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Beyond the Niche: Tissue-Level Coordination of Stem Cell Dynamics

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Abstract

Adult animals rely on populations of stem cells to ensure organ function throughout their lifetime. Stem cells are governed by signals from stem cell niches, and much is known about how single niches promote stemness and direct stem cell behavior. However, most organs contain a multitude of stem cell–niche units, which are often distributed across the entire expanse of the tissue. Beyond the biology of individual stem cell–niche interactions, the next challenge is to uncover the tissue-level processes that orchestrate spatial control of stem-based renewal, repair, and remodeling throughout a whole organ. Here we examine what is known about higher order mechanisms for interniche coordination in epithelial organs, whose simple geometry offers a promising entry point for understanding the regulation of niche number, distribution, and activity. We also consider the potential existence of stem cell territories and how tissue architecture may influence niche coordination.

Contents

THE CHALLENGE OF STEM CELL COORDINATION ACROSS A TISSUE

Adult stem cells are the linchpins of tissue renewal, repair, and remodeling in mature organs. By calibrating their divisions to meet the changing needs of their resident tissues, stem cells enable lifelong optimization of organ form and function. The ability of stem cells to sense and respond to tissue needs derives, in large part, from their intimate association with niches. In its essence, a niche is a microenvironment that maintains stemness and directs stem cell behavior (Schofield 1978). The niche microenvironment often takes the form of specialized cells that directly contact stem cells and secrete stem cell regulatory factors (Losick et al. 2011, Morrison & Spradling 2008, Nystul & Spradling 2006). Over the past three decades, our understanding of the stem cell–niche relationship has expanded dramatically—from the elemental simplicity of the distal tip cell, which provides a unicellular niche for the *Caenorhabditis elegans* germline, to the intricate choreography of the mammalian hair follicle, where multiple types of stem cells and niches cooperate to control the cyclic production of new hairs.

The stem cell–niche unit will remain a rich area of investigation for many years to come. Nonetheless, most self-renewing organs are supported by a multitude of stem cell–niche units, distributed throughout the expanse of the tissue. Moreover, growing evidence points to surprising heterogeneity in molecular profiles, division patterns, and population sizes of stem cells and niches within a given tissue (Greco & Guo 2010, Li & Clevers 2010, Mascré et al. 2012, Ousset et al. 2012, Powell et al. 2012, Simons & Clevers 2011, Van Keymeulen & Blanpain 2012, Van Keymeulen et al. 2011). Organs therefore face the considerable challenge of regulating not only each individual niche but also, critically, the collective output of all the niches in an organ. When summed over

a large number of niches, even small perturbations in the activity of single niches could produce excessive tissue growth or atrophy. Thus, beyond the dynamics of the stem cell–niche unit lies the question of whether and how multiple, heterogeneous, and spatially dispersed units are coordinated throughout the expanse of a tissue (**Figure 1**).

In our view, the next challenge in stem cell biology is to understand the higher order processes that coordinate stem cell–niche units at the whole-organ level. Of all self-renewing tissues, epithelial organs offer particular promise for starting to shed light on these issues. The tissue structure of epithelia is geometrically straightforward and relatively well understood in both simple (intestine, lung, mammary gland, prostate) and stratified (skin, bladder, esophagus) epithelial organs (see **Figure 2**; see also sidebar, Tissue Organization of Stem-Based Epithelial Organs). Intriguingly, the stem cells that support different epithelia have common structural features, such as basal adhesion to a basement membrane and lateral adhesions to surrounding progeny, that integrate the stem cells into epithelial tissue architecture (**Figure 3**). These shared features raise the possibility that the structure-based mechanisms play a role in epithelial stem cell coordination.

Must there be communication between niches within an organ, or is it possible that homeostasis could reliably arise from mechanisms that are autonomous to each stem cell–niche unit? Can the challenges of tissue maintenance be met by each niche responding independently to tissue-extrinsic cues, in the absence of active coordination between niches? The answers to these questions for any system are currently unknown. Despite the importance of the question to tissue biology, explicit investigation of intraniche communication is just beginning, and few studies have directly investigated how niches may coordinate. Here we follow the hypothesis that communication between niches does exist. We consider how this communication can give rise to tissue-level properties, such as spatially efficient replacement of lost cells, that would not emerge from an uncoordinated system.

Tissue-level, supraniche mechanisms could come into play in four major contexts: (*a*) establishing the number and spacing of niches within an organ, (*b*) coordinating collective stem cell output during tissue renewal to maintain cellular and morphologic equilibrium, (*c*) spatially targeting stem cell and niche behaviors during tissue repair, and (*d*) remodeling organs to accommodate alterations in physiologic demand. In this review, we frame central questions that arise from recognition of the necessity of supraniche coordination and glean existing information from the best-understood models of mammalian stem-based organs, such as epidermis and intestine, as well as a rapidly emerging invertebrate model, the *Drosophila* midgut.

SETTING NICHE NUMBER AND SPACING

In organs maintained by stem cells, the number of stem cells is constrained by the number of niches, and the spatial dynamics of renewal reflect the spacing of these niches. Thus, to consider how stem cell–niche units are coordinated across an epithelium, we must first consider how the number and spacing of niches are controlled. It is striking that stem cells in all self-renewing epithelia are spatially dispersed, not clustered in a few concentrated sites. But despite this commonality, the mechanisms that establish niche spacing vary widely. For skin appendages, such as hair and feather follicles, spatial patterning is permanently fixed during embryonic development. For gastrointestinal crypts, patterning is established in development but continually revised throughout life. And for epithelia without dedicated anatomic niches, such as the lung and *Drosophila* midgut, niches may be improvised ad hoc in maturity, using inherent architectural elements of the tissue. These different mechanisms of niche specification carry implications for tissue-wide spatial regulation of stem cell and niche populations.

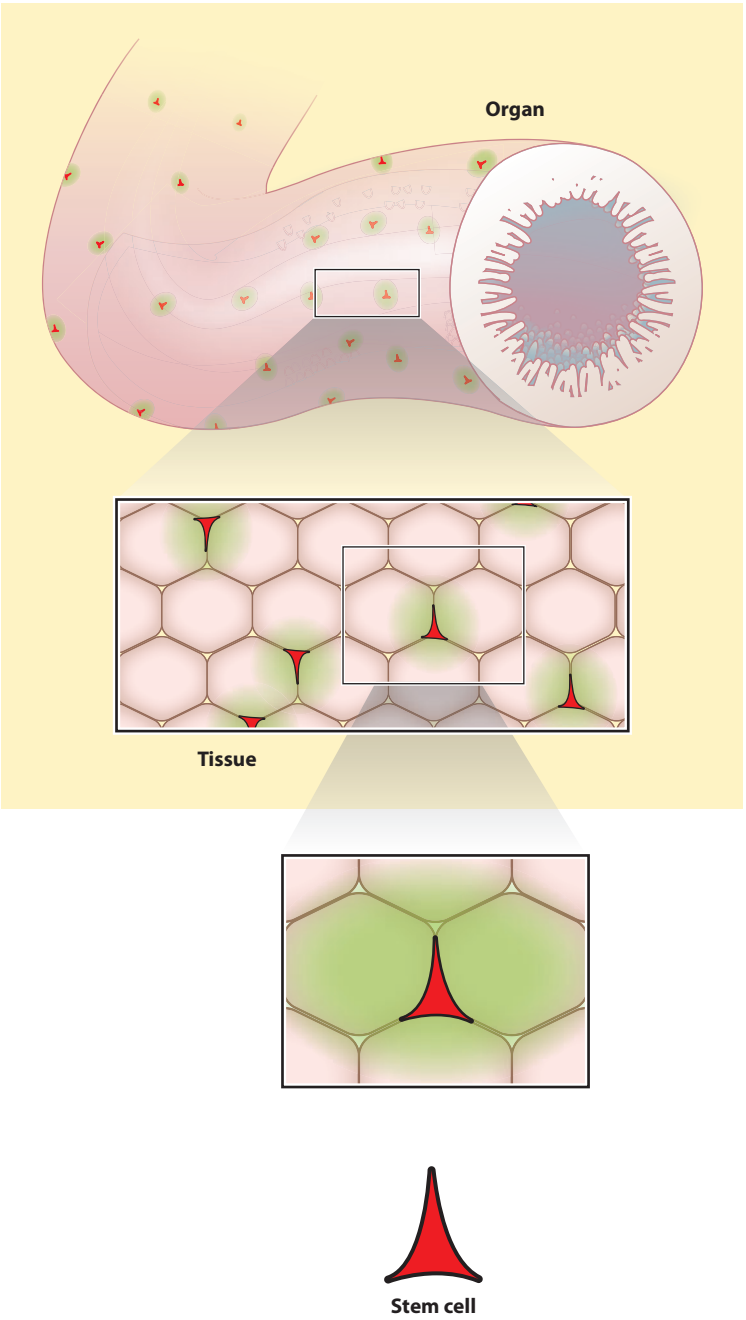


Figure 1

Scales of stem cell regulation in self-renewing tissues. Regulation of stem cell behavior can be considered at a range of biological scales, from stem cells (*red*) and their intrinsic properties, to the stem cell–niche unit (*red* and *green*), to stem cell–niche units within a localized tissue region, to the entire population of stem cell–niche units in the whole organ. This review focuses on coordination of stem cell–niche units within tissue regions and organs (*top two panels*).

TISSUE ORGANIZATION OF STEM-BASED EPITHELIAL ORGANS

Three of the best-understood stem-based epithelial organs, in order of increasing complexity, are schematized in **Figure 2** and described below:

Drosophila midgut (simple epithelium with dispersed stem cells). The fly midgut is functionally equivalent to the vertebrate stomach and small intestine. A single layer of epithelial cells lines the midgut tube. Midgut stem cells are dispersed throughout the entire epithelium, with each stem cell driving renewal of its surrounding tissue region. Other simple epithelia with dispersed stem cells include lung, mammary gland, and prostate.

Small intestine (simple epithelium with spatially segregated stem cell–niche units). The mammalian small intestine, like the fly midgut, is lined by a single layer of epithelial cells. In small intestine, the epithelial layer is further shaped into millions of luminal projections, called villi, and invaginations, called crypts. Villi expand the epithelium's surface area for nutrient absorption. They are composed exclusively of differentiated, postmitotic cells; cell extrusion and death occur at villus tips. Crypts form the niches for intestinal stem cells, which are interspersed between specialized cells at crypt termini. New cells, generated by stem cell divisions in crypt bottoms, collectively migrate up the crypt-villus axis. This migration is accompanied by loss of proliferative potential and progressive differentiation into terminal lineages. Stomach and colon exhibit a similar tissue organization and partitioning of terminal and proliferative cells, although the analog of the crypt-villus axis in these organs is smaller. In all these gastrointestinal epithelia, the spatial segregation of terminal and proliferative cell populations requires long-distance coordination of cell death and cell division.

Skin (complex, heterogeneous epithelium with multiple stem cell systems). Skin comprises multiple stem-based tissues. Its major component is epidermis, a stratified epithelium that forms the primary barrier to the outside world. In epidermis, the basal-most layer contains stem and progenitor cells that drive epidermal renewal. New cells are displaced to suprabasal layers and eventually to superficial layers. This upward progression is accompanied by gradual differentiation into mature keratinocytes; when they reach the outermost layer, dead keratinocytes are sloughed off. A similar tissue organization characterizes other stratified, stem-based epithelia, such as esophagus and bladder.

Another major skin component is the follicles that produce hair and feathers. Follicles are continuous with the basal layer of the epidermis and invaginate into underlying dermis. Stem cells that give rise to new hairs or feathers localize toward the follicle base; these cells cycle through active hair/feather production and inactivation. In mammals, additional stem-based skin components include sebaceous glands and sweat glands. Sebaceous glands bud off from the hair follicle neck. These epithelial glands, shown in **Figure 2** without cellular detail, are renewed by stem and progenitor cells found within the follicle neck and the glands themselves. Sweat glands are also distributed throughout the skin and are renewed by their own, distinct stem cell populations.

Establishing Niche Spacing Through Turing Patterning in Embryogenesis

An obvious feature of skin appendages—hair, feathers, fur, and scales—is their arrayed distribution over the body's surface. The periodic spacing of these appendages reflects the spacing of their follicular niches. During embryonic development, patterning of niches in the epidermis may arise through a self-organizing Turing mechanism of reacting and diffusing morphogens. As articulated in 1952 by mathematician Alan Turing, periodic patterning can emerge through autonomous amplification of small initial fluctuations in an otherwise homogeneous mixture of a diffusible activator and its inhibitor, if the two factors have particular characteristics (Headon & Painter 2009). Indeed, establishment of hair and feather follicles arguably represents the best-validated Turing system in biology.

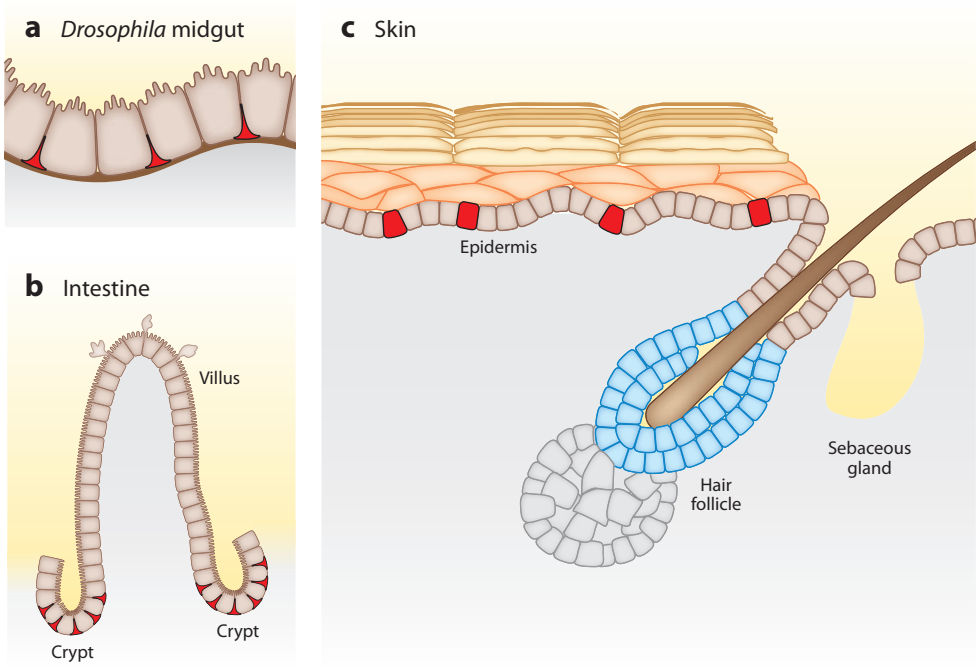


Figure 2

Schematic illustrations of model stem-based epithelial organs, shown in order of increasing complexity: (*a*) *Drosophila* midgut, a simple epithelium with dispersed stem cells (*red*); (*b*) mammalian small intestine, a simple epithelium with spatially segregated stem cells (*red*); and (*c*) mammalian skin, a complex, heterogeneous epithelium with multiple stem-based tissues. These include epidermis, a stratified epithlium with stem cells (*red*) in its basal layer; hair follicles, which contain epithelial-like stem cells (*blue*) in the follicle termini, and sebaceous glands (cellular detail not shown). Illustrations depict cross-sectional views through the epithelial plane. Gray shading represents stroma on the basal side of this plane, and yellow shading represents the outside world, or luminal contents on the apical side.

During embryogenesis, the two layers of developing skin—epithelial (future epidermis) and mesenchymal (future dermis)—cooperate to make follicles. Whereas the epithelial layer determines the competence to form follicles, the mesenchymal layer controls their initiation, spacing, and differentiated identity (Hughes et al. 2011, Jiang et al. 1999). Together, these two layers produce Turing activator:inhibitor pairs: FGF4/Shh:BMPs for avian epidermis and WNTs:DKKs and Edar:BMPs for mammalian epidermis (Jung et al. 1998, Mou et al. 2006, Schlake & Sick 2007, Sick et al. 2006). The activators instigate the earliest stages of follicle morphogenesis, whereas the inhibitors oppose the activators. Owing to differing ranges of action and feedback control of the activator:inhibitor circuit, signaling instabilities in the skin layers resolve over time to create local foci where the activating signal prevails, amid a global background where the inhibitory signal prevails (Painter 2012). These foci of activation predict where follicles will form, and the inhibited space between them becomes interfollicular epidermis (**Figure 4*a***). Similar follicle patterns arise in culture—even with dissociated cells—demonstrating the robust self-organizing qualities of Turing patterning (Jiang et al. 1999).

Spacing between adjacent follicles varies with the relative concentrations and diffusion rates of activator:inhibitor pairs, and the periodicity of follicle spacing can be predicted by computational Turing models (**Figure 4*b***) (Jung et al. 1998; Mou et al. 2006, 2011; Sick et al. 2006). As one

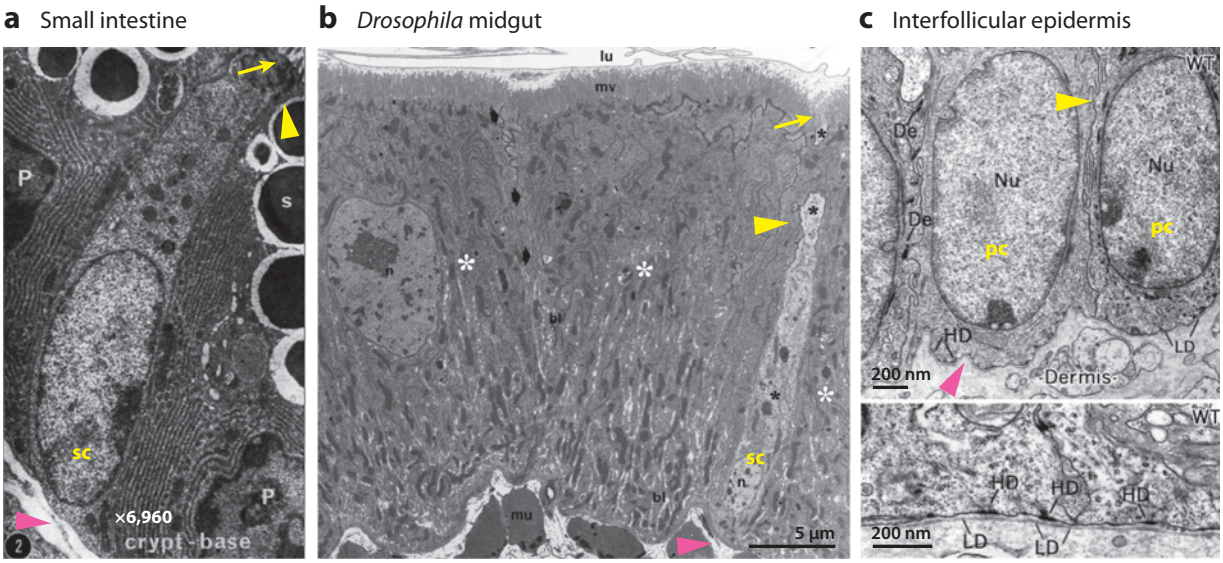


Figure 3

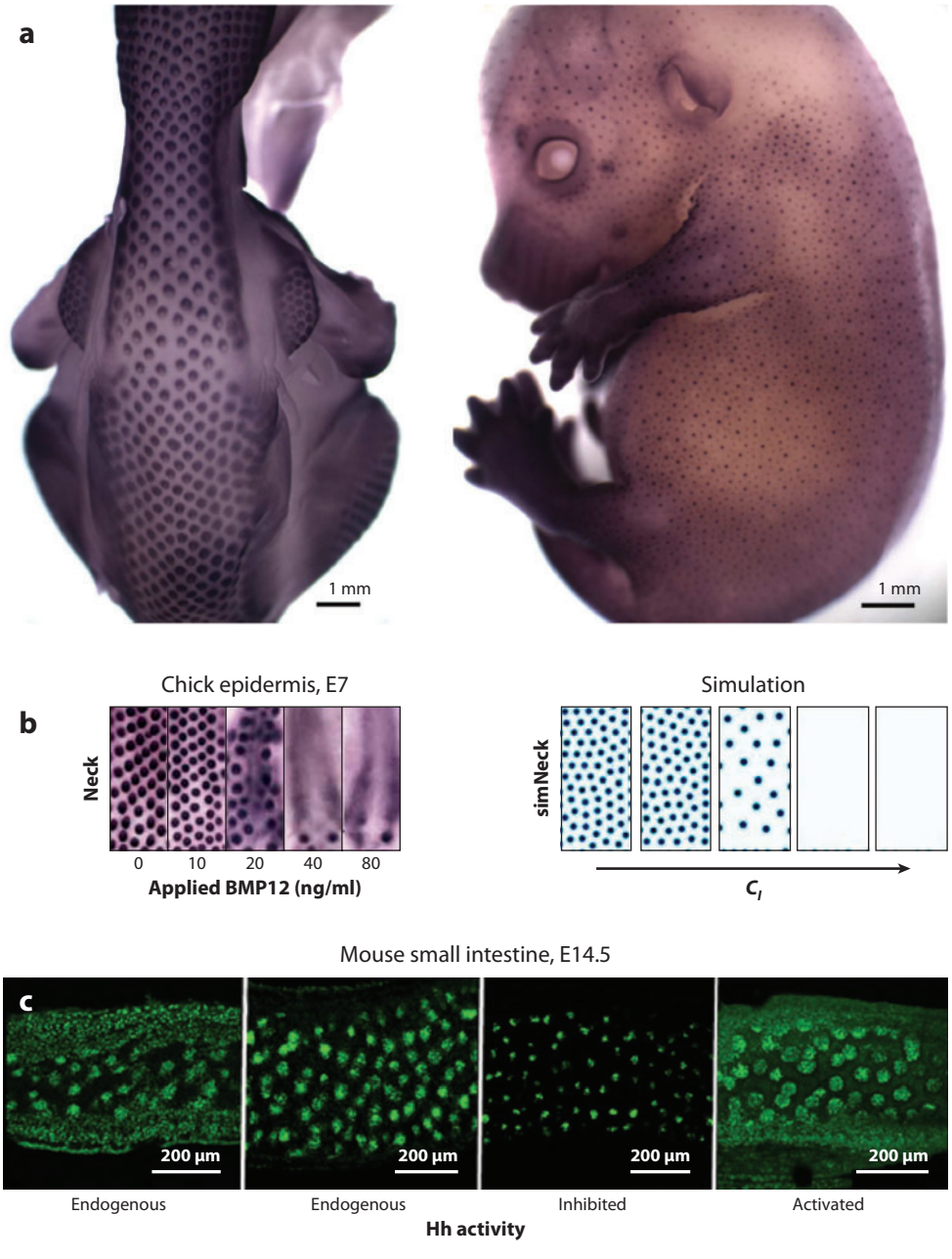
Stem cells are structurally integrated into the architecture of epithelial tissues. Electron micrographs of stem cells (sc) in the simple epithelia of (*a*) the mouse small intestine and (*b*) the *Drosophila* midgut, and of progenitor cells (pc) in the basal layer of stratified mouse epidermal epithelium. In all cases, stem/progenitor cells form junctional adhesions with neighboring epithelial cells (*yellow arrowheads*) and directly contact a basal basement membrane (*pink arrowheads*). In simple epithelia, stem cells also exhibit apical microvilli (*yellow arrows*). Adapted with permission from (*a*) Cheng H, Leblond CP. 1974. *Am. J. Anat.* 141:461–79; (*b*) Baumann O. 2001. *Exp. Cell Res.* 270:176–87; and (*c*) Raghavan S, Bauer C, Mundschau G, Li Q, Fuchs E. 2000. *J. Cell Biol.* 150:1149–60. Abbreviations: bl, basolateral; De, desmosome; HD, hemidesmosome; LD, lamina densa; lu, lumen; mv, microvilli; n, nucleus; P, Paneth cell; s, secretory granule; WT, wild type.

example, mouse dorsal skin normally contains ~30 follicles per mm^2 at E14, but exogenous expression of the Eda activator raises follicle density to ~50 per mm^2. Exogenous expression of Eda in Eda$^{-/-}$ skin raises follicle density still further, to ~90 per mm^2, because the Eda$^{-/-}$ skin has not been primed by endogenous Eda. Finally, exogenous expression of both Eda and the BMP inhibitor Noggin in Eda$^{-/-}$ skin produces a maximum of ~140 follicles per mm^2 (Mou et al. 2006). In domestic chickens, a spontaneous genomic insertion in the BMP12 locus results in elevated levels of the BMP12 inhibitor and a consequent reduction in feather density, thus offering a naturally occurring test of Turing patterning (Mou et al. 2011).

Hair follicle formation occurs in three distinct waves. The first follicles appear as placodes in the epithelial layer at E14, following initial establishment of the Turing equilibrium. However, continued embryo growth destabilizes this initial equilibrium. By E16–17, a new Turing equilibrium has been established, leading to a second wave of follicles interspersed with the first. (Once created, follicles become refractory to activator:inhibitor signals, and thus the initial follicles persist.) Continued growth resets the Turing equilibrium a third time, generating a final wave of follicle formation at E18–P1 (Schlake & Sick 2007, Sick et al. 2006). After the final wave, follicle distribution remains fixed for life. Of note, stasis is not an inherent endpoint for all Turing equilibria; indeed, other biological Turing patterns, such as pigmentation in fish, exhibit continual change. In epidermis, the mechanisms that prevent later waves of follicle formation are not currently clear. However, computational models and some experimental evidence suggest that

neonatal induction of a factor that inhibits the Turing inhibitor may fix follicle patterning after birth (Headon & Painter 2009, Lo Celso et al. 2004).

Like epidermal follicles, crypts in the gastrointestinal (GI) tract exhibit periodic spacing. But in contrast to epidermis, where the patterned elements are follicular niches, in the GI tract it is villi that are directly patterned. Villar patterning then determines the initial development of cryptal niches. Villi first emerge as a regular array during embryonic gut development. Crypts appear after birth as shallow indentations in the spaces between villi, and they acquire their full depth and



tubular morphology over the first two weeks of postnatal life (Calvert & Pothier 1990, Cheng & Bjerknes 1985). Suggestive parallels exist between villus and epidermal follicle development. Villus formation, like follicle formation, involves cooperative interactions between juxtaposed epithelial and mesenchymal layers. The action of Hh and BMPs in villar development bears comparison to the Turing activator:inhibitor pair Shh:BMPs in feather follicles. In the gut, uniform secretion of Hh at E15 induces regularly spaced epithelial placodes that predict where villi will form, and altering levels of Hh signaling alter the spacing of these placodes (**Figure 3*c***) (Walton et al. 2012). BMP inhibitors are induced by Hh in the gut (Madison et al. 2005), analogous to BMP induction by Shh during feather patterning (Jiang et al. 1999, Jung et al. 1998). Expression of the BMP inhibitor Noggin has a similar effect in developing gut as in epidermis, producing a smaller number of enlarged villi or follicles (Batts et al. 2006, Jiang et al. 1999). Another parallel is that villus formation occurs in four distinct waves, at E15.5, 16.5, 17.5, and 18.5, and that each wave intersperses new villi between older villi. Akin to hair follicle formation, these waves of villus formation could arise through destabilization and reestablishment of a reaction-diffusion equilibrium by organ growth. Whether a true Turing equilibrium underlies the patterning of villi and their associated crypts, or whether these similarities to follicle patterning arise by an alternative mechanism, will require directed future inquiry.

Expanding Niche Populations During Postnatal Growth

Despite their commonalities during embryonic development, the GI crypt population, unlike the epidermal follicle population, continues to expand after birth. During the first weeks of postnatal growth, crypt numbers increase dramatically—from ~4,600 to ~425,000 in the rat colorectal epithelium (Maskens & Dujardin-Loits 1981). By contrast, villus numbers stay constant; instead, the cross-sectional area of each individual villus increases. Consequently, in postnatal jejunum, the number of crypts per villus rises from 3 to 11, but crypt density per cm^2 at week 4 is similar to that at birth (Cheng & Bjerknes 1985). This burst of postnatal crypt production may reflect an underlying need to accommodate more stem cells as the organ grows; unlike hair and feather stem cells, which produce discrete appendages, GI stem cells support organ-wide cell renewal and may require a minimum ratio of stem cells to differentiated cells. Rapid crypt production ends with weaning and the ingestion of solid food, but new crypts continue to be generated at a slow rate throughout life (Cheng & Bjerknes 1985, Totafurno et al. 1987).

The cellular mechanism for addition of postnatal crypts is entirely different from the neonatal generation of initial crypts. Instead of de novo invagination of the intervillus epithelium, new crypts arise in later life through longitudinal fission of preexisting crypts (Cheng & Bjerknes 1985, Maskens 1978). Fission initiates through bifurcation of the crypt base and moves progressively

Figure 4

Modulation of niche spacing in developing epithelial tissues. (*a*) In the skin of chick and mouse embryos, regular arrays of placodes (β-catenin in situ expression) foreshadow the spacing of feather and hair follicles, respectively. Reproduced with permission from Headon & Painter (2009). (*b*) (*Left*) In E7 chick epidermis, application of recombinant BMP12 inhibitor increases the spacing of feather placodes in a dose-dependent manner. (*Right*) Simulated Turing reaction-diffusion dynamics with increasing concentrations of inhibitor generates patterning similar to that observed in vivo. Adapted with permission from Mou et al. (2011). (*c*) In E14.5 mouse intestine, varying levels of Hh signaling alter the density and size of epithelial placodes that prefigure where villi will form (E-cadherin, *green*). (*Left to right*) Control (vehicle only), control (tomaditine, an inactive analog of cyclopamine), inhibited Hh signaling (cyclopamine), and activated Hh signaling (SAG, Smoothened agonist). Adapted with permission from Walton et al. (2012).

toward the crypt-villus junction (Cairnie & Millen 1975, Cheng et al. 1986a). In steady-state adults, 3–10% of jejunal crypts are in the process of splitting; during postnatal growth, this proportion reaches as high as 38% (Cheng & Bjerknes 1985). Whereas initial crypt formation reflects synchronous, tissue-wide patterning, crypt fission appears regulated at the level of the individual crypt (Totafurno et al. 1987). Crypts split asynchronously and without an apparent spatial pattern. Their bifurcation planes orient randomly with respect to the crypts' longitudinal axis. Indeed, the single parameter that has been correlated with fission probability is a crypt-autonomous feature, cell number. The volume of individual crypts varies eightfold in adult mouse jejunum, but only crypts within the upper 25% of this size distribution will split (Totafurno et al. 1987). Indeed, an ~100-day crypt cycle has been postulated, in which crypts progressively accumulate cells until they reach an unstable threshold size and then split (Bjerknes 1986). The molecular and cellular basis for crypt fission is currently unknown, although cultured intestinal organoids, in which crypts also undergo fission (Sato et al. 2010), may facilitate mechanistic investigation. Higher frequencies of fission in colitis, Crohn's disease, and familial adenomatous polyposis patients, as well as in mouse APC mutants, might also provide clues (Bjerknes et al. 1997, Cheng et al. 1986a). Thus, in the switch from embryonic to postnatal growth, size control of the cryptal niche population shifts from uniform, tissue-wide regulation to localized, niche-based control.

Improvising Niches Ad Hoc in Maturity

For epidermal appendages and the GI tract, the spatial distribution of niches is readily apparent. However, many epithelia lack discrete, identifiable niches, raising the question of whether their stem cells employ a functional substitute. The list of niche-less epithelial organs includes both the simple epithelia of lung, mammary gland, prostate gland, and *Drosophila* midgut and the stratified epithelia of interfollicular epidermis, esophagus, and bladder (Clayton et al. 2007, Doupé et al. 2012, Mascré et al. 2012, Micchelli & Perrimon 2006, Ohlstein & Spradling 2006, Ousset et al. 2012, Rawlins et al. 2009, Rock et al. 2009, Shin et al. 2011, Van Keymeulen et al. 2011, Wang et al. 2009). In simple epithelia, individual stem cells typically intercalate between the differentiated workhorse cells, with two to four columnar epithelial cells surrounding a stem cell. Generally smaller than the epithelial cells, these stem cells reside in the epithelium's basal domain and contact the epithelial basement membrane. In stratified epithelia, stem cells localize to the basal-most layer, surrounded by their immediate, undifferentiated progeny, and are also in contact with the basement membrane. Stem cell populations are spatially dispersed throughout their particular organ—a pattern that, at least in the *Drosophila* midgut, is established during development (Jiang & Edgar 2009). Although different organ regions can show stereotyped differences in stem cell density—for instance, scale edges in tail skin epidermis or distal hairpin in *Drosophila* midgut (O'Brien et al. 2011, Roshan & Jones 2012)—within these broad domains, no distinct anatomic microenvironments house individual stem cells. Thus, to understand how stem cell spacing and number are controlled, we must first understand how the roles of archetypal niches like crypts and follicles are met in these other systems.

As a starting point, we consider that canonical niches serve two general functions: They create a signaling microenvironment that regulates stemness, and they use adhesive contacts to keep stem cells in signal-receiving range (Losick et al. 2011, Morrison & Spradling 2008, Nystul & Spradling 2006, Ohlstein et al. 2004). In epithelia without canonical niches, these basic needs are met, at least in part, by hallmark features of the epithelium itself: polarized, columnar epithelial cells and the epithelial basement membrane. Stem cells without a dedicated niche adhere to neighboring epithelial cells via E-cadherin, akin to the E-cadherin junctions that anchor stem cells to canonical

niches in *Drosophila* gonads and in GI crypts (Choi et al. 2011, Maeda et al. 2008, Song & Xie 2002, Song et al. 2002, van der Flier et al. 2009, Wang et al. 2006). Within this stem cell–epithelial interface, juxtacrine Notch signals regulate stem cell–fate decisions in the simple epithelia of the lung, mammary gland, prostate, and fly midgut, again reminiscent of Notch regulation in canonical niches (Bouras et al. 2008, Ohlstein & Spradling 2007, Perdigoto et al. 2011, Rock et al. 2011, Valdez et al. 2012). Secreted regulatory factors such as Wnts, BMPs, cytokines, and EGF ligands are shared by stem cells both with and without canonical niches, although whether their spatial range of action in the latter case recapitulates their short-range specificity in the former remains to be determined. Besides their contacts with neighboring epithelial cells, stem cells also form integrin-mediated adhesions with the basement membrane, thus tethering the stem cells to the epithelium's basal domain (Goulas et al. 2012, Jones et al. 1995, Taddei et al. 2008). This basal localization appears to be important for stem cell identity, at least in interfollicular epidermis and fly midgut (Goulas et al. 2012, Lechler & Fuchs 2005). Thus, in the absence of dedicated niches, inherent features of the epithelium itself take on a facultative role, fulfilling at least some of the adhesive and signaling functions of canonical niches. In other words, stem cells could be viewed as improvising a niche ad hoc by using workhorse epithelial cells and other architectural elements of the tissue.

From this vantage, the widespread distribution of stem cells in epithelia without dedicated niches may reflect the broad availability of ad hoc niches. At least in simple epithelia, niche number and spacing could become a function of epithelial tissue organization: the number of columnar epithelial cells and the packing arrangements of these epithelial cells with stem cells. The architectural requirements of sheet formation in the epithelial plane constrain the cellular packing geometries that are possible (Farhadifar et al. 2007); the mosaic tiling of larger, typically five- or six-sided epithelial cells with smaller, pyramidal stem cells will create geometric patterns. These packing geometries may influence the number of stem cells that the epithelium can accommodate. During tissue renewal, cell loss and replacement will dynamically alter packing geometry, with concomitant changes in the availability and positioning of ad hoc niches. Because newborn epithelial daughters tend to retain junctional contacts with their stem cell of origin (reflected in the coherence of epithelial stem cell clones), stem cells must rearrange their cell-cell junctions after each division to accommodate at least one new neighbor. Thus, the geometry of a stem cell's ad hoc niche evolves concomitantly with cell production, perhaps giving immediate feedback to influence cell cycle progression or fate decisions. By coupling stem cell placement to the local dynamics of epithelial organization, ad hoc niches present a means for stem cell control that is both flexible and responsive to local needs.

In the above sections, we have discussed three different types of mechanisms for achieving a distributed population of stem cells during tissue development. Despite their diversity, these mechanisms all exhibit some degree of self-organization. Interestingly, the scale of self-organizing properties seen in each tissue inversely correlates with the niches' capacity for remodeling. For epidermal follicles and GI crypts, patterning across the entire expanse of the tissue establishes the niche during embryonic development. In epidermis, the pattern established in development is irrevocable. In the GI tract, flexibility in niche spacing after birth occurs through a shift in control from the entire tissue to the local neighborhood of an individual niche. In the intestinal crypt population, the correlation between crypt cell numbers and fission propensity suggests an autoregulatory mechanism that is sensitive to niche size. For organs with ad hoc niches, the scale of niche control becomes smaller still—to the two to four epithelial cells that immediately surround an individual stem cell. Spacing of ad hoc niches is a by-product of the self-organizing property of differentiated epithelial cells. This coupling of stem cell regulation

to tissue structure may serve to relay information about local cellular fluctuations directly to stem cells in the immediate vicinity, enabling a rapid and precise stem cell response. The details of these niche-spacing mechanisms, and their sensitivity to local conditions within the tissue, hold implications for the spatial range of action of niche-coordinating mechanisms and for the plasticity of niche populations as a means to influence stem cell dynamics, as discussed below.

NICHE COORDINATION IN TISSUE RENEWAL

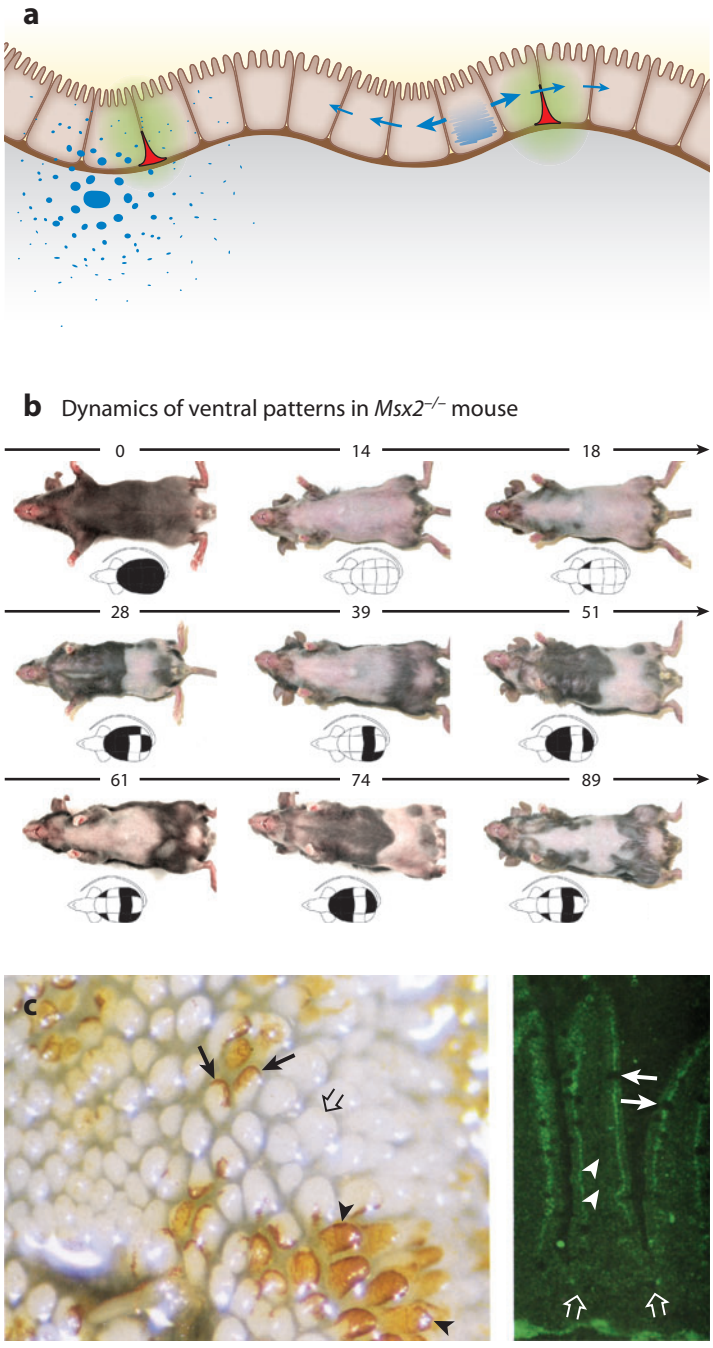
In most adult epithelia, cellular loss occurs regularly and at a small scale throughout the tissue; continuous and reliable replacement is required to prevent breaches of the epithelial barrier. The niches that instruct stem cell behavior must be coordinated across the tissue's spatial expanse such that new cells are generated at the right time and place to maintain homeostasis. Tissue-wide coordination of stem cell outputs can require that niches act in sync, or that they act in distinct but complementary modes. It can also involve different physical scales—from two adjacent niches separated by a few epithelial cells, as in the fly midgut, to large subsections of an organ that exhibit characteristically different rates of proliferation and renewal, as with the ileum and jejunum of the small intestine (Altmann & Leblond 1970, Clarke 1970). Irrespective of scale, coordination of epithelial stem cell–niche units can arise through two general types of signals: (*a*) soluble growth factors that diffuse through stromal or luminal environments and (*b*) propagated signals that relay information from cell to cell or through the extracellular matrix. The topic of tissue-wide coordination becomes particularly germane with recent studies showing that stem cell divisions in numerous simple and stratified epithelia exhibit stochastic, neutral drift dynamics (Simons & Clevers 2011). These findings raise the question of how tissue homeostasis is upheld if new cell production is stochastic. Here, we discuss the diffusible and propagated signals that could provide spatial coordination of stem cell–niche units (**Figure 5*a***). We then consider how such signals might bias neutral drift dynamics to maintain cellular equilibrium at the whole-tissue level.

Figure 5

Mechanisms for spatial coordination of epithelial renewal. (*a*) Two general types of mechanisms coordinate stem cell–niche units across space. (*Left*) Diffusion of signaling molecules, such as soluble growth factors, through stromal or luminal environments can create gradients of activity that spatially modulate stem cell–niche outputs. (*Right*) Relay of propagated signals, such as mechanical force, from cell to cell or through extracellular matrix can provide spatially directed feedback to stem cell–niche units. (*b*) Coordination through diffusible signals. Zones of synchronized hair follicle cycling arise through staggered waves of diffusible Wnt, FGF, and BMP signals in adult mouse skin. Zone dynamics are revealed by changes in visible pigmentation of a *Msx2* mutant with cyclic alopecia. The same individual was followed over 89 days. Adapted with permission from Plikus & Chuong (2008). (*c*) Coordination through propagated signals. In chimeric-transgenic small intestine, perturbing tissue homeostasis through local disruption of cell-cell adhesion induces a compensatory response in adjacent, unperturbed regions. (*Left*) Whole mount of small intestine from an adult B6←→129/Sv chimeric-transgenic mouse. Three types of villi are observed: wholly B6 derived (*white; open arrow*), wholly 129/Sv derived (*brown; solid arrowheads*), and chimeric (*brown/white; solid arrows*). (*Right*) 129/Sv-derived cells contain a transgene under control of a FABP promoter fragment. Within 129/Sv-derived tissue, transgene expression (*green*) occurs only in postmitotic enterocytes (*closed arrowheads*), not in crypts (*open arrows*) or goblet cells (*closed arrows*). Expression of dominant-negative E-cadherin in these cells accelerates their migration and shedding from villus tips. This perturbation elicits compensatory proliferation and migration of nonexpressing progenitor cells specifically in neighboring crypts, thus maintaining overall cellular equilibrium. Adapted with permission from Hermiston & Gordon (1995).

Diffusible Signals that Coordinate Renewal

Perhaps the best-understood example of spatial niche coordination is the fluctuating patterns of regional hair follicle activity that occur in the skin of adult rodents. Each individual hair follicle undergoes cycles of stem cell activation and inactivation during which hair growth initiates and stops (Hsu & Fuchs 2012). From follicle to follicle, the timing of these cycles is neither random



nor globally synced. Rather, the skin exhibits discrete spatial zones of follicle activation and inactivation. Within a given zone, all follicles are in phase. At the same time, each zone is exactly out of phase with its neighboring zones. Zone boundaries are irregular and vary from animal to animal. Within the same animal, they change over days to weeks, moving across the skin in a wavelike fashion (**Figure 5*b***) (Plikus & Chuong 2008).

The molecular basis of hair follicle zones shows how staggered production of diffusible activators and inhibitors can dynamically coordinate niche activity. A new activated zone is nucleated by a small, random cluster of hair follicles that stochastically expresses a threshold level of Wnt ligands. These initiating follicles then activate adjacent follicles, likely through secretion of FGFs, and thereby instigate a wave of activation that travels through the skin (Enshell-Seijffers et al. 2010, Greco et al. 2009, Plikus et al. 2011). The wave halts when it reaches a zone of follicles that have recently been inactivated. In these refractory zones, high levels of inhibitory BMPs make follicles inert to activating Wnts and FGFs (Plikus et al. 2008, 2011). This juxtaposition of activated and refractory follicles produces a sharp boundary between zones and explains why neighboring zones are necessarily out of phase. What generates these refractory zones of high BMPs? The activating wave sets into motion its own extinction by inducing BMPs in dermal cells surrounding the follicles. Over time, BMP signaling builds to a point where hair follicles in the activated zone no longer respond to Wnts/FGFs, tipping them into the inactivated, refractory phase of the cycle (Plikus & Chuong 2008).

In the GI tract, crypts do not appear to exhibit zonal waves of activation, arguing against an analogous mechanism of spatial niche control. Nonetheless, certain intestinal pathologies may indicate that soluble factors from one region diffuse through stroma to affect niche activity in another. At the margin of overgrowing colon tumors, colossal crypts develop from genotypically normal tissue (Bjerknes & Cheng 1999). These structures grow up to ten times longer than normal crypts and display frequent branching, but they retain a monolayered, tubular architecture and do not progress to cancer. One appealing explanation for colossal-crypt formation is that the tumors secrete diffusible mitogens that activate proliferation in normal crypts nearby, producing crypt hyperplasia (Bjerknes & Cheng 1999).

Another type of diffusible signal influences GI stem cells from the luminal side of the epithelium: factors secreted by the gut's commensal microflora. In both mice and *Drosophila*, indigenous bacteria help set normal rates of GI proliferation and epithelial turnover through activation of Toll and JAK-STAT pathways, respectively (Buchon et al. 2009a, Rakoff-Nahoum et al. 2004, Walker et al. 2009). In *Drosophila*, this pathway proceeds through enterocytes in the ad hoc niche, which sense microbial ligands and respond by secreting cytokines that signal directly to stem cells (Buchon et al. 2009b, Jiang et al. 2009). Could microbial regulation serve to spatially coordinate gut stem cell activity? This question is an open one, but characteristic differences in the resident microbiome along the length of the gut tube may hint at such a scenario. These patterns could generate proximal-distal gradients of microbial ligands in the gut lumen that influence coordinated regional differences in stem cell proliferation.

Propagated Signals that Coordinate Renewal

In the small intestine, the spatial arrangement of cell turnover implies a major role for adhesive signals that are propagated through the epithelial layer. In this tissue, differentiated cells are shed from the tip of a villus and are replaced by cells generated in the crypts surrounding that villus. Thus, a spatially restricted population of niches compensates for a localized cellular loss. Importantly, new cell production by crypts is not an invariant, hardwired behavior but rather a

specific response to decreased cell numbers in contiguous villi. When cell migration and shedding are slowed by E-cadherin overexpression, crypt cell production slows down to compensate (Hermiston et al. 1996). Conversely, accelerating migration and shedding through expression of dominant-negative E-cadherin results in compensation through accelerated cell production (Hermiston & Gordon 1995). Tellingly, when dominant-negative E-cadherin is restricted to just upper villi, cell production still accelerates in the genetically unaffected crypts below (**Figure 5*c***) (Hermiston & Gordon 1995). Altogether, these findings argue that rates of crypt cell proliferation are actively calibrated to match cell loss through the relay of information about cell density or number to spatially distant niches. A next step toward understanding these mechanisms would be to elucidate their effective range of action. For instance, given a chimeric villus in which just half of the upper region expresses dominant-negative E-cadherin, is compensatory proliferation observed in all its surrounding crypts, or only in the crypts that directly gave rise to the altered cells?

That altering E-cadherin can modulate the kinetics of intestinal turnover suggests that cell-cell adherens junctions are likely a central component of the relay that coordinates crypt behavior. A simplified version of this scenario occurs in the fly midgut. Following a midgut stem cell division, the new daughter cell remains attached to its mother stem cell via a distinctive adherens junction that transiently inhibits the stem cell's ability to reenter the cell cycle (Choi et al. 2011, Maeda et al. 2008, Ohlstein & Spradling 2006). And in interfollicular epidermis, individual, slow-cycling stem cells are surrounded by their immediate, fast-cycling progeny (Potten 1974), raising the possibility that similar adhesion-based feedback may inhibit stem cell division rate.

Contact inhibition in cultured epithelial cell monolayers provides insight into how cell-cell adhesion could provide spatial feedback on the need for new cell production. In culture, E-cadherin cell-cell contacts propagate compressive forces throughout the epithelial plane (McClatchey & Yap 2012). As the density of cells per unit area increases, compressive forces also increase. Cells interpret these forces as an indicator of crowding; thus, cell cycle entry is inhibited when compression is high (McClatchey & Yap 2012). A similar mechanism might operate in the intestinal epithelium to spatially couple cell loss and proliferation. Information about cell crowding in villi could be relayed through junctional E-cadherin to crypts, where it would influence rates of progenitor cell division. Along these lines, it is notable that the intestinal stem cell marker Lrig1 directly associates with E-cadherin in cultured cells and that this association is required for density-dependent proliferative control (Lu et al. 2012b, Powell et al. 2012).

Could crowd sensing be a general strategy for spatial coordination of epithelial stem cells? A key mediator of crowd sensing is the Hippo pathway. For both cultured epithelia and in vivo epidermis, Hippo signaling links E-cadherin signaling at junctions to cell cycle control in the nucleus, with high junctional adhesion inhibiting nuclear translocation of the downstream transcription factor YAP (McClatchey & Yap 2012, Schlegelmilch et al. 2011, Silvis et al. 2011). Evidence also suggests that Hippo/YAP can respond directly to cell loss. Nuclear YAP is activated by the extrusion signal sphingosine-1-phosphate (Miller et al. 2012), a bioactive lipid that causes dying cells to be squeezed out of the epithelium (Eisenhoffer et al. 2012, Gu et al. 2011). In epidermis, YAP-dependent crowd sensing is mediated by stem cells (Schlegelmilch et al. 2011). YAP also regulates stem cell divisions in the small intestine and fly midgut (Barry et al. 2012, Cai et al. 2010, Karpowicz et al. 2010, Ren et al. 2010, Shaw et al. 2010, Staley & Irvine 2010), although a role in density-dependent proliferation remains to be shown. Altogether, crowd sensing in stem-based epithelia may be a means to convert spatial information about cell density into nuclear control of stem cell divisions, with Hippo/YAP as the mechanism's molecular linchpin.

Neutral Drift Dynamics and Spatial Control of Stem Cells: Creating Equilibrium from Stochasticity

Under conditions of steady-state renewal, stem cells were traditionally believed to generate progeny at uniform rates. Recently, however, quantitative analyses of stem cell clone dynamics have argued that this is often not the case. In the small intestine, interfollicular epidermis, esophagus, and *Drosophila* midgut, stem cell populations exhibit an emergent behavior termed neutral drift (Clayton et al. 2007; de Navascués et al. 2012; Doupé et al. 2010, 2012; Lopez-Garcia et al. 2010; Mascré et al. 2012; Snippert et al. 2010b). In neutral drift systems, the division patterns of individual stem cells are stochastic; each stem cell cycles at variable rates, and after division, each of the two daughters adopts either a stem cell or a terminal fate with variable frequency (Klein & Simons 2011). (The phrase "neutral drift" refers to unbiased, population-level turnover of the stem cells themselves, which occurs as a consequence of these division patterns.) Chance dictates that some stem cells generate large numbers of progeny, whereas other stem cells generate few or none. But when summed over the entire stem cell population, the collective rate of new cell production matches the rate of cell loss, and cellular equilibrium is maintained.

If the behavior of individual stem cells is stochastic, how is collective stem cell output coordinated to achieve tissue-level homeostasis? One scenario would be stem cell autonomous: Intrinsic regulatory circuits that control division and fate may be noisy by design, and this noisiness would generate particular division patterns with chance frequencies that, in aggregate, meet the tissue's needs for cell replacement (Klein & Simons 2011). However, a strictly stem cell–autonomous mechanism appears incompatible with the ability of stem cells to modulate cell production to compensate for altered rates of cell loss (Hermiston & Gordon 1995).

An alternative scenario posits nonautonomous influences on stem cell stochasticity. Extrinsic signals that bias the frequency of particular division patterns could provide a link between tissue needs and division outcomes (Klein & Simons 2011). An appealing source of such extrinsic signals would be the differentiated progeny of stem cells themselves. Because epithelial stem cells form lateral adhesions with their differentiated progeny—some of which even become part of the niche—the opportunity exists for direct and immediate feedback control. As one possible scenario, chance fluctuations in the spatial abundance of niche progeny could bias daughters toward symmetric or asymmetric fates, which might then feed back to correct the original, local imbalance. By biasing neutral drift dynamics in a spatially targeted way, local feedback regulation could help maintain tissue-wide cellular equilibrium, even in the face of stochasticity.

NICHE COORDINATION IN INJURY REPAIR

Epithelial stem cells not only drive constitutive renewal but also can restore tissue lost to injury. The stem cell response to injury is inherently spatial. Following localized damage, such as a punch wound, stem cells within a given distance from the wound edge mobilize, whereas stem cells outside this zone continue their usual business. Tellingly, stem cell–niche units themselves do not have to be damaged to activate repair. Removing superficial layers of epidermis by applying adhesive tape and then peeling it off or exposing the apical-most layer of the bladder epithelium to pathogens provokes a proliferative response in basal stem cells located on the opposite side of the stratified epithelium (Ito et al. 2005, Shin et al. 2011). Similarly, denuding villi in the intestine through chemical treatments activates stem cells that lie at the crypt base (Silen & Ito 1985). These observations imply that injury signals propagate through space to reach stem cell–niche units at some distance from the wound.

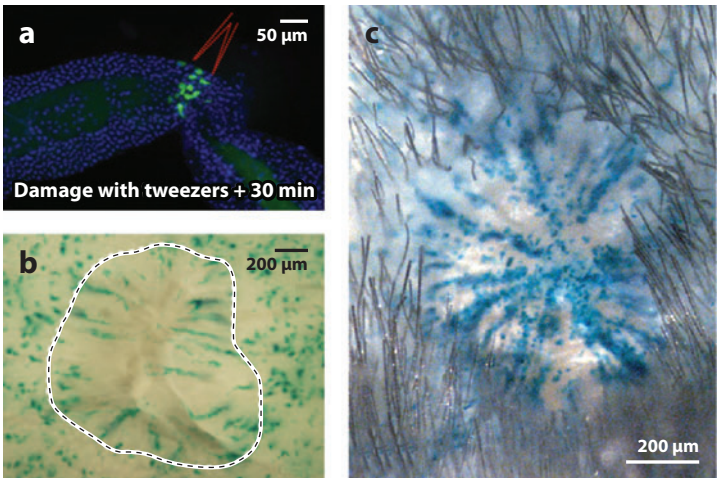


Figure 6

Spatial mobilization of stem cells for epithelial wound repair. (*a*) In *Drosophila* midgut, localized wounding induces localized secretion of stem cell mitogens. Within 30 min of pinch wounding, damaged cells turn on expression of the cytokine Unpaired 3 (*green*), which activates stem cell proliferation. Adapted with permission from Buchon et al. (2009a). (*b*,*c*) Radial migration of stem and progenitor cells from undamaged tissue toward wound sites. (*b*) In mouse colonic epithelium, labeled cells leave neighboring crypts and converge upon a punch wound to populate new crypts (adapted with permission from Miyoshi et al. 2012). (*c*) In mouse interfollicular epithelium, labeled stem cells and their progeny migrate from nearby hair follicles toward a punch wound to regenerate epidermis (adapted with permission from Ito et al. 2005).

What delimits the spatial range of the epithelial stem cell response to injury? Few studies have explicitly examined this question, but the response zone is presumably set by the extent to which injury signals travel through the tissue. Insight into these processes may be gleaned from a wealth of research on the signaling and cellular processes that repair epithelia in vivo and in culture. Here we review mechanisms that help define the spatial range of stem cell–mediated repair and discuss how cellular repair responses converge upon the wound site. Finally, we compare regeneration of stem cell–niche units following injury to establishment of niches during development.

Mechanisms for Localized Stem Cell–Niche Activation

The best-understood signals that activate local injury repair are growth factors secreted either by the damaged cells themselves or by immune cells recruited for acute damage control. Some of these signals are specific to wounding, such as the cytokines released by dying enterocytes in the fly midgut or by infiltrating macrophages in the mammalian colon (Buchon et al. 2009b, Jiang et al. 2009, Pull et al. 2005, Seno et al. 2009). Other signals function in both repair and renewal but are induced in a distinct manner. For instance, Wnts in the intestine and epidermis are produced at higher levels during injury (Davies et al. 2008, Ito et al. 2007). EGF ligands in the fly midgut, which are secreted constitutively by visceral muscle cells, are also induced in enterocytes specifically after damage (**Figure 6*a***) (Buchon et al. 2010, Jiang et al. 2011). As they diffuse away from the wound site, these signals establish a zone of stem cell repair activity.

Do direct cell-cell relays act in conjunction with secreted growth factors to define the injury response zone? One intriguing possibility is that niches are sensitive to mechanical forces created during epithelial restitution, the tissue's emergency response to plug the breached epithelial

barrier before the stem-based proliferative response can be mounted. In restitution, differentiated epithelial cells on the wound edge become mesenchymal in character, migrate toward the denuded region, and flatten out to create a makeshift covering (Moore et al. 1989, Potten & Allen 1975, Silen & Ito 1985, Van Winkle et al. 1995). At the same time, cellular contractility condenses the tissue to minimize the open area (Buchon et al. 2010, Day & Bennetts 1953, Jiang et al. 2009, Mammen & Matthews 2003, Sonnemann & Bement 2011). The molecular events that underlie restitution—including loosening of cell-cell junctions, remodeling of the basement membrane, and heightened epithelial tension—produce a zone of altered mechanical properties in the epithelium surrounding the wound. Because niches are integral with the epithelium, these mechanical stresses can be transmitted to niches within the altered zone. Perhaps niches, via architecture-sensitive pathways such as Hippo/YAP, interpret such stresses as signposts to the wound site and respond by activating their resident stem cells. Such a mechanism could explain, for instance, the dichotomous response to longitudinal versus transverse incisions in the epithelial lining of the uterus. Longitudinal incisions, which sever the subepithelial muscle sheath in an orientation that can be countered by muscle contraction, are repaired perfectly; by contrast, transverse incisions, oriented 90° perpendicular, completely fail to repair (Selye & McKeown 1934).

Spatial Behaviors of Activated Stem Cells

Within the injury response zone, repair elicits stem cell and niche behaviors that are distinct from renewal behaviors. Injury signals not only activate stem cell divisions beyond steady-state renewal rates but also can lead remote stem cells to exit their niches and migrate to the wound site (**Figure 6*b***). One example comes from punch wounds in the colonic epithelium. After—perhaps even activated by—restitution, streams of progenitor cells migrate from nearby, uninjured crypts to the wound site, their lines of travel forming channels in the intercrypt stroma (Miyoshi et al. 2012, Seno et al. 2009). Even more striking, injury in interfollicular epidermis activates the exodus of heterologous hair follicle stem cells from their niches to contribute to repair (Ito et al. 2005, Levy et al. 2007, Lu et al. 2012a, Snippert et al. 2010a). The streaming of hair follicle stem cells to the wound site involves polarized assembly of microtubules caused by local inhibition of phosphorylation of the microtubule-actin crosslinker protein ACF-7 by GSK3b at the leading edge (Wu et al. 2011). At the wound site, hair follicle–derived cells help epidermal stem cells to repopulate the wound. Hair follicle progeny adopt epidermal identities, and a subset is even reprogrammed to become basal epidermal stem cells (Levy et al. 2007, Snippert et al. 2010a). This heterologous activation occurs even when only superficial epidermal cells are damaged (Ito et al. 2005), which suggests that the injury signal either diffuses through the dermis or mechanically propagates through the epithelium (continuous with the outer layer of the hair follicle). Hair follicle–derived cells can represent up to 40% of cells in the repopulated wound. Without their heterologous contribution, healing is slowed and requires epidermal stem cell activation from a region four times greater in surface area (Langton et al. 2008), pointing to feedback mechanisms that locally link niche activation to required cellular output.

Although wound proximity is a major determinant in stem cell activation, it is not necessarily the sole determinant. In tissues such as interfollicular epidermis, small intestine, and bronchioles, the stem cell population that drives constitutive renewal is distinct from the population that drives repair. Instead, small populations of reserve or facultative stem cells, spatially segregated and with distinct biomarker profiles, are specifically mobilized by injury (Greco & Guo 2010, Li & Clevers 2010). At least in small intestine and bronchioles, reserve stem cells resist damage better than constitutive stem cells, which are killed by treatments like irradiation (intestine) or naphthalene (lung) (Patt & Quastler 1963, Rawlins et al. 2009, Yan et al. 2012). Less active during renewal

(Buczacki et al. 2013, Zhu et al. 2013), a single reserve intestinal stem cell generates multiple contiguous crypt-villus units over the course of one repair event (Yan et al. 2012). Reserve stem cells inhabit niches that are either directly adjacent to or overlapping with constitutive stem cells, and interconversion between the two populations can occur (Buczacki et al. 2013, Giangreco et al. 2009, Mascré et al. 2012, Rawlins et al. 2009, Tian et al. 2011, Yan et al. 2012). This juxtaposition of constitutive and reserve niches, and the fuzzy boundary between the two, adds a spatial dimension to the question of how injury selectively activates the reserve population. Is physical segregation required for the reserve population to maintain itself? Does sudden depletion of stem cells from the constitutive niche activate the adjacent, reserve population? Does the microenvironment of the reserve niche enable its stem cells to resist damage? The answers to these questions await future investigation.

Regeneration of Stem Cell–Niche Units

When tissue damage destroys not only differentiated cells but also stem cell–niche units, the repair response can include the formation of new stem cell–niche units. Here, the complexity of architectural niche restoration may be inversely related to the dynamism of niches during renewal. For ad hoc niches in epithelia like the fly midgut and mammalian trachea, new niches arise simply through the generation of new epithelial cells. However, where the stem cells that populate these niches come from—whether symmetric divisions of stem cells that escaped damage, reprogramming of heterologous stem cells, or dedifferentiation of terminal cell types—and how they find or perhaps make their niches have not been explicitly examined. In the mammalian intestine, once a wound has become reepithelialized, new crypts form through invagination of the epithelial monolayer, reminiscent of crypt fission during postnatal growth and renewal (Miyoshi et al. 2012). The repopulation dynamics of stem cells in new crypts is not currently known. Finally, in skin, where hair follicles are normally fixed for life, punch wounds that penetrate the dermis induce the formation of new, functioning hair follicles—but only when the wounded area exceeds a critical diameter (Ito et al. 2007). These new follicles are formed after epithelialization, from epidermal cells in situ, rather than through migration of preexisting hair follicle cells from other regions (Ito et al. 2007). Interestingly, postinjury follicle formation recapitulates embryonic follicle development, suggesting that injury resets the epithelium to a naïve developmental state. The regeneration of niches seen in intestine and epidermis suggests that simply producing more progeny from a reduced population of niches may be inadequate for long-term maintenance in the face of cell turnover. Instead, specific mechanisms exist to restore the density and spatial distribution of niches to maintain organ homeostasis.

STEM CELL AND NICHE POPULATIONS DURING TISSUE RESIZING

Tissue renewal and repair are homeostatic processes, in which stem cell populations act to preserve a constant organ size. Although homeostasis is a common tissue state, it is not the only mode that normal tissues can exhibit. Many mature organs naturally fluctuate in size in response to fluctuations in functional workload (Meyers & Bull 2002). Epithelia that exhibit adaptive resizing include the vertebrate GI tract, *Drosophila* midgut, urinary bladder, and mammary gland (Goss 1964, O'Brien et al. 2011, Piersma & Lindström 1997, Van Keymeulen et al. 2011). Described as an "economy of nature" (Johnson et al. 1990), adaptive resizing allows organs to tune their functional capacity to different levels of physiologic demand (Piersma & Lindström 1997). This demand-driven growth and shrinkage breaks the allometry of the body plan that was established

during development and, unlike developmental growth, can be reversed and repeated throughout adult life.

The cellular dynamics of adaptive resizing make resizing a compelling phenomenon for examining the spatial coordination of stem cells and niches. Classical studies suggest that, in at least some cases, adaptive growth and shrinkage involves changes in total cell number and stem cell activity (Goss 1964). We are just beginning to understand how physiologic inputs can alter the equilibrium between cell production and loss to alter organ size. Open questions include how information about functional demand is communicated to stem cell–niche units, how organ size and stem cell numbers are calibrated to meet different workloads, and whether particular organ subregions—such as those that bear the brunt of physiological activity—or particular populations of stem cells selectively respond to resizing signals. In many tissues, endocrine hormones convey information to stem cells about the body's physiological state (reviewed in Gancz & Gilboa 2013, in this volume). Below, we examine how heightened workload elicits tissue-level responses from stem cells and niches during adaptive growth of three epithelial organs: small intestine, *Drosophila* midgut, and mammary gland.

Altering Organ-Wide Niche Capacity

The vertebrate intestine, arguably the best-understood epithelial model for adaptive resizing, exhibits size fluctuations that are highly sensitive to changes in dietary load. This sensitivity may reflect the intestine's high cellular and digestive demands, which require ten times more energy per unit mass than does the body as a whole (Johnson et al. 1990). In laboratory rodents, cycles of starvation and refeeding cause up to threefold changes in intestinal mass, disproportionately larger than changes in overall body mass (Dunel-Erb et al. 2001). In the wild, where diverse animal species undergo regular periods of feast and famine, extreme fluctuations in intestinal size are an ordinary occurrence (Carey 1990, Secor & Diamond 1998).

Changes in overall intestinal mass reflect changes in the cell numbers of individual crypts and villi. Prolonged withdrawal of food causes the number of cells per crypt to diminish by ~20% (Aldewachi et al. 1975, Altmann 1972). A similar reduction occurs even when the organism's metabolic needs are met through intravenous nutrition, implying that food must be ingested for the intestine to perceive workload (Li et al. 1982, Menge et al. 1975). At the cellular level, crypts are smaller owing to the culling of transit-amplifying cells (Yilmaz et al. 2012); interestingly, numbers of Lgr5-expressing stem cells and Paneth cells increase slightly, perhaps to enable rapid regrowth upon refeeding (Yilmaz et al. 2012). The combined effect of fewer proliferating cells, longer cell cycle times, and slowed migration of cells from crypt to villus results in an ~40% decrease in the number of cells per villus (Aldewachi et al. 1975, Brown et al. 1963). As overall numbers of crypts and villi are not altered (Clarke 1972), the reduced cell numbers of individual crypts and villi are likely the chief cause of intestinal shrinkage when food is scarce.

When food availability is not limiting, how are the sizes of individual crypts and villi determined? A key factor appears to be the total digestive capacity needed to meet the organism's caloric needs. When 30–70% of the small intestine's length has been surgically removed, the organ compensates not by regenerating the missing part but by augmenting the functional throughput of the portion that remains. Thus, crypts in shortened guts acquire ~40–50% more cells than controls with the same dietary load (Cheng et al. 1986b, Hanson et al. 1977). In addition, the rate of crypt fission increases by 33%, resulting in a 30% increase in crypt density and a 35% increase in the number of crypts per jejunal villus (Cheng et al. 1986b). This heightened generative capacity produces a supranormal, 40–80% increase in the number of cells per villus. The expansion of crypts and villi restores the mass of the shortened intestine—and presumably also its digestive

throughput—to that of nonresected controls. Altogether, the fact that both limited nutrients and limited nutrient delivery trigger resizing argues that intestinal input and output are integrated across the expanse of the tissue to cooperatively determine overall organ size.

Scaling and Redistribution of Stem Cell Populations During Resizing

Despite the prevalence and importance of adaptive organ resizing, the mechanisms that underlie it remain unknown. We recently investigated these mechanisms by using the *Drosophila* posterior midgut, whose stem cell lineages and digestive physiology make it a reductionist version of the mammalian small intestine. We found that the midgut, like the small intestine, exhibits a reversible and repeatable growth response to increased dietary load (O'Brien et al. 2011), with total cell numbers becoming approximately fourfold greater in well-fed animals compared with fasted animals. Interestingly, molecular regulation of midgut adaptive growth is controlled by the midgut itself: Feeding induces midgut visceral muscle to secrete an insulin-like peptide, dILP3, in advance of systemic insulin upregulation. This local production of dILP3 is both necessary and sufficient for the growth response.

Adaptive resizing in the fly midgut is directly driven by stem cells, and the midgut's relative simplicity allows a precise analysis of dynamics of the stem cell population. During growth, the kinetics of stem cell divisions change dramatically: Over four days, an average stem cell in a growing gut gives rise to 15–17 new progeny, whereas in a nongrowing gut it gives rise to only 1–3 (O'Brien et al. 2011). Importantly, stem cells also exhibit a switch in division fate outcomes. We directly measured the frequency of symmetric and asymmetric stem cell divisions by differentially labeling the two daughters of a stem cell division and tracking their fates (Yu et al. 2009). When the midgut is at cellular equilibrium—either homeostatically fed or homeostatically fasted—70–80% of stem cell divisions are asymmetric (de Navascués et al. 2012, O'Brien et al. 2011), producing primarily differentiated daughters. But, at the peak of growth, ∼70% of stem cell divisions are instead symmetric (O'Brien et al. 2011), leading to a fourfold expansion of the stem cell population within the tissue. This burst of new stem cell production is reminiscent of postnatal murine intestinal crypts, where "bang-bang" proliferation jump-starts crypt formation through initial symmetric divisions that enlarge the stem cell pool (Itzkovitz et al. 2012). At the whole-midgut level, these symmetric divisions have vital significance: They maintain a constant proportion of one stem cell for every approximately four differentiated cells, even as absolute numbers of cells fluctuate (O'Brien et al. 2011). Thus, the stem cell population scales with the size of the tissue, keeping itself at ∼20% of the total population. Whereas the mammalian intestine adjusts the size of its transit-amplifying population for adaptive resizing (Yilmaz et al. 2012), the fly midgut, which lacks transit-amplifying cells, accomplishes a similar feat by adjusting its stem cell population. Stem cell scaling may reflect an intrinsic limit in the numbers or spatial expanse of differentiated cells that a single stem cell can support; as the tissue grows, a larger stem cell pool would decrease the renewal burden of each individual stem cell.

Stem cell scaling has also been reported during adaptive resizing of the mammary gland, although whether this scaling is precisely isometric, as in the midgut, is currently unclear. The mammary gland dramatically grows and shrinks to fit changing needs for milk production throughout pregnancy, lactation, and involution. Mammary tubules comprise two adjacent cell layers separated by a basement membrane. The inner, luminal epithelium synthesizes milk, and the outer myoepithelium contracts to expel milk. Each epithelium houses its own stem cell population, and the two populations coordinately activate to expand the mammary tubular network during pregnancy (Van Keymeulen et al. 2011). Coordination is achieved, at least in part, because luminal cells secrete cytokines that activate myoepithelial stem cell proliferation (Asselin-Labat et al.

2010, Joshi et al. 2010). A 10–15-fold increase in numbers of myoepithelial stem cells occurs during pregnancy; after involution, myoepithelial stem cell numbers return to prepregnancy levels (Asselin-Labat et al. 2010, Joshi et al. 2010). Interestingly, luminal stem cells do not appear to undergo scaling, even though their proliferation is also activated during growth. Future work to understand this difference between the myoepithelial and luminal stem cell populations will shed light on the functional significance of stem cell scaling.

How does such striking scaling behavior emerge? These mechanisms await further investigation; however, some clues might be suggested by the relationship between fly midgut stem cells and their ad hoc niche. One possible scenario would link epithelial packing geometry to niche availability and division fate outcomes. Even as the stem cell population expands during growth, the scattered distribution seen in homeostasis is upheld; regions that lack stem cells are not observed in the enlarged tissue (O'Brien et al. 2011). Scattering is maintained, at least in part, because after a symmetric division, the two newly generated stem cells often lose contact and are physically separated by a distance of one or more differentiated cells (O'Brien et al. 2011). It is not yet known whether the geometry of the ad hoc niche might accommodate only a single stem cell, causing the other to be squeezed out, or alternately if stem cells actively crawl apart. Regardless, the inherent properties of the ad hoc niche create the potential for autoregulation of stem cell scaling and dispersal during adaptive growth.

STEM CELL TERRITORIES: SPATIAL UNITS OF TISSUE-LEVEL COORDINATION?

This review has discussed how a dispersed population of stem cell–niche units is collectively regulated within an epithelium during renewal, repair, and resizing. Although our knowledge of the molecular and cellular mechanisms that coordinate stem cell–niche units is still in its infancy, it is evident that many of these higher order processes are inherently spatial. To advance further, we will need to develop a clearer understanding of these mechanisms' spatial ranges of action—a physical mapping of the dynamic relationship between cell loss and cell production throughout a tissue, and how this relationship adjusts to changing tissue needs.

The individual and collective responses of stem cell–niche units to spatially localized perturbations in differentiated cells are fundamental to organ function in vivo, and yet very little is known. Recent technical developments in genetic lineage tracing, live tissue imaging, and organoid culture will facilitate future investigation (Rompolas et al. 2012, Sato et al. 2011, Van Keymeulen & Blanpain 2012). As a framework for such inquiry, we posit that the notion of stem cell territories could prove useful for considering the basis of tissue-wide coordination. We define the stem cell territory as the spatial field in an organ that is under the surveillance of a particular stem cell–niche unit (**Figure 7**). Functionally speaking, a stem cell–niche unit would respond to cellular events within its territory and be indifferent to events outside its territory. As we envision it, tissue-wide coordination of stem-based renewal, repair, and resizing would occur at the level of territories. Based on the nature of higher order control mechanisms discussed above, the borders of individual territories are likely to (*a*) be dynamic rather than fixed, (*b*) overlap with one another, and (*c*) reflect graded probabilities rather than absolute predictions of stem cell behaviors. The mechanisms that delimit territory boundaries would be a means to coordinate the activity of dispersed stem cell–niche units. Interactions between territories could guide collective stem cell–niche behaviors to achieve tissue-wide objectives, such as steady-state cellular equilibrium or adaptive growth.

The tissue organization of epithelia is an appealing entry point to probe how stem cell territories are defined. The coherent, tiled array of cells in planar epithelial sheets creates an architecture in which distance in the cellular plane is a primary component. Moreover, the hallmark

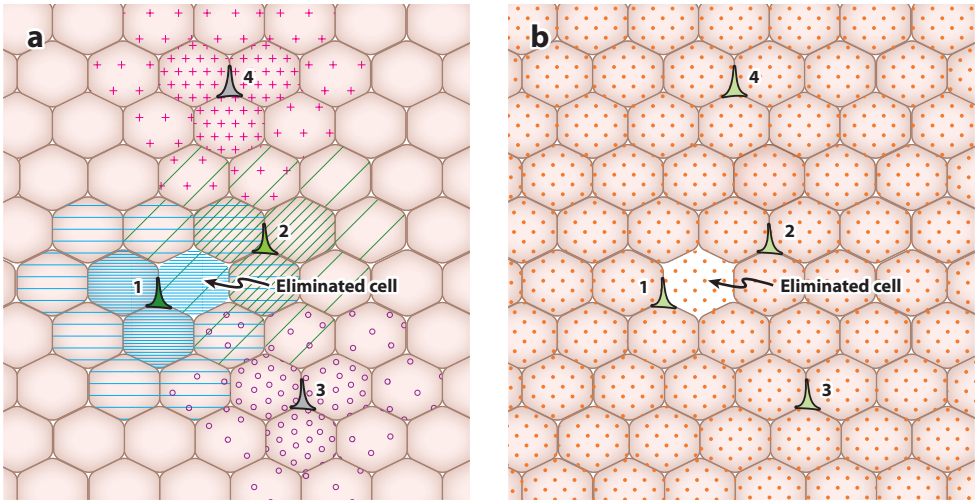


Figure 7

Contrasting models of (*a*) spatially biased and (*b*) spatially unbiased stem cell responses to a localized perturbation in the epithelium. (*a*) Stem cell territories. The spatial relationship between a given stem cell and a localized perturbation determines the probability that the stem cell will respond to the perturbation. The map of this probability distribution defines the territory of that particular stem cell. The cartoon depicts one possible such model. Colored patterns represent territories of stem cells (*triangles*) within the epithelium (*hexagons*). The density of each pattern reflects the probability of a response from the central stem cell. Following elimination of a differentiated cell (indicated by labeling), the adjacent stem cell 1 (*dark green triangle*) will divide with high probability. Stem cell 2 (*light green triangle*), which is near but not adjacent, will divide with lower probability. Stem cells 3 and 4 (*gray triangles*), which are distant, will be unresponsive. Numbers of cells shown in territories and their uniformity are currently speculative. Territory size and shape likely vary with tissue type; systemic factors, such as organismal age; and the nature of the perturbation, such as acute injury, stochastic loss, or stochastic overcrowding. (*b*) Spatially unbiased response. Instead of being determined by territories, the probability that a given stem cell (*pale green triangle*) responds to elimination of a differentiated cell is independent of the stem cell's proximity to the lost cell.

epithelial features through which these signals are transduced—including apicobasal cell polarity, lateral junctions for cell-cell adhesion and barrier formation, and basal adhesion to the basement membrane—extend throughout stem cell–niche units themselves, making these units integral components of the planar cellular network. In the above sections, we discussed examples of both secreted, diffusible factors and juxtacrine, cell-cell relays that transduce information to and from stem cell–niche units in the context of the epithelial plane. These mechanisms all share the property that they propagate through space, as opposed to being spatially isotropic or cell autonomous. They can therefore convey distance and directionality, allowing a gradient of response that could define territory boundaries. Thus, despite the diversity of mechanisms that regulate epithelial stem cell–niche units, many could yield an outcome akin to stem cell territories.

Is there currently evidence for the existence of stem cell territories? The localized activation of stem cell–niche units following acute injury could be viewed as the response of stem cells whose territories encompass the wound site. Consistent with the idea that territories delimit the injury response zone, tissue-wide repair of extensive damage, such as that caused by chemical or pathogenic agents, resembles a composite of many local repair events proceeding simultaneously. However, explicit inquiry into how injury response zones are established in stem-based tissues remains to be performed. During renewal and resizing, whether analogous stem cell territories

define spatial patterns of stem cell–niche activity is an open question. For all three tissue states, an informative first step would be to map the boundaries of putative stem cell territories. Direct evaluation of territory boundaries could be attained through real-time, tissue-wide monitoring of stem cell divisions and differentiated cell loss. If stem cell territories do exist, the prediction would be that the cellular output of a given stem cell–niche unit is biased by the dynamics of differentiated cells and other stem cell–niche units nearby, with the spatial extent of this influence forming the territory boundary. Such a technical feat is at the moment beyond the reach of in vivo systems, although organoid culture may represent a tractable alternative.

What might stem cell territories look like? At this point, we can only speculate; although much stem cell–niche regulation is spatial, the physical distances involved are virtually unknown. Nonetheless, many intriguing questions arise: Are the sizes and shapes of territories uniform or uneven, both within a particular epithelium and between different types of epithelia? If the latter, does territory size or shape correlate with tissue features such as niche density, turnover rate, or stem cell–division frequency? Are individual territories stable or changeable over time? Do territories evolve when the tissue switches between renewal, repair, and resizing? Finally, is there a relationship between stem cell lineages and territory boundaries? The answers to these questions hold profound implications for how tissue-wide coordination of stem cells and niches ensures lifelong optimization of organ form and function.

SUMMARY POINTS

1. Epithelial tissues contain a distributed population of niches whose activity must be spatially and quantitatively coordinated for proper organ function.
2. Niche numbers and spacing are initially set through a spectrum of mechanisms, from fixed prepatterning to ad hoc improvisation. These mechanisms influence their flexibility in adulthood.
3. Both diffusible and propagative mechanisms can enable communication among stem cell–niche units and between differentiated cells and niches to regulate stem cell behavior.
4. Tissue damage elicits a spatially graded stem cell response that involves mechanisms distinct from those that operate during tissue renewal.
5. Some epithelia can dynamically alter their stem cell populations in response to physiological cues, creating tissue growth or shrinkage. This adaptive resizing may provide insight into mechanisms of tissue–wide stem cell coordination.
6. Certain epithelia create new niches during repair or adaptive growth, which suggests the existence of feedback mechanisms that monitor and maintain niche spacing. A minimum density of stem cell–niche units may be required to support the tissue.
7. The concept of stem cell territories may prove useful in considering how dispersed populations of stem cell–niche units collectively promote lifelong optimization of organ form and function.

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