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Stem Cell Fate in Proliferating Tissues: Equal Odds in a Game of Chance

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Quantitative lineage tracing reveals stem cell fate in vivo. A new study in a recent issue of *Cell* shows intestinal crypt stem cells are functionally equivalent, with equal odds of differentiation. Differentiating stem cells are replaced by the symmetric division of adjacent stem cells.

Many adult tissues have to work hard to stand still. Differentiated cells are lost rapidly from tissues, such as the intestinal epithelium, epidermis, and testis, and intense proliferation is required to maintain homeostasis. The balance between loss and production is critical: a small excess of cell loss will result in progressive tissue failure, while excessive cell production is a hallmark of cancer. Understanding how stem and progenitor cell behavior is tuned to achieve the exquisite balance required for lifelong tissue maintenance is a central issue in stem cell biology.

How might cell production be organized in homeostasis? A long-held hypothesis is that long-lived, self-renewing tissue stem cells divide asymmetrically to generate a stem cell and a "transit-amplifying" cell, which in turn divides a limited number of times after which its progeny differentiate. In this "invariant asymmetry" model, stem cell behavior is fixed: each and every stem cell generates one stem cell and one differentiating daughter. An alternative paradigm, "population asymmetry," proposes a pool of stem cells with the potential to divide to generate either two stem cells, two differentiating cells, or one of each. If the probability of differentiation is matched by that of a self-duplicating stem cell division, homeostasis is achieved (Watt and Hogan, 2000).

In an innovative study in a recent issue of *Cell*, Snippert et al. (2010) apply the powerful approach of quantitative cell-fate analysis to investigate how stem cells support intestinal epithelial homeostasis. The epithelium is organized into pits called crypts that feed differentiated cells onto projections, called villi, from which they are shed. Genetic lineage tracing has demonstrated that cells highly expressing the marker Lgr5 contribute to all intestinal lineages throughout life, arguing that this population includes stem cells (Barker et al., 2007). Each crypt contains about 12–16 Lgr5^{hi} cells, found near the crypt base, a subset of which coexpresses a second candidate stem cell marker, BMI1 (Sangiorgi and Capecchi, 2008). The majority of mitotic spindles in this region are oriented perpendicular to the crypt basement membrane and may partition newly synthesized DNA asymmetrically. According to the "immortal strand" hypothesis, these findings indicate crypt stem cells divide asymmetrically (Quyn et al., 2010). These results raise two questions: are all Lgr5^{hi} cells functionally equivalent stem cells and do they indeed divide with "invariant asymmetry"?

To resolve these issues, Snippert et al. used a "confetti" mouse, which allowed inducible genetic labeling of Lgr5^{hi} cells and their progeny in multiple colors at clonal frequency. When all the Lgr5^{hi} cells in a clone differentiated, the clone was lost by migration. Thus, as time went on, the number of clones per crypt fell progressively. In the persisting clones, the number of Lgr5^{hi} cells increased progressively, the labeled cells expanding around the circumference of the crypt in a continuous band. This indicates that when an Lgr5^{hi} cell leaves the crypt through differentiation, it is replaced by the division of an adjacent Lgr5^{hi} cell. The data also reveal that the rate at which Lgr5^{hi} cells divide, once per day, is almost the same as the rate at which differentiated cells are replaced by division. It follows that almost all Lgr5^{hi} cell divisions generate two Lgr5^{hi} daughters and, hence, that spindle orientation and the location of newly synthesized DNA strands do not correlate with cell fate (Quyn et al., 2010).

In a second set of experiments, labeling was induced at high frequency; the number of clones/crypt decreased progressively until eventually crypts became monoclonal. In the past, such observations have been used to argue that each crypt is supported by a "hierarchy" comprising a single long-lived, slowly cycling stem cell and shorter-lived progenitors: the crypt clones which only last a short time derive from the labeled progenitors and only clones rooted in the stem cell persist long term (Winton and Ponder, 1990). However, there is an alternative possibility, that all Lgr5^{hi} cells are functionally identical stem cells with stochastic fate: at long time points one clone takes over the crypt by *chance*. This process is analogous to "neutral drift" in population genetics, where an allele with no survival advantage becomes predominant in a population. Snippert et al. showed that their clone fate data set bears the mathematical signature of "neutral drift": the stochastic model has an excellent *quantitative* fit to the entire data set, a conclusion supported by an independent study using a different genetic-labeling system (Lopez-Garcia et al., 2010).

The picture of stem cell behavior in the intestinal crypt is startling in its simplicity (Figure 1). All Lgr5^{hi} stem cells are functionally equivalent with the same "life chances." In a day, a given stem cell has a 50% chance of differentiating and being lost from the crypt base. The differentiating cells are replaced by the division of a neighboring stem cell into two stem cells, so the pool of crypt stem cells exhibit population asymmetry.

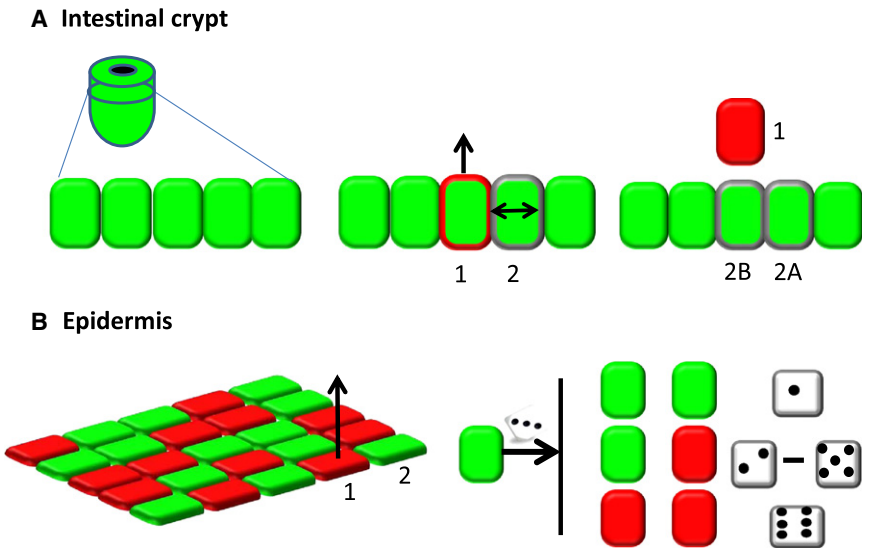

Figure 1. Stem Cell Fate in Homeostatic Tissues
(A) Intestinal epithelium: the base of the intestinal crypt (inset) contains stem cells (green) interspersed between postmitotic Paneth cells (not shown). The differentiation of stem cell 1 and its migration out of the crypt is linked to the division of a neighboring stem cell, 2, into two stem cell daughters, 2A and 2B. Every stem cell has a 50% chance of differentiating per day.
(B) Epidermis: progenitor (green) and differentiating, postmitotic (red) cells lie scattered over the proliferative basal cell layer. The migration of postmitotic cell 1 is linked to the division of nearby progenitor cell 2. The three possible outcomes of this cell division, and their associated probabilities, are shown. Note that the probabilities of both daughters remaining as progenitors or both becoming postmitotic are equal.

How do these findings compare with other tissues? Quantitative cell-fate tracking has shown that murine testis germline stem cells are also functionally equivalent. As in the intestine, when a neighboring stem cell commits to terminal differentiation, an adjacent stem cell divides symmetrically, generating two stem cell daughters (Klein et al., 2010b).

Clone fate in murine interfollicular epidermis also exhibits "neutral drift." Epidermis is maintained by a single population of progenitor cells with balanced stochastic fate, but in contrast to intestine and testis, daughter cells may both be progenitors, both may differentiate, or one may remain as a progenitor while the other differentiates (Clayton et al., 2007). The probabilities of these three outcomes are balanced to achieve homeostasis across the progenitor population (Figure 1). Unlike stem cells in intestine and testis that respond to the loss of a neighbor, epidermal progenitor fate appears cell autonomous. Thus, epidermal progenitors cannot generate excess cells in response to injury without either mobilizing a separate stem cell population in the hair follicles or altering their behavior. In contrast, the rules followed by intestinal and testis stem cells enable them to both maintain and regenerate their respective tissues.

Intestinal epithelium, male germ line stem cells, and interfollicular epidermis have been held up as textbook examples of tissues maintained by asymmetric division of long-lived, slowly cycling stem cells. Quantitative lineage tracing has rigorously excluded this model in all three cases and revealed simple but distinct patterns of cell fate, each of which bears the signature of "neutral drift." Excitingly, the experimental approaches and analytical methods used by Snippert et al. are applicable to understanding homeostasis in many other organs and organisms and have the potential to disclose how cell fate changes in response to injury, aging, mutation, and drug treatment (Klein et al., 2010a).

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