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Universal patterns of stem cell fate in cycling adult tissues

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Summary

In cycling tissues that exhibit high turnover, tissue maintenance and repair are coordinated by stem cells. But, how frequently stem cells are replaced following differentiation, aging or injury remains unclear. By drawing together the results of recent lineage-tracing studies, we propose that tissue stem cells are routinely lost and replaced in a stochastic manner. We show that stem cell replacement leads to neutral competition between clones, resulting in two characteristic and recurring patterns of clone fate dynamics, which provide a unifying framework for interpreting clone fate data and for measuring rates of stem cell loss and replacement in vivo. Thus, we challenge the concept of the stem cell as an immortal, slow-cycling, asymmetrically dividing cell.

Key words: Stem cells, Stochastic cell fate, Tissue homeostasis

Introduction

In adults, the maintenance and repair of tissue is coordinated by stem cells, which maintain the ability to self-renew throughout adult life. Underlying their extraordinary proliferative capacity is a fundamental asymmetry in stem cell fate: on average, exactly half of all stem cell progeny must differentiate to maintain homeostasis whereas the remaining half must maintain their stem cell identity (Watt and Hogan, 2000). In actively cycling (or renewing) tissues (Leblond, 1981), the proliferative burden on stem cells is considerable.

For the vast majority of adult tissues, it remains unclear how stem cells succeed in maintaining a precise balance between proliferation and differentiation in steady state. To explain their long-term viability, it has been argued that tissue stem cells are maintained in a long-lived quiescent state, with the majority of divisions in a tissue supported by differentiating progenitor cells that ultimately exit the cell cycle and are replaced by stem cell progeny (Cairns, 1975; Cotsarelis et al., 1990; Lajtha, 1979; Potten and Loeffler, 1990). An alternative view, promoted in early studies, posits that all cycling cells in tissues have the capacity to self-renew (Bullough, 1962), but the fate of individual cells is unpredictable (Elgjo, 1969; Epstein and Maibach, 1965; Leblond et al., 1964; Marques-Pereira and Leblond, 1965). A recent series of papers have now provided evidence that again calls into question the paradigm of tissue maintenance. These papers found that, in the mouse epidermis (Clayton et al., 2007; Doupe et al., 2010), gut (Lopez-Garcia et al., 2010; Snippert et al., 2010) and male germ line (Klein et al., 2010b), stem cells divide frequently and that, rather than being long-lived, they are lost and replaced by neighbouring stem cells in a manner that can be accurately described by a stochastic process.

With stochastic patterns of stem cell self-renewal found in several cycling tissues, it is tempting to speculate that variability in cell fate might have a far more general role in adult tissue homeostasis, and perhaps in development and organ growth. Indeed, some evidence indicates that developing tissues can take advantage of transcriptional noise to generate stochastic fate outcomes (Losick and Desplan, 2008). In *Drosophila* proneural clusters, for example, stochastic fate arises from gene expression heterogeneity in the Delta-Notch signalling pathway, which ensures that just one proneural cell commits to a neuroblast fate during the development of the nervous system (Campos-Ortega, 1997). During retina development, cone photoreceptors also differentiate in a stochastic manner to ensure a uniform random distribution of red and green photoreceptors (Smallwood et al., 2002; Wernet et al., 2006). Similarly, the cell fate choice of olfactory neurons is stochastic to ensure a uniform representation of different receptor types (Mombaerts, 2004). However, at present, little is understood about the mechanisms that drive stochastic stem cell fate in adult tissue homeostasis.

In this Hypothesis article, we draw together recent experimental evidence that supports a role for stochastic fate choice in cycling adult tissues, in particular the choice between stem cell proliferation and differentiation. Using theoretical analysis, we argue that, where such stochasticity appears, it is limited to just two possible classes of behaviour that lead to universal patterns of stem cell fate across different tissues. As we shall show, these patterns can be seen clearly in quantitative lineage-tracing experiments. Based on the current evidence, we predict that stochastic stem cell fate will prove to be ubiquitous in cycling tissues. Finally, we propose mechanisms that might be responsible for generating stochastic stem cell fate outcomes.

Models of stem cell self-renewal

It is often held that tissue stem cells represent a rare population of slow-cycling cells, which divide asymmetrically to produce differentiating progeny (Cotsarelis et al., 1990; Potten and Loeffler, 1990). This pattern of self-renewal (Fig. 1A) provides a mechanism for protecting stem cells from damage and loss throughout adult life. Clear evidence for asymmetric stem cell divisions is found in invertebrates, with the best-studied examples found in the developing nematode worm *Caenorhabditis elegans* and in the fruit fly *Drosophila* (for reviews, see Gonczy, 2008; Knoblich, 2008). Division asymmetry could arise through polarisation of the stem cell prior to division, or through cues from the local microenvironment; for example, by allowing only one daughter cell to maintain contact with the stem cell niche (Li and Xie, 2005; Voog and Jones, 2010). The latter mechanism might not lead to invariant asymmetry, however, if both daughter cells receive similar environmental cues. Asymmetric division also often occurs in mammalian adult tissues, such as in haematopoietic cells

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(Schroeder, 2007; Wu et al., 2007) and muscle satellite stem cells (Kuang et al., 2007). However, in all cases, both asymmetric and symmetric divisions can be observed, and evidence for invariant asymmetric stem cell fate is lacking. In haematopoiesis, experiments looking for evidence of asymmetric segregation of chromosomes, for example, suggest that no such asymmetry occurs (Kiel et al., 2007).

Another mode of self-renewal can occur through 'population asymmetry' (Fig. 1B), whereby some stem cells differentiate and are lost, whereas others divide symmetrically into two stem cells to replace them (Morrison and Kimble, 2006; Watt and Hogan, 2000). Although the average cell fate outcome is the same as in the case of division asymmetry, the regulation required for this mode of self-renewal can be strikingly different. The idea that tissues might self-renew through population asymmetry goes back several decades to the pioneering works of McCulloch, Till and Siminovitch on transplantation in haematopoiesis (Till et al., 1964), and of Charles Phillipe Leblond on epithelial turnover. By tracking [^{3}H]-thymidine-labelled cells in the intestinal crypt, Leblond concluded that all cell types in the crypt arise from a homogeneous population of crypt base columnar cells that either divide or differentiate (Cheng and Leblond, 1974). Similar analysis of labelled cells in the mouse oesophagus suggested that all basal layer oesophageal cells have the capacity to exit the tissue at random and independently of the fate of their sister cells (Marques-Pereira and Leblond, 1965).

Until recently, the identification of the mode of self-renewal in vivo in mammals has not been possible, owing to the challenge of identifying stem cells within tissues and tracking their fate. Yet, the study of patterns of stem cell fate has important implications for stem cell regulation: if some stem cells differentiate while others multiply, what then determines the survival and proliferative potential of each stem cell? Is stem cell fate dependent on transient or hereditary factors? How is the entire stem cell population kept constant, such that only one stem cell multiplies for each stem cell that is lost? As we describe below, the recent development of lineage-tracing techniques has opened up the possibility of characterising the fate of long-lived, self-renewing clones in a manner that provides a clear indication of which of these two patterns of stem cell fate occurs in different tissues.

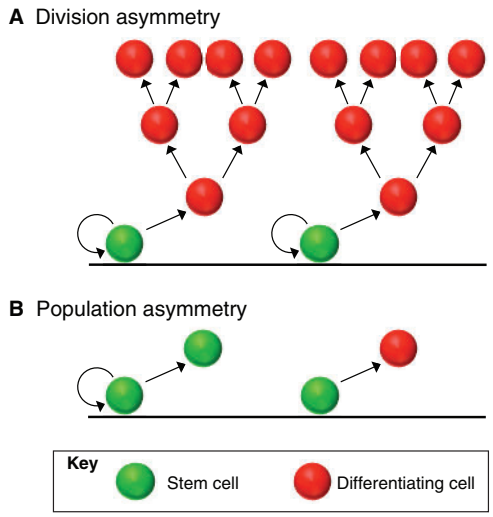


Fig. 1. Models of tissue self-renewal. (**A**) In division asymmetry, slow-cycling stem cells (green) divide to give rise to one daughter stem cell (in the process of self-renewal, indicated by circular arrow) and one differentiating cell (red) that may undergo a limited number of rounds of cell division (as drawn) or it may exit cycle. (**B**) In population asymmetry, half of the stem cells differentiate, and hence are lost, and the remainder divide symmetrically to give rise to stem cells that can replace the lost cells.

Analysing stem cell renewal by lineage-tracing: spermatogenesis

An instructive system in which to study the pattern of stem cell self-renewal is the mouse male germ line. Spermatogenesis occurs in the testes along seminiferous tubules, with germline stem cells residing on the basement (outer) membrane of the tubules in a vasculature-associated niche (Yoshida et al., 2007) (Fig. 2A). Upon division, these stem cells give rise to spermatogonia that might subsequently produce up to 4000 mature spermatids within just five weeks of spermatogonial differentiation (de Rooij and Russell, 2000; Russell et al., 1990). Thus, although the spermatogonial system has a specialised physiology and ontogeny, it is nonetheless representative of mammalian stem cell systems with its considerable proliferative burden, a differentiating transit-amplifying compartment and a small stem cell population.

Perhaps more than in somatic tissues, it is expected that self-renewal of germline stem cells should be carefully regulated to protect against mutations and proliferative exhaustion (Cairns, 1975; Rubin, 2002). In a recent study, this hypothesis was examined using long-term clonal labelling of spermatogonia, which revealed the size of long-lived clones over a period of up to 14 months (Nakagawa et al., 2007). If germline stem cells self-renew through infrequent asymmetric division, one would predict that each stem cell would support a stable patch of differentiating spermatocytes and spermatids throughout adult life.

The results of the long-term spermatogonial clone fate labelling study were, therefore, surprising. Rather than observing a stable population of long-lived clones of typical size, it was discovered that the male germ line was maintained by ever-fewer clones of ever-increasing size, with increasingly heterogeneous sizes (Fig. 2B-E). This behaviour occurred independently of the age of the mice at the start of the experiment, indicating that it is not a transient feature of young mice, but rather occurs continuously during homeostasis.

One hypothesis that could explain the variety in clone size and persistence is that the experiment labels various members of a hierarchy of stem and progenitor cells with decreasing proliferative potential. In this scenario, the few cells at the top of the hierarchy could give rise to ever-expanding clones that gradually replace the rest of the cell population. However, this hypothesis does not explain why long-lived clones should become increasingly disparate in size over time (Fig. 2E), nor why stem cells fail to generate a clone of stable size within 14 months, which is a considerable fraction of the lifetime of the organism. Furthermore, throughout the 14-month post-labelling period, the size of the smallest clones remained constant (Fig. 2E), providing no indication that persisting clones are enriched with stem cells of high proliferative potential. It therefore seems that, even if it exists, a stem cell hierarchy provides no simple explanation for the observed clonal dynamics. In that case, what leads to the increasingly diverse clone sizes and to their ongoing loss? To answer this question, one can search for clues in the quantitative clone fate data.

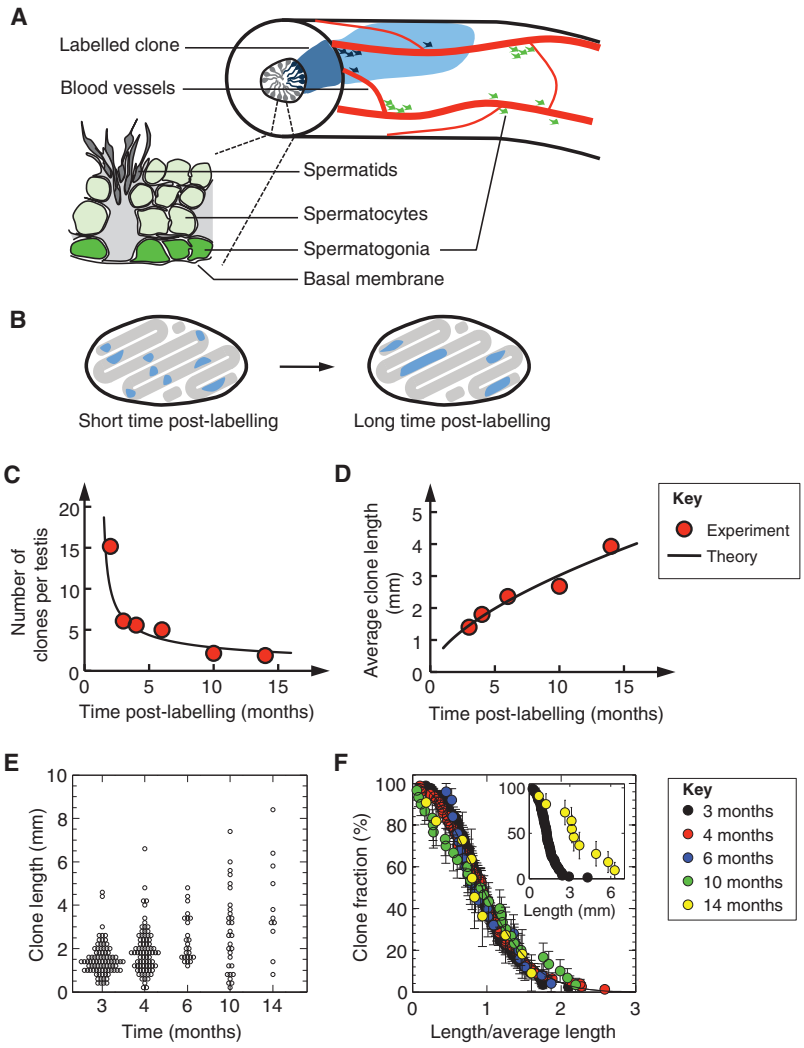


Fig. 2. Clonal analysis in the mouse male germ line. (**A**) A schematic of a mouse seminiferous tubule (ST), in side view and cross section, showing blood vessels in red. As shown in the cross section, stem cells (green) reside along the basal (outer) membrane of the STs in vasculature-associated niches. After differentiation, they undergo multiple rounds of division to form differentiated spermatids that collect in the lumen. Clonal labelling can be used to follow the fate of labelled (depicted here in dark blue) spermatogonial stem cells. If stem cells self-renew through infrequent asymmetric division, each stem cell should support a stable and long-lived labelled clone (light blue) of differentiating spermatocytes and spermatids that spans the full thickness of the tubules throughout adult life. (**B**) Schematic of a testis showing STs (grey) with labelled clones (blue) observed after a short (left) and a long (right) time post-labelling. In long-term labelling experiments, the number of labelled clones per testis decreases steadily over time, and the average clone length grows to maintain homeostasis. (**C**,**D**) Analyses of clone fate dynamics demonstrates that the number of clones decreases with time, whereas the size of the remaining clones increases. Red circles show experimental data; the 'theory' curve shows clone growth and decay proportional to the square-root of the time post-labelling (see first and second predictions in text). (**E**) A plot showing that the distribution of clone sizes broadens over time. (**F**) The cumulative clone size distributions from all time points (3, 4, 6, 10 and 14 months) plotted with the clone length divided by their average length, a process known as 'rescaling'. All the distributions rest on a single Gaussian 'universal' curve, $F(x)=\exp[-\pi x^2/4]$, which is predicted by the neutral drift hypothesis (see Box 1 and third and fourth predictions in the text). Inset: the same data at 3 and 14 months before rescaling. Error bars show s.e.m. Panels C-F are modified with permission from Klein et al. (Klein et al., 2010b).

Neutral drift and stochastic stem cell turnover in spermatogenesis

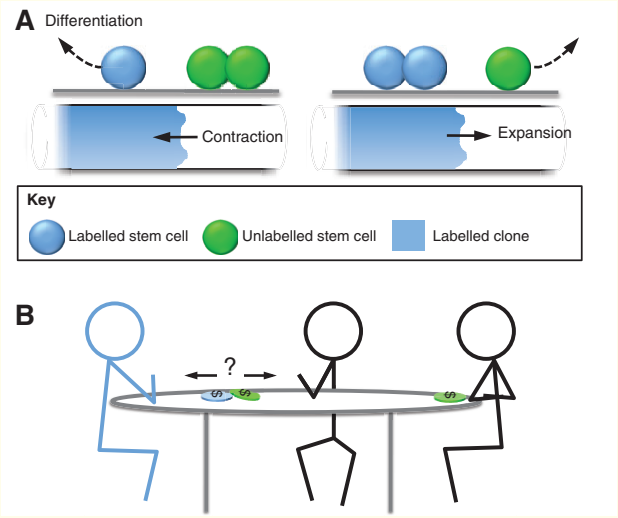
An alternative hypothesis that could explain the ongoing depletion of clones and their increasing heterogeneity is that all stem cells have equal proliferative potential, but that some stem cells are lost in a stochastic manner and others multiply to replace them. Here, clonal dynamics can be likened to a gambling game with equal odds (Box 1) in which stem cells represent the 'cash' won or lost in successive rounds between neighbouring 'players' (clones). In this game, 'equal chance' does not guarantee 'equal outcome': some players will become rich whereas others, through bad luck, will lose their money and leave the game. Games of chance such as this have been studied extensively in the fields of mathematical finance and probability theory, giving simple predictions for the dynamics of clones over time. Perhaps the best-known example of these games in biology is the neutral theory of molecular evolution in population genetics (Kimura, 1983), which proposes that competition between alleles of equal fitness leads to 'neutral drift' of the alleles within a population. Applied to the case of stem cell dynamics, where we deal with isogenic individuals, the clonal label takes the place of a mutant (but neutral) allele. A clone, in this context, consists of the descendants of an ancestor, now marked by labelling rather than by allelic variation. In spermatogenesis, the hypothesis of stochastic neutral competition makes the following predictions (Klein et al., 2010b):

First, the fraction of surviving clones should decrease in proportion to the inverse square root of time, $1/\sqrt{\lambda t}$, where λ is the stem cell replacement rate. With regards to the gambling analogy, this means that, at each round, a few more players lose their final stake and exit the game.

Second, to ensure a fixed stem cell population, the average number of stem cells per surviving clone should increase over time according to a square-root power law $\langle n(t)\rangle \sim \sqrt{\lambda t}$. In terms of the gambling game, the surviving players become increasingly richer (clones expand) because the total amount of money in the game is fixed.

Third, the clone size distributions are predicted to have a special property, known as 'scaling', whereby their shape is preserved over time post-labelling, despite the average clone size growing steadily. This effectively means that the clone size distributions should 'stretch out' over time. The scaling property corresponds to the probability of finding a clone with n or more stem cells at a time t post-labelling of the general form $P_n(t) = F(n/\langle n(t)\rangle)$, where $F(x)$ is known as the 'scaling function'. In terms of the gambling game,

Box 1. Neutral competition between stem cells



In lineage-tracing experiments, the loss of a labelled stem cell by differentiation (dashed curved arrow) leads to clonal contraction (panel A, left), whereas stem cell multiplication can lead to clonal expansion (panel A, right). The number of stem cells in the tissue remains the same in both cases, but the labelled clone size changes. Stem cell replacement is, therefore, analogous to a game of chance, giving powerful predictions of clone size statistics. In this analogy (panel B), each gambler starts with one dollar, corresponding to one stem cell. At every round, the players bet one dollar against a neighbour: they either lose their dollar or double it. If each dollar represents a stem cell, then stem cell loss from a clone (e.g. through differentiation) corresponds to 'losing a dollar', whereas stem cell division corresponds to 'winning a dollar'. Stem cells are thus effectively 'exchanged' between clones, which can be thought of as 'gamblers'. Clones that lose their last stem cell are 'bankrupt' and leave the game.

The resulting clone size statistics depend on the relative fitness advantage of the clones, in the same way that the outcome of gambling depends on the odds of winning. If the long-term potency of all stem cells is the same (i.e. the odds are equal for all players), then competition between clones is neutral. In this case, the size of each clone expands and contracts at random, with future growth being independent of previous performance. The resulting statistics give the five predictions described in the main text. By contrast, if the gambling odds favour some players over others, corresponding to long-term proliferative heterogeneity of stem cells, then these predictions should fail (Klein et al., 2010b). Further, if stem cells are effectively immortal (the players decide not to gamble), then the size of all clones should remain stationary over time.

the scaling property means that the wealth of the richest 10% of gamblers, for example, maintains a fixed ratio to the wealth of the poorest 10%. To visualise the scaling property, if the (inverse) cumulative clone size distributions are plotted with the number of stem cells n divided by the average, $n/\langle n(t)\rangle$, then the entire family of cumulative clone size distributions at different times should rest on a single curve (Fig. 2F).

Fourth, the fraction $P_n(t)$ of clones containing at least n stem cells at some time t post-labelling acquires a distinct shape that satisfies a Gaussian (or normal) distribution with a fixed coefficient of variation of $\sqrt{\pi/2}$ (Fig. 2F). This shape is particular to the tubular anatomy of the seminiferous tubule, which allows long-term clonal expansion only in one dimension along the tubule length. We will see shortly that, for other geometries, there are just two possible clone size distributions that arise from neutral stem cell competition. However, the Gaussian shape only emerges if competition between stem cells is strictly neutral, so it can be clearly distinguished from any non-neutral model of stem cell replacement.

Fifth, if stem cell replacement were restricted to a niche of limited size, the predictions above would hold for a short time following clonal labelling, but would ultimately break down once a single clone comes to dominate the niche. By analogy, when the number of gamblers is limited, eventually all but one gambler must lose their money and the game ends with a single winner. However, in the case of spermatogenesis, the stem cell niche extends along the entire length of the seminiferous tubules and exhibits no such limitation. Therefore, the scaling behaviour can be observed throughout adult life.

When these predictions are compared with the entire clone fate data set, they are found to be in strikingly good agreement. By contrast, models of non-neutral stem cell replacement, such as the 'hierarchical' model discussed in the previous section, predict neither the scaling behaviour, nor the Gaussian shape of the clone size distribution, nor the square-root dependence of the clone growth curve (Klein et al., 2010b).

How deep is the analogy between stem cell turnover and the game of chance? In the latter, the fate of each bet is determined entirely at random, with equal odds for all players. The clone fate data appear to imply that stem cell fate is also determined in a seemingly unpredictable or stochastic manner. But note that, by 'stochastic', we simply mean that the long-term potency of any individual cell cannot be determined by its clonal history; biomarkers, such as gene and protein expression levels, might correlate with short-term behaviour, but they provide no long-term prediction of stem cell potency or even survival. Indeed, this behaviour is confirmed in spermatogenesis. Spermatogonia that express high levels of two spermatogonial stem cell markers, glial cell line-derived neurotrophic factor (GDNF) family receptor alpha-1 (GFRα1; Gfra1 – Mouse Genome Informatics) and Nanos homolog 2 (Nanos2), are primed for self-renewal, whereas cells expressing high levels of the transcription factor neurogenin 3 (Ngn3; Neurog3 – Mouse Genome Informatics) are more likely to differentiate (Nakagawa et al., 2010). Yet, long-term lineage-tracing studies show that both of these groups of cells are capable of generating long-lived clones that contain both Nanos2-GFRα1 and Ngn3-positive cells, and all clones have the same long-term survival characteristics irrespective of the identity of the cell initially labelled (Klein et al., 2010b; Nakagawa et al., 2007; Sada et al., 2009). By analogy, in the *Drosophila* testis, spermatogonia normally committed to differentiation might re-enter the niche and give rise to stem cells of equal long-term potency (Brawley and Matunis, 2004; Kai and Spradling, 2004; Sheng et al., 2009). Therefore, in spermatogenesis it appears that stem cell fate is ultimately determined in a stochastic manner with the progeny of persisting stem cells maintaining no memory of their parent's short-term predisposition to either loss or self-renewal.

Predicting stem cell dynamics in other tissue types: three patterns of fate

Spermatogenesis occurs in a highly specialised tissue, so one might wonder whether other tissues also exhibit stochastic stem cell behaviour. A more stereotypical tissue is the interfollicular

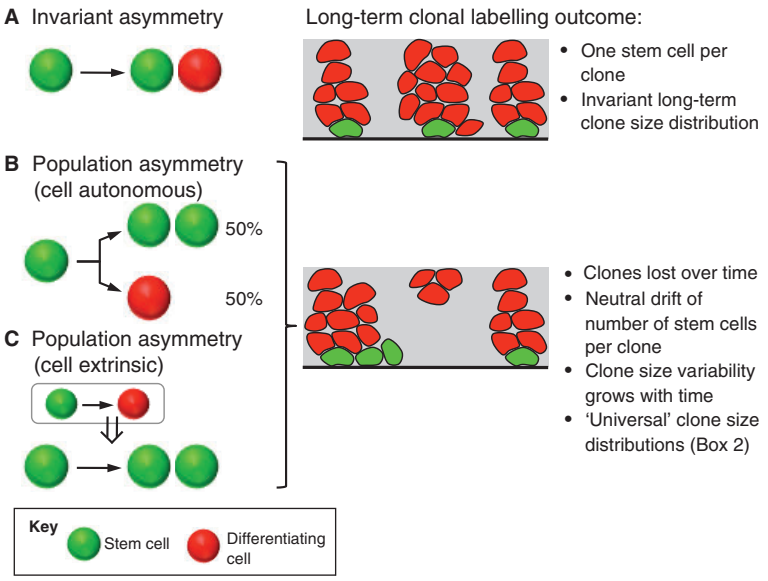


Fig. 3. Patterns of stem cell self-renewal and their clonal behaviour. In homeostasis, the long-term clonal evolution of an equipotent stem cell population belongs to one of three universal classes of behaviour characterised by the following fate characteristics: (**A**) invariant asymmetry, in which each and every division of a stem cell (green) results in asymmetric fate, giving rise to another stem cell and a differentiating daughter cell (red); (**B**) population asymmetry derived from intrinsic (cell-autonomous) regulation, in which stem cells either divide to give rise to two stem cells (green) or are lost (as indicated by differentiation to a red cell) with equal probability; and (**C**) population asymmetry derived from cell-extrinsic regulation, in which stem cell multiplication is compensated for by the loss of a neighbour. The outcome of clonal labelling in each case is indicated, showing examples of the stem cell-derived clones that can be observed. Both patterns B and C of population asymmetry are characterised by neutral drift dynamics of the clonal population leading to long-term scaling behaviour of clone size distributions.

epidermis (IFE), which, until recently, was thought to represent a classic example of the stem/transit-amplifying cell paradigm, with tissue maintained by long-lived, slow-cycling stem cells following a pattern of invariant asymmetry. However, direct evidence of invariant asymmetry was lacking. Using a similar statistical analysis to that used in the studies of spermatogenesis, two recent lineage-tracing studies (Clayton et al., 2007; Doupe et al., 2010) have revealed that, here too, the tissue is maintained by population asymmetric self-renewal, as well as by asymmetric divisions. In normal homeostasis, the IFE was found in these studies to be maintained by a single equipotent cell population that followed a pattern of balanced stochastic fate, in which cell division can give rise to two dividing cells, one dividing and one non-dividing cell, or two non-dividing cells.

Should one be surprised at the evidence that similar patterns of stochastic stem cell fate exist in tissues as different as the IFE and spermatogonia? To understand whether stochastic stem cell fate could be a common outcome of tissue homeostasis, it is helpful to consider first what would happen in a 'homogeneous' tissue in which all stem cells have sustained access to the same range of internal or environmental cues.

In this case, there can be just three classes of stem cell fate, each of which can be identified from clone size distributions measured by inducible hereditary cell labelling (Fig. 3). In the first class of stem cell fate, a tissue is maintained by cells that follow an invariant pattern of asymmetric division (Fig. 3A) in which each and every stem cell division gives rise to one daughter that maintains stem cell identity while the other daughter differentiates. Clonal labelling in this case would reveal a mosaic of 'proliferative units', each supported by a long-lived stem cell. If, however, self-renewal does not involve strict division asymmetry, then it must belong to just one of two classes of population asymmetry: if the stem cells are not confined by anatomical constraints to isolated niches, they might adopt a balanced stochastic fate, the product of cell-autonomous (or uncoordinated) regulation (Fig. 3B); alternatively, self-renewal might rely upon cell-extrinsic (or coordinated) regulation (Fig. 3C), in which stem cell multiplication is compensated for by the loss of neighbours. Significantly, any mechanism of stem cell self-renewal, however complex, will lead to clonal evolution that is indistinguishable from one of the three fate behaviours shown in Fig. 3 (Bramson and Griffeath, 1980).

Although invariant asymmetry allows individual stem cells to persist long term, both mechanisms of population asymmetry lead to neutral competition between stem cells, as seen in spermatogenesis. Therefore, the clone size distributions in any homeostatic cycling tissue in which stem cells might be lost (Fig. 3B,C) should converge onto one of just a few patterns of neutral stem cell competition that are 'universal': they are independent of stem cell number and of their rate of loss or division and differentiation, and depend only on the spatial organisation of stem cells in a tissue (Box 2).

Evidence of stochastic and neutral stem cell replacement in other tissues

How do the 'universal' patterns predicted compare with the quantitative clone statistics obtained from different tissues? Figure 4 compares the clone fate data acquired from studies of two tissue types in addition to the male germ line: the murine IFE and the intestine. These tissues are each representative of stem cell-supported tissues, in that they rapidly turn over and can undergo long-term maintenance and repair. In both cases, lineage tracing performed using inducible genetic labelling shows that each tissue is maintained by an ever-diminishing clone number of ever-increasing size (Fig. 4A,C), a hallmark of neutral drift dynamics. Moreover, as with spermatogenesis, both tissues reveal long-term scaling behaviour, with cells in the IFE belonging to the class of cell-autonomous regulation (Fig. 3B), in which stem cell fate is independent of the fate of neighbouring cells (Clayton et al., 2007). By comparison, both spermatogenesis and intestinal crypt maintenance rely upon cell-extrinsic regulation (Fig. 3C), in which the division of a stem cell occurs in response to the loss of a neighbour (Klein et al., 2010b; Lopez-Garcia et al., 2010). In the intestinal crypts, the expansion of surviving clones progresses, as predicted by neutral drift, until all the stem cells within the crypts are monoclonal. At late times (6-8 weeks post-labelling), this 'monoclonal conversion' of crypts leads to a cessation of scaling behaviour, as discussed in the fifth prediction above. However, the timing of monoclonal conversion is also predicted with quantitative accuracy (Lopez-Garcia et al., 2010).

Box 2. Clone size statistics used to detect neutral stem cell turnover

Here, we outline the characteristic clone size statistics that emerge from the two general patterns of population asymmetric stem cell self-renewal.

Cell-autonomous self-renewal

In this scenario, cell fate is specified randomly with each stem cell division leading to stem cell multiplication or loss with equal probability [a mathematical process known as a critical birth-death process (Bienayme, 1845; Harris, 1948; van Kampen, 2007)]. With a division rate λ, the long-term ($t>1/\lambda$) clone survival probability diminishes as $P^{(surv.)}\approx 1/\lambda t$, whereas the average size of surviving clones grows linearly as $\langle n(t)\rangle \equiv 1/P^{(surv.)}(t)\approx \lambda t$. The clone size distribution acquires a scaling property (third prediction in the main text), with the scaling function $F(x)=\exp[-x]$.

Cell-extrinsic self-renewal

In this scenario, the loss of a stem cell correlates with the multiplication of a neighbour. Such behaviour is encountered in games of chance, but also in other fields of probability, where it is known as the 'stepping stone model' of neutral drift in population genetics (Kimura and Weiss, 1964), a 'Moran process' in population dynamics (Moran, 1962), and (inspired by treatments in which labelled and unlabelled stem cells represent voters with different political opinions) a 'voter model' (Holley and Liggett, 1975), which has received much attention from both mathematicians and physicists (Ben-Naim et al., 1996; Liggett, 1985; Sood and Redner, 2005). Although this process also leads to long-term scaling behaviour, the particular form of the scaling function $F(x)$ and the average growth curve $\langle n(t)\rangle$ depend on the number of dimensions in which a clone may expand in the tissue. In tubular and glandular tissues, clones may expand only along one dimension; in epithelial tissues, clones may expand in two dimensions, and so on. With a stem cell loss rate, λ, the average size of persisting clones asymptotes to (Bramson and Griffeath, 1980; Sawyer, 1979; Sudbury, 1976),

$$\langle n(t)\rangle = \begin{cases} \sqrt{\lambda t} & \text{1D} \\ \lambda t / \ln(\lambda t) & \text{2D} \\ \lambda t & \geq \text{3D} \end{cases},$$

corresponding to 'tubular' (1D) tissues, 'epithelial' (2D) tissues, 'volumnar' tissues (3D) and 'distributed' (>3D) tissues. Moreover, the asymptotic clone size distributions also vary with dimension $F(x)=\exp[-\pi x^2/4]$ in 1D and, as with the cell autonomous process, $F(x)=\exp[-x]$ in higher dimension.

The signature of stochastic stem cell fate can also be revealed using mosaic or multi-colour cell labelling without resorting to clonal analysis. Stem cells undergoing stochastic turnover will lead to the progressive 'coarsening' and aggregation of labelled domains (Fig. 5). In particular, the clonal diversity, or 'heterozygosity', of the tissue (Frachebourg and Krapivsky, 1996; Korolev et al., 2010; Krapivsky, 1992) will decrease over time t as $\sim 1/\sqrt{\lambda t}$ in tubular and glandular tissues (in which clones may expand only along one dimension), and as $1/\ln(\lambda t)$ in epithelial tissues (where clones may expand in two dimensions). Once again, the dynamics of coarsening provide quantitative hallmarks of the underlying pattern of stem cell turnover (Snippert et al., 2010). Curiously, this coarsening pattern has also been observed in the cornea (Mort et al., 2009), but has been associated with stem cell depletion. It is tempting to speculate that, instead, the cornea undergoes population asymmetric self-renewal consistent with the above examples of rapidly cycling epithelial tissues.

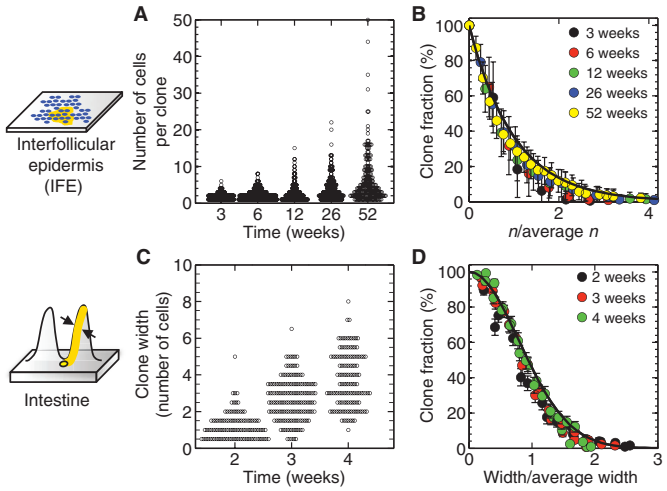


Fig. 4. Neutral drift in long-term clone evolution in the murine interfollicular epidermis and intestine. Studies of clonal evolution following inducible genetic labelling of two additional stem cell supported tissues: (**A**,**B**) the murine interfollicular epidermis (IFE); and (**C**,**D**) the intestine (both shown in yellow in the schematics on the left). These studies reveal scaling behaviour (Box 2) consistent with neutral stem cell competition. (A) The size of surviving clones in murine IFE following inducible genetic labelling. Clonal fate data was acquired at single cell resolution over a 12-month period (Doupe et al., 2010). (B) Rescaled cumulative clone size distributions calculated from A, which describe the probability of finding a surviving clone with more than n/(average n) cells. If stem cells self-renew through population asymmetry, we predict (Box 2) that the curves will sit on the same universal curve (or 'scaling function') for all time points. The particular scaling function that fits the data in B, $F[x]=e^{-x}$, combined with the linear growth of surviving clones, places IFE stem cells in the cell-autonomous regulation class of population asymmetry. (C) The size of surviving clones in intestinal crypts (Lopez-Garcia et al., 2010). (D) Rescaled cumulative clone size distributions calculated from C, showing a fit to the scaling function predicted for cell-extrinsic regulation of self-renewal as predicted in Box 2.

Intrinsic and extrinsic origins of stochastic stem cell fate

It is tempting to speculate that the universal patterns of stem cell fate seen in different tissues might result from conserved mechanisms of fate regulation. Yet, mathematical analysis shows that the dynamics of neutral drift [see the five predictions above, and Box 2] are extremely robust: even if stem cells are lost and replaced just a few times during an experiment, the same long-term predictions hold irrespective of the detailed anatomy of a tissue, the drivers of stem cell loss, or the mechanism coordinating their replacement. Therefore, long-term clonal statistics are largely insensitive to the molecular mechanisms responsible for stochastic stem cell decisions.

Nevertheless, a comparison of clone size distributions across the different tissues (Fig. 2E,F and Fig. 4) does provide indications of the mechanisms of cell fate regulation, particularly by extracting the frequency of stem cell replacement for each tissue (denoted λ, see Box 2). In spermatogenesis, stem cells are replaced in less than two weeks on average (Klein et al., 2010b), a time scale comparable to the cell division rate in the seminiferous tubules. In the intestinal crypt, the replacement rate was even higher, but again was comparable to the cell division rate (~1/day) (Lopez-Garcia et al., 2010; Snippert et al., 2010). In the epidermis, clonal analysis

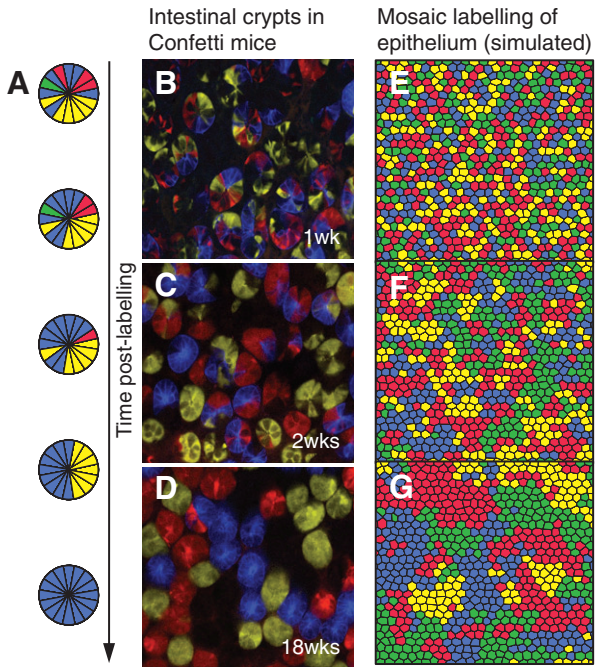


Fig. 5. Neutral stem cell competition leads to coarsening of mosaic labelling. (**A**) Model of stochastic turnover of mosaic-labelled stem cells in an intestinal crypt. Stem cells are lost and replaced by their neighbours, leading to the growth of same-colour domains and a 'coarsening' of the mosaic pattern. The clones are analogous to gamblers sitting around a table, with stem cells 'exchanged' between neighbouring clones in one dimension around the crypt circumference. The gambling ends when a single player 'wins' (i.e. crypts become monoclonal). Stem cells continue to be replaced by their neighbours, but this cannot be seen as the entire crypt is now labelled in a single colour. (**B-D**) Cross-sections of small intestine glandular crypts from mosaic-labelled (Confetti) mice reveal coarsening at 1, 2 and 18 weeks post-labelling (Snippert et al., 2010). (**E-G**) In simulations of self-renewal in a mosaic epithelium, coarsening is also observed. In the simulations, cells in the basal layer are removed (i.e. they 'differentiate') at random and are immediately replaced through division of a randomly selected nearest neighbour. The rate of coarsening of the coloured domains discloses the rate of stochastic cell turnover.

reveals that daughter cells acquire a symmetric fate every few cell divisions (Clayton et al., 2007). Stem cell replacement is not a rare event in these different tissues, and is, therefore, unlikely to be the result of stem cell damage or senescence. Instead, it appears to characterise routine tissue stem cell activity.

What, then, leads to stochastic fate decisions during routine tissue maintenance? One possibility is that gene expression in stem cells is noisy, leading to cell-to-cell variability in their response to extrinsic tissue signals (Enver et al., 2009). Evidence in support of this hypothesis is seen in a haematopoietic stem cell line, in which levels of a key transcriptional regulator, stem cell antigen 1 (SCA1), were found to fluctuate over time (Chang et al., 2008). SCA1 levels correlate with cell fate decisions in response to retinoic acid treatment, indicating that noise in gene expression can lead to a stochastic cell fate response. Similarly, fluctuations of several genes in mouse embryonic stem cells have been shown to influence cell fate decisions: fluctuations in the key stem cell gene Nanog regulate the probability of stem cell self-renewal and propagation in vitro (Kalmar et al., 2009); oscillations in the level of the murine helix-loop-helix factor Hairy and enhancer of split 1 (Hes1) (Kobayashi et al., 2009) affect the probability of stem cell differentiation into either neuronal fates (Hes1 low) or mesoderm fates (Hes1 high); and fluctuations in the endodermal marker haematopoietically expressed homeobox (Hex; Hhex – Mouse Genome Informatics) affect the probability of the stem cells contributing to extra-embryonic endoderm (Hex-high) or epiblast cells (Hex-low), following re-introduction into mouse embryos (Canham et al., 2010). However, it remains to be seen whether these fluctuations also occur in vivo and whether they affect normal tissue homeostasis.

A second possible driver of stochastic cell fate decisions is spatial heterogeneity in the signals arising from the stem cell niche, which could result in different environmental cues affecting neighbouring stem cells. The majority of adult tissues are characterized by heterogeneous niche structures in which extrinsic factors, such as intercellular matrix proteins and signals from niche cells, are believed to actively influence cell proliferation and differentiation (Voog and Jones, 2010). Niche location might, therefore, ensure that particular cells are primed for multiplication, quiescence or loss.

It remains for future studies to establish the degree to which cell-intrinsic factors or environmental cues regulate stochastic cell fate decisions in different tissues. Single-cell techniques, such as flow sorting of stem cells from dissociated tissue, combined with single-cell gene expression profiling, might reveal whether cell-to-cell variability is significant in stem cell populations in vivo, with particular focus on the levels of key growth factor receptors and transcription factors. Further, transplantation experiments provide a functional assay to test differences in self-renewal efficiency of the sorted stem cells. By contrast, evidence of cell-extrinsic heterogeneity could be investigated by looking for patterns of proliferation or differentiation that affect groups of neighbouring cells and which correlate with gene expression levels and protein levels of niche cells.

A new paradigm for stem cell self-renewal?

In light of the central role of population asymmetry in homeostasis, it is interesting to revisit some common assumptions of stem cell biology that are rooted in a classical 'immortal stem cell' view of homeostasis. First, one might challenge the concept of the slow-cycling stem cell: until recently, quiescence was considered a hallmark of adult stem cell behaviour. Cells capable of retaining DNA labels such as [^{3}H]-thymidine or 5-bromo-2′-deoxyuridine (BrdU) are suggested to belong to a stem cell pool (Braun and Watt, 2004; Cotsarelis et al., 1990; Quyn et al., 2010). The finding that equipotent stem cells are replaced within a remarkably short time (days or weeks), and that they are in cycle, appears to conflict with these findings. It is possible that stem cells occasionally enter a quiescent state to undergo repair, as recently suggested by the discovery of quiescent telomerase-expressing cells in intestinal crypts that can generate long-lived clones (Montgomery et al., 2011). However, tissue maintenance in all of the cycling tissues examined here appears to be supported by actively cycling stem cells and does not require a quiescent population. Indeed, to give rise to neutral competition, quiescent stem cells in the tissues studied can have no long-term proliferative advantage over their shorter-lived cycling brethren.

A second implication of population asymmetry is that clonal heterogeneity does not necessarily imply heterogeneity in stem cell potency. In the corneal epithelium, the appearance of large clonal regions is suggested to result from stem cell depletion with age, with the surviving stem cells supporting larger numbers of

differentiating progeny (Mort et al., 2009). This interpretation ignores the possibility of neutral competition maintaining a fixed stem cell population with only the number of clones decreasing. Similarly, clonal expansion in the mouse pancreas is proposed to result from mutations leading to a fitness advantage of large clones (Wiktor-Brown et al., 2008). However, large clones might also arise naturally during neutral competition, and do not necessarily arise from fitter, or mutant, stem cells. To correctly determine whether a mutation influences stem cell potency, it is necessary to contrast mutant clone size distributions with the predictions from neutral drift. For example, p53-mutant clones in UV-irradiated mouse epidermis are found to expand exponentially, whereas wild-type clones in epidermis are predicted to grow linearly with size (see Box 2) (Klein et al., 2010a).

Finally, the shift away from an invariant model of asymmetric stem cell fate prompts new questions about the molecular mechanisms of stem cell regulation. First, if the survival of individual stem cells is stochastic, then what fixes the total population size? Second, what controls the dice? In the intestinal crypt, it has recently been shown that stem cell contact with a Paneth cell (a cell traditionally associated with immune response) is necessary for self-renewal in vitro, providing a short-term predictor of stem cell fate, whereas long-term potency remains unpredictable. In spermatogenesis, gene expression correlates strongly with stem cell fate for approximately seven days post-labelling, with Ngn3-expressing stem cells more likely to differentiate, whereas GFRα1 or Nanos2-expressing cells are more likely to self-renew. Understanding stem cell fate regulation in homeostasis will probably depend on experiments that aim to uncover such transient factors that correlate with short-term cell fate decisions. Assays of mutant clone growth in vivo might provide insight into the role of particular genes, and comparison of clonal growth in young and aging tissue might also reveal novel behaviours. As stem cells ultimately lose their proliferative capacity during aging, one might observe a non-neutral expansion of surviving clones. Aging could, therefore, be accompanied by a large drop in clonal diversity with enrichment for age-resistant mutations or epigenetic modifications.

Conclusions

For many, the stereotypical picture of stem cells as long-lived, slow-cycling, asymmetrically dividing cells has informed the search for the molecular regulatory mechanisms controlling their fate. Label-retaining assays have been combined with specific molecular markers to resolve stem cell identity, and emphasis has been placed on characterising the extrinsic, niche-based, regulatory factors that maintain stem cell competence and promote the differential segregation of fate determinants. However, recent lineage-tracing studies have shown that, in several cycling tissues, stem cells behave as an equipotent population in which the balance between proliferation and differentiation is achieved through frequent and stochastic stem cell loss and replacement. In such tissues, the long-term self-renewal potential rests with the population but the lifetime of individual stem cells is not defined.

Here, we have shown that strategies for the long-term self-renewal of homeostatic tissues can be separated into three possible classes of behaviour. Alongside the mode of invariant division asymmetry, there exist just two patterns of population asymmetry, discriminated by the nature of the underlying regulation – cell-autonomous versus external – and characterised by neutral drift dynamics of the clonal population. Mechanisms that lead to population asymmetric self-renewal leave behind universal and robust signatures in lineage-tracing data, resulting in a simple, long-term scaling behaviour of clone size distributions. As well as providing the means to identify the underlying pattern of stem cell self-renewal, this classification scheme provides a quantitative platform with which we can assess the effects of drug delivery and explore factors that lead to stem cell dysregulation in disease and aging.

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Competing interests statement

The authors declare no competing financial interests.

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