriction implies that differentiated daughters somehow "remember" their mother stem cell; this cellular memory may help keep regions distinct during renewal.

Stereotypic differences in regional stem cell populations, reported by Marianes, may perhaps contribute to regional autonomy. Between compartments, stem cell division rate and abundance vary up to 5-fold. Furthermore, stem cells in particular subregions are predisposed to form tumors, whereas in other—even adjacent—subregions, they are resistant. Whether regional tumorigenicity is determined by differential signaling factors, metabolic activity, or microbial interactions will be a topic of great interest. Of note, these regional biases in midgut tumorgenicity are reminiscent of spatial biases exhibited by certain human GI cancers (San Roman and Shivdasani, 2011). All together, these findings in the *Drosophila* midgut draw the exciting suggestion that in humans, future clinical interventions might exploit regional genetic biases to elicit specialized stem cell responses for targeted therapies.

Looking forward, a crucial test will be whether regional identity can be reassigned by forced expression of compartment-specific transcription factors or localized reprogramming of stem cells. Given the chicken-and-egg relationship between stem cells and their progeny, is one or the other dominant for compartment specification? Other compelling avenues of investigation include the mechanisms that underlie regional autonomy of stem cells and the origins of differential tumor susceptibility. Buchon et al. and Marianes and Spradling provide a strong platform for probing these fundamental issues.

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