View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Apollo

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Cell Biology

Clonal analysis of stem cells in differentiation and disease

Bartomeu Colom[1] and Philip H Jones[1,2]

Tracking the fate of individual cells and their progeny by clonal analysis has redefined the concept of stem cells and their role in health and disease. The maintenance of cell turnover in adult tissues is achieved by the collective action of populations of stem cells with an equal likelihood of self-renewal or differentiation. Following injury stem cells exhibit striking plasticity, switching from homeostatic behavior in order to repair damaged tissues. The effects of disease states on stem cells are also being uncovered, with new insights into how somatic mutations trigger clonal expansion in early neoplasia.

Addresses
[1] Wellcome Trust Sanger Institute, Hinxton CB10 1SA, UK
[2] MRC Cancer Unit, University of Cambridge, Hutchison-MRC Research Centre, Box 197, Cambridge Biomedical Campus, Cambridge CB2 0XZ, UK

Corresponding author: Jones, Philip H (pj3@sanger.ac.uk)

Current Opinion in Cell Biology 2016, **43**:14–21

This review comes from a themed issue on **Differentiation and disease**

Edited by **Tom Misteli** and **Graham Warren**

http://dx.doi.org/10.1016/j.ceb.2016.07.002

0955-0674/© 2016 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Many adult tissues are continually turned over. New cells must be made at a rate that exactly matches cell loss. This balance is critical, as if slightly too few cells are made the tissue will fail while excess cell production is a feature of cancer. New cells of each lineage are produced by stem cells. Clonal analysis to resolve the fate of individual rather than bulk populations of stem cells has revealed the cellular mechanisms by which stem cells sustain a variety of lineages throughout life. The proliferative diversity between tissues and the dynamic and adaptable nature of the cells that sustain them makes defining the term 'stem cell' ever more challenging. Here we will adopt a purely functional definition, stem cells are cell populations that maintain and/or regenerate adult tissues or lineages [1,2]. We discuss the rapidly developments in clonal analysis in three adult stem cell systems, intestine, squamous epithelium and blood, and consider how recent results have revised the stem cell paradigm.

Stem cells in homeostasis

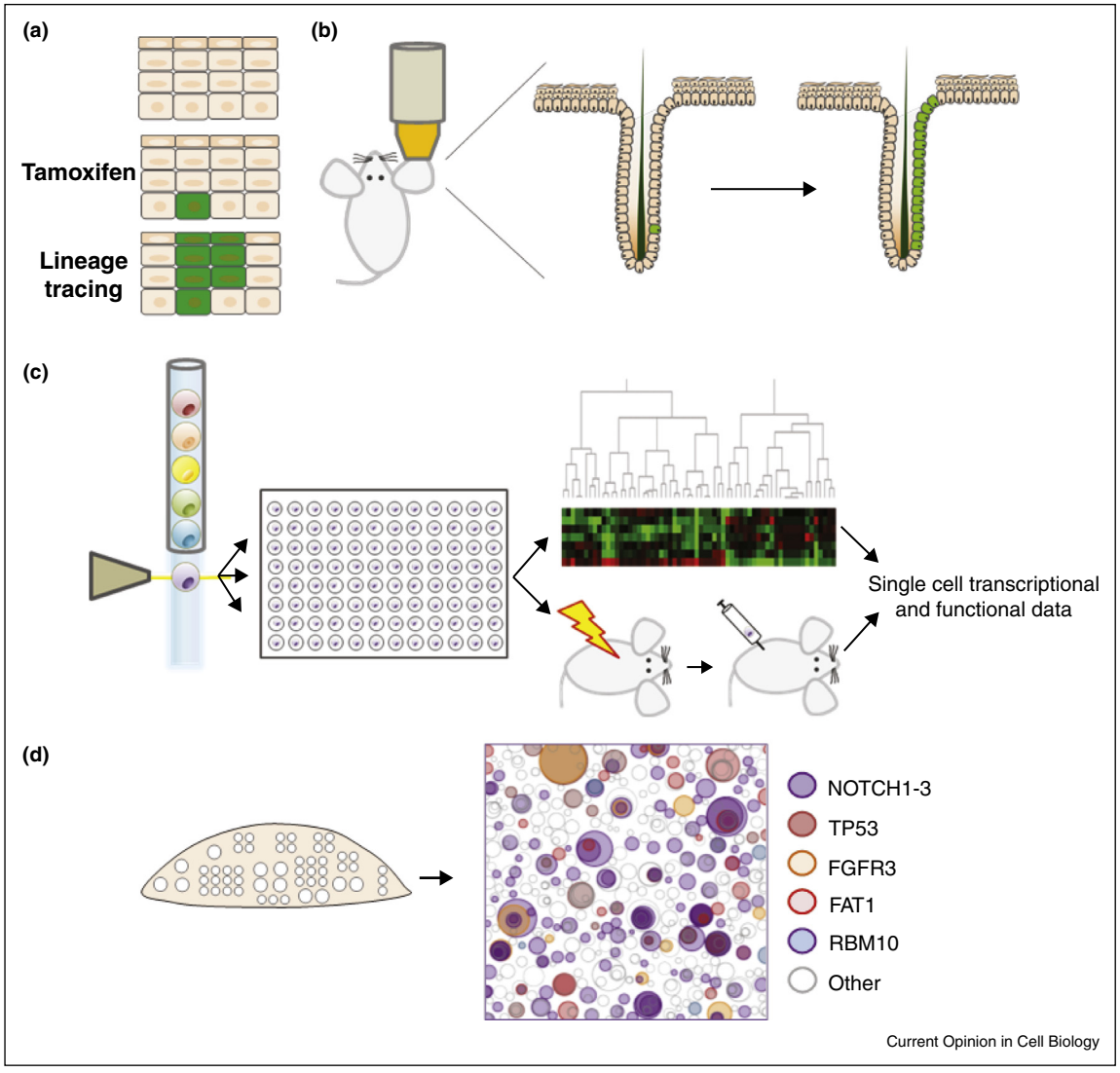
Intestinal epithelium

Our understanding of the stem cells of the epithelium that lines the intestine has been transformed by the application of clonal analysis in transgenic mouse models (Figure 1a, b) [3]. The tissue is rapidly turned over. Differentiated cells on the finger-like villi are continually shed and replaced by proliferating cells located in pits, known as crypts, which lie adjacent to the villi [4]. Inducible genetic lineage tracing of single cells expressing the *Wnt* target gene *Lgr5* reveals clones containing the four differentiated cell lineages of the epithelium, some of which persist long term (Figure 2a) [5]. This indicates that the Lgr5+ population both sustains itself and maintains the epithelium. *In vitro* clonal analysis, in which LGR5+ cells were cultured revealed that the progeny of single cells could self assemble into intestinal like 3 dimensional structures termed organoids that contained the four differentiated cell types and could be serially propagated, as long as the media contained *Wnt* ligands [6]. *In vivo*, the source of WNT is the Paneth cells that lie adjacent to the *Lgr5*+ cells at the crypt base (Figure 2a). [7]. Fluorescent tagging of WNT3 in a transgenic mice reveals that the restricted distribution of WNT signaling at the crypt base is due to the protein remaining bound to cell membranes and being diluted when cells divide [8••]. This mechanism restricts stem cells to the crypt base, as once they leave the niche, stem cells receive less WNT signal and undergo differentiation [9,10]. In each crypt stem cells compete neutrally with their neighbours, with the result that, purely by chance, the crypt will eventually become colonized by the progeny of one stem cell [9,10]. It was thought that all Lgr5+ cells contributed equally to tissue maintenance, but more recent studies have shown that only a third of the cells in the crypt are proliferating at any one time. Combined intravital imaging with genetic lineage tracing has shown that the cells in the uppermost part of the niche are the most likely to differentiate [11].

Squamous epithelia

The outermost layer of the skin, the epidermis, and the lining of the oesophagus consist of layers of keratinocytes (Figure 2b, c) [2,12]. Proliferation is confined to the basal layer of cells. On commitment to terminal differentiation, cells exit the cell cycle and leave the basal layer, migrating to the tissue surface from which they are shed. There are conflicting models of how the epidermis is maintained.

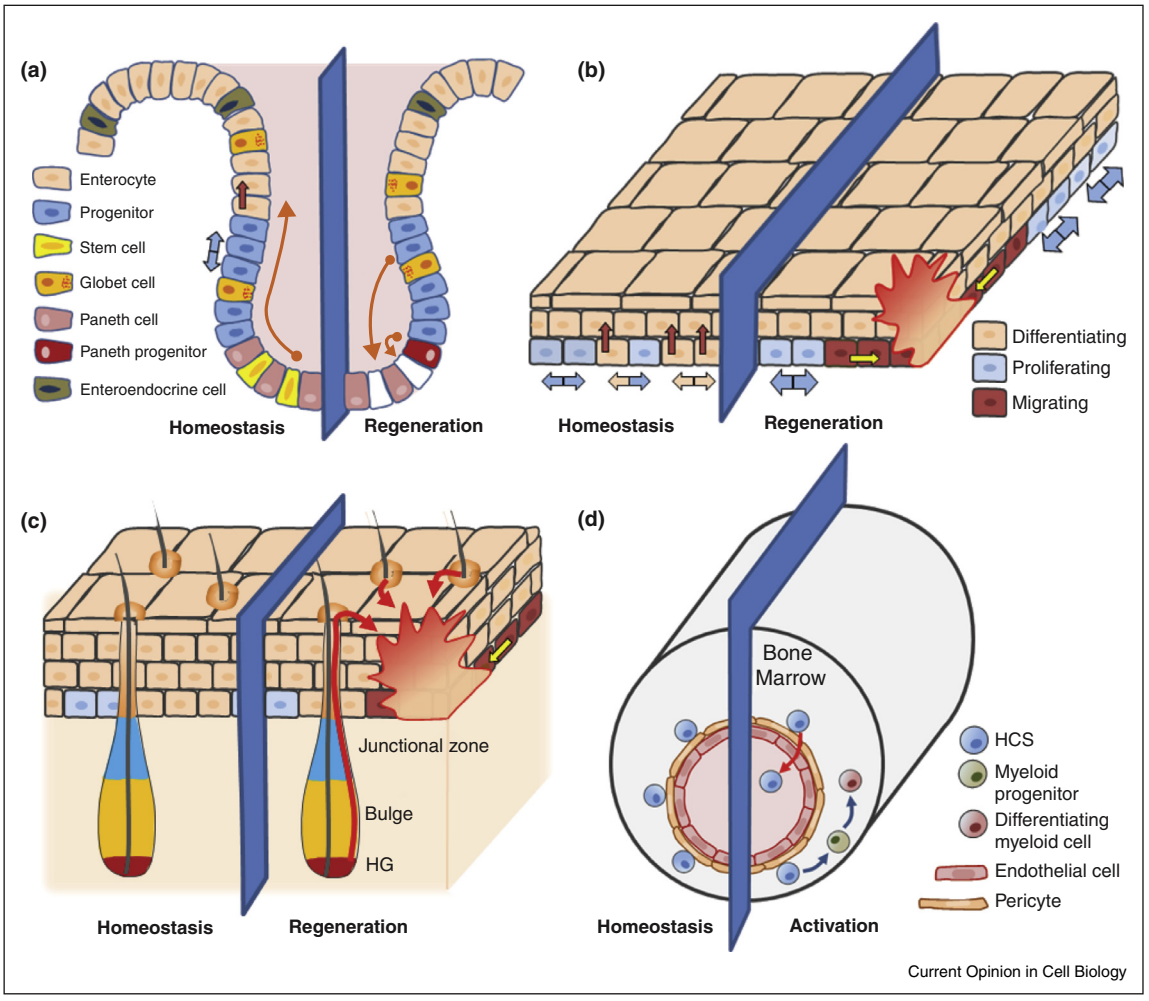
Figure 1



Methods of clonal analysis. **(a)** In this example, genetic lineage tracing in the epidermis is activated by tamoxifen induced cre recombination, leading to the reporter gene (GFP, green) being expressed in scattered cells in the basal cell layer. Expression of the label is inherited by the progeny of the labelled cell (green), revealing the fate of the labelled cell and its daughters over the time since induction. **(b)** Lineage tracing can be combined with intravital imaging to study stem cell biology in live animals, for example in tracking single stem cells within the hair follicle during homeostasis and regeneration. **(c)** Index sorting allows parallel transcriptional and functional analysis of individual cells in a population sharing the same surface markers and may reveal unexpected heterogeneity at the single cell level. **(d)** Deep targeted exome sequencing of small samples of normal sun-exposed human skin has revealed a high burden of clones carrying oncogenic driver mutations (represented by shaded circles). Measured clone areas are projected onto a simulated 1 cm^2 area of skin, open circles indicate neutral mutations.

Statistical analysis of inducible genetic lineage tracing argues that the proliferating cells in the basal layer of the epithelium contribute equally to tissue maintenance [13–17]. The outcome of individual cell divisions is unpredictable, producing two differentiating daughters, two dividing cells or one cell of each type. However, the probabilities of generating dividing or differentiating daughter cells are balanced so homeostasis is achieved across the population of dividing cells. The case for this single progenitor model has been strongly reinforced by a recent study of ear and paw epidermis which combines intravital imaging with transgenic lineage tracing to show that there are no slow cycling stem cells at these sites and that measurement of proliferating cell behavior is entirely consistent with a single cell type [18••]. It appears that not all body sites are the same however. Tail epidermis does contain a slow cycling stem cell population in addition to progenitors that is mobilized following injury [19]. Another report fails to find evidence for slow cycling cells in back skin but argues, in contradiction to earlier work, that there are two populations of rapidly dividing progenitor cells dividing at different rates in different regions of the

Figure 2



Stem cell dynamics during homeostasis and injury. **(a)** Intestinal epithelium. In homeostasis (left side) a self-sustaining population of Lgr5$^+$ stem cells (yellow) located at the base of the intestinal crypt generates the four differentiated lineages of the epithelium via progenitor cells in the upper crypt. Differentiated cells leave the crypt, migrate on to the villi and are lost by shedding or apoptosis. Regeneration (right side): following ablation of the Lgr5$^+$ population, progenitor cells migrate to the lower crypt and regenerate Lgr5$^+$ stem cells. **(b)** Squamous epithelia. Mouse esophageal epithelium consists of layers of keratinocytes with proliferating stem cells located in the basal layer. Homeostasis (left side) is maintained by stem cells whose divisions have three possible outcomes: two stem cells, two differentiating daughters or one cell of each type. The result of an individual division is unpredictable but the probabilities of the symmetric outcomes are balanced so that on average, across the stem cell population, equal proportions of stem and differentiating cells are produced. Regeneration (right side). In response to injury, cells neighbouring the damaged area stop proliferation and migrate towards the wound. Behind this migrating front, progenitor cells reversibly switch their fate to produce more stem than differentiating progeny until the tissue is repaired. **(c)** Skin epidermis is similar to the oesophagus in homeostasis (left) and regeneration (right), but also contains hair follicles which can be functionally split into three distinct compartments: junctional zone, bulge and hair germ (HG); all of these containing populations of stem cells that maintain each compartment during homeostasis. After injury, cells derived from the hair follicle migrate into the epidermis to support wound repair. **(d)** Hematopoietic stem cells (HSC). During homeostasis (left) HSC are confined to the bone marrow microvasculature and divide infrequently. HSC are activated by stress signals (e.g. released cytokines such as M-csf) to generate multiple cell lineages. Activated HSC may also enter the blood stream.

epidermis [20]. Intravital imaging of tail and back skin will hopefully resolve the basis of epidermal homeostasis at these sites. In the mouse oesophagus, transgenic lineage tracing makes a compelling case for tissue maintenance by a single progenitor population [15,21].

The skin also contains hair follicles, complex organs with multiple cell types that undergo cyclical growth and contraction (Figure 2c). Lineage tracing in this system in which there is extensive cell death is challenging. However it is clear that the upper parts of the hair follicle, the junctional zone and infundibulum, are maintained by a separate population of stem cells from the lower follicle (the bulge region and areas beneath it) [22–25]. As with the intestine, the combination of intravital imaging with transgenic tools to track cells has

begun to reveal the dynamics of stem cells in hair follicles [23,26].

Hematopoiesis

For decades hematopoietic stem cells (HSC) have been assayed by flow sorting bone marrow cells for multiple surface markers and transplanting them into recipients whose hematopoietic system has been destroyed by radiation [27]. HSC are defined by their ability to reconstitute hematopoiesis in the long term, a property demonstrated even by single cells [28]. Allowing single HSC to divide once in culture and then separating and transplanting the individual daughter cells revealed that single stem cells generate a diversity of progeny, from two HSC daughters to pairs of more differentiated cells with some divisions with one HSC and one differentiating cell, findings which argue against hematopoietic stem cell fate being predetermined [29].

Single cell analysis is also challenging the classification of HSC and early progenitors based on combinations of cell surface markers. Individual cells from a population isolated by flow cytometry that appears pure in terms of surface marker expression have been subjected to 'index sorting' (Figure 1c). Some single cells are transcriptionally profiled by RNA sequencing while parallel functional assays including clonal *in vivo* lineage tracing are performed on other individuals from the same sorted population [30••]. This 'approach has revealed substantial functional and transcriptional heterogeneity in what was thought to be a single common myeloid progenitor population based on surface marker expression and bulk rather than clonal assays [31••,32••,33••]. These results challenge a long held model in which HSC derived progenitors progressively lose the capacity to generate multiple cell lineages as differentiation proceeds. The combination of index sorting, single cell transcriptomics and genetic lineage tracing may have wide application in the study of other types of stem cells that appear pure but have divergent fates.

While transplantation is a powerful technique, it tests the ability of cells to survive the stress of transplantation as well as regenerating the blood system, and arguably gives limited insight into the how the cells function in homeostasis (Figure 2d) [34]. Genetic lineage tracing argues that adult hematopoiesis in unperturbed animals is very different from transplants, with self renewing populations of lineage committed progenitors sustaining the blood system while transplantable HSC are almost quiescent and make negligible contribution to maintenance [35••,36,37••].

Stem cell plasticity following injury

Alongside maintaining cellular turnover, adult stem cells also have to regenerate tissues following injury. Clonal analysis has uncovered remarkable flexibility in the responses of stem cells and their progeny to tissue damage.

Intestine: dedifferentiation to regenerate stem cells

A series of innovative studies has revealed that contrary to what has long been assumed, cellular differentiation is not a 'one way street'. Normally when stem cells leave their niche at the base of the crypt they differentiate into lineage restricted progenitors and migrate through the crypt to become terminally differentiated cells [9]. However, clonal genetic lineage tracing combined with single cell transcriptional analysis reveals that following ablation of LGR5+ stem cells both lineage committed Paneth and enterocyte precursor cells can reenter the niche and reconstitute the LGR5+ stem cell population (Figure 2a) [38–40]. Such plasticity explains how the intestine is able to restore homeostasis after transgenic LGR5+ cell deletion unless this is combined with an additional insult such as irradiation that also destroys precursor cells [41].

Squamous epithelia: crossing compartments and switching proliferation

The skin epidermis is frequently injured. In response to wounding, stem cell progeny migrate across boundaries of tissue compartments that are not normally crossed [25,42]. Stem cells in the hair follicle and sweat ducts contribute cells to the interfollicular epidermis after injury [25,43,44]. Quiescent epidermal stem cells that are mobilized by wounding have also been described in mouse tail epidermis [19]. Lineage tracing reveals clonal streams of genetically marked cells entering the epidermis until the injury is repaired. In addition intravital imaging shows that if stem cells in hair follicle bulge are ablated cells from the upper hair follicle regenerate them [23].

Studies in mouse oesophagus have shown that a 'reserve' population of slow cycling stem cells is not essential for wound repair. Clonal lineage tracing argues that the stem cells close to a wound switch from producing equal proportions of stem cells and differentiating cells to transiently produce an excess of stem cell daughters until the defect in the tissue is healed [15,16]. This ability to flip between 'maintenance' to 'wound' mode and back again in response to loss and recovery of local cell confluence has recently been visualized directly by reconstructing clonal cell lineages from live imaging of primary cultures of human keratinocytes [45••]. Such plasticity provides a rapid and robust mechanism to restore the integrity of frequently wounded squamous tissues [12]. It remains to be seen whether such behavior contributes to wound healing in mouse epidermis.

Hematopoiesis: lineage bias

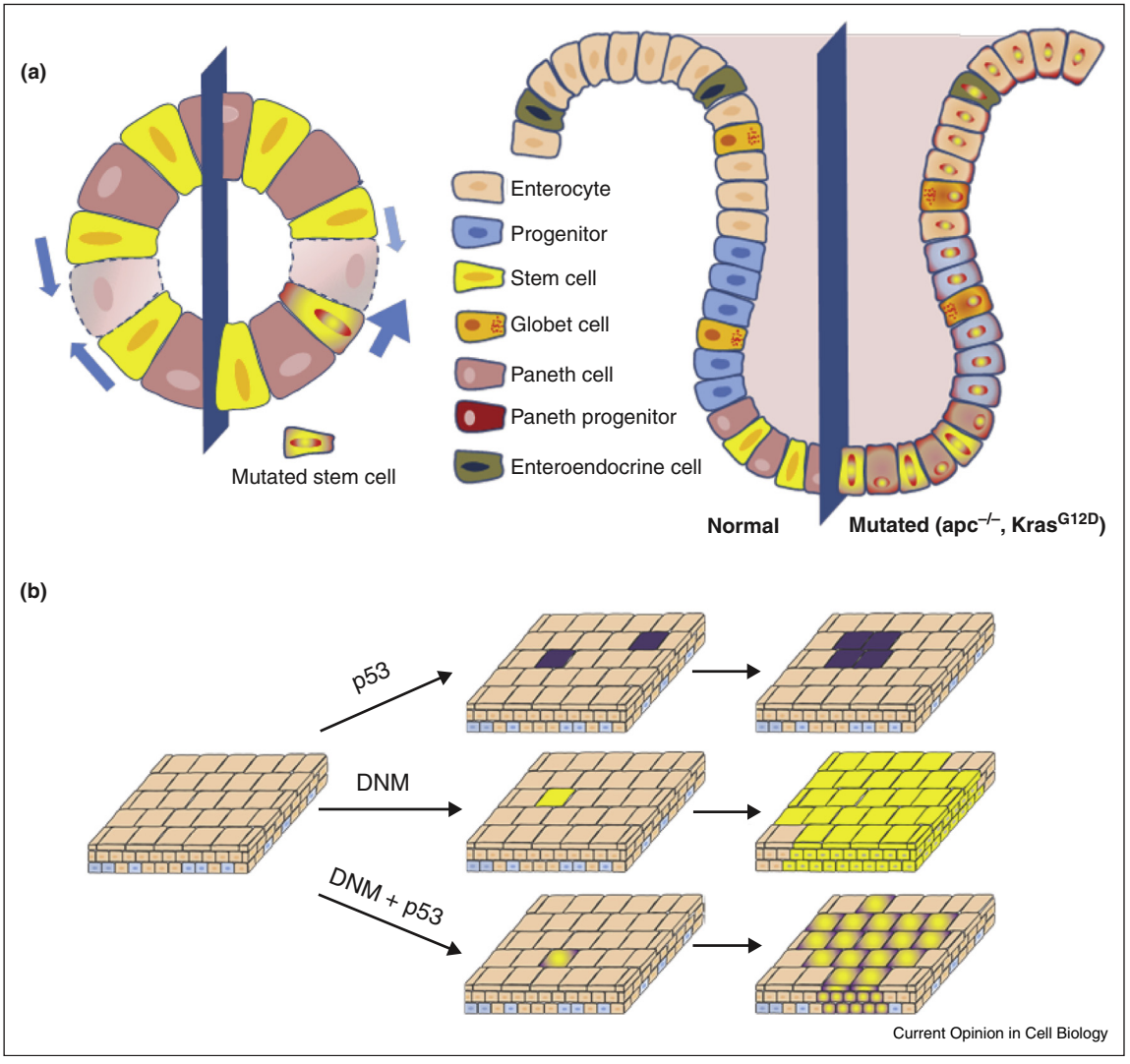
HSC generate multiple cell lineages and may need to increase production of a particular lineage to meet sys-

temic challenges [2]. For example, an increase in the levels of myeloid cells is required during infection. To meet these requirements normally slow cycling are HSC may be mobilized in response to circulating factors (Figure 2d). For example, live imaging and transcriptional analysis of individual HSCs in culture reveals that the cytokine MCSF promotes myeloid fate [46].

Stem cell mutation and clonal competition

All cells are subject to somatic mutation either from environmental agents or from 'clock' like processes that generate mutations at a rate proportional to age [47]. Stem cells may generate clones of cells carrying the accumulated mutations of the founder cell. While the stochastic differentiation of stem cells eliminates many mutant clones, those clones that persist long term will accumulate further mutations. Should a mutation result accelerate the rate cell division or result in more stem cell than differentiating progeny being produced, the clone will outcompete its wild type neighbours and begin to colonize the tissue [48]. Recently the cellular processes by which clones carrying cancer driver mutations become established in mouse and human tissues, the first step towards the development of cancer, have been revealed by clonal analysis [49].

Figure 3



Stem cell mutation, clonal competition and field change. Somatic mutations conferring a competitive advantage over neighbouring cells will promote clonal expansion. **(a)** In the intestine stem cells null for *Apc* or carrying *Kras* mutations have a competitive advantage (arrowed) over wild type cells and may colonize the entire crypt, after which they will persist long term. **(b)** In oesophagus a Notch inhibiting mutation (DNM, yellow), leads to clonal expansion by accelerating cell division rate, producing more dividing than non-dividing cells at each division and promoting differentiation of neighbouring wild type cells. p53 mutations (black) generate small clones, but double mutant cells with mutant p53 and DNM expand into large double mutant regions, phenomenon known as 'field change'.

Intestine: competing for crypts

Stem cells with a neutral mutation compete with their unmutated neighbours on even terms. However clonal lineage tracing reveals that stem cells lacking the *Apc gene*, which is frequently inactivated in colorectal cancers, have an substantial competitive advantage over wild type cells and a high probability of colonizing an entire crypt (Figure 3a) [50]. Once no wild type cells remain in a crypt the mutant stem cells become permanent residents of the epithelium and may go on to acquire the additional mutations required to develop cancer [49].

Squamous epithelia: short term expansion, long term constraint

Deep sequencing of small areas of normal human sun exposed skin has identified a remarkably high density of clones carrying mutations in genes such as *NOTCH1* and *TP53* that are frequent in squamous skin cancer [51••]. The largest of these clones will have evolved over decades yet they are only slightly larger than clones carrying neutral (synonymous) mutations. This argues that the cells with driver mutations have a short-term advantage over their neighbours, after which their growth is constrained and they revert to homeostatic behavior. This hypothesis is supported by lineage tracing of cells carrying a Notch inhibiting mutation in mouse esophageal epithelium (Figure 3b) [52••]. Initially the mutant clones expand exponentially due to an increase in the cell proliferation and a tilt in cell fate, so more mutant stem cells than differentiating cells result from the average cell division. Later, once all the wild type cells have been expelled from the tissue, mutant cell divisions revert to producing equal proportions of stem and differentiating cells, establishing a new steady state within the tissue [52••].

Blood: aging and mutation

The relentless acquisition of mutant clones with age is also seen in the hematopoietic system. Clones carrying oncogenic genes such as DNMT3A, which promotes myeloid leukemia, are rare in humans under 60, but are found in one in five people over 90 years of age [53••,54].

Concluding remarks

The application of genetic lineage tracing to track stem cells within tissues has revised the concept of the stem cell. In combination with intravital imaging, stem cell dynamics within tissues can be resolved with unparalleled precision. The development of index sorting and single cell transcriptomics may not only allow cells to be assigned to lineages but also may begin to reveal the molecular basis of stochastic self renewal and differentiation of the stem cell populations that sustain adult tissues. Finally deep sequencing has detected an unexpectedly high burden of cells carrying oncogenic mutations. Aging human tissues appear to be a patchwork of mutant clones. This insight may provide a basis for therapies to purge tissues of cells with specific mutations before they transform into cancer.

Acknowledgements

We thank Maria Alcolea for illuminating discussions. BC and PHJ are supported by a core grant from the Wellcome Trust to the Wellcome Trust Sanger Institute. PHJ acknowledges support from a Cancer Research UK Programme Grant (C609/A17257).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Clevers H: **STEM CELLS. What is an adult stem cell?** *Science* 2015, **350**:1319-1320.

2. Wabik A, Jones PH: **Switching roles: the functional plasticity of adult tissue stem cells**. *EMBO J* 2015, **34**:1164-1179.

3. Alcolea MP, Jones PH: **Tracking cells in their native habitat: lineage tracing in epithelial neoplasia**. *Nat Rev Cancer* 2013, **13**:161-171.

4. Clevers H: **The intestinal crypt, a prototype stem cell compartment**. *Cell* 2013, **154**:274-284.

5. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M, Haegebarth A, Korving J, Begthel H, Peters PJ *et al.*: **Identification of stem cells in small intestine and colon by marker gene Lgr5**. *Nature* 2007, **449**:1003-1007.

6. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ *et al.*: **Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche**. *Nature* 2009, **459**:262-265.

7. Sato T, van Es JH, Snippert HJ, Stange DE, Vries RG, van den Born M, Barker N, Shroyer NF, van de Wetering M, Clevers H: **Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts**. *Nature* 2011, **469**:415-418.

8. •• Farin HF, Jordens I, Mosa MH, Basak O, Korving J, Tauriello DV, de Punder K, Angers S, Peters PJ, Maurice MM *et al.*: **Visualization of a short-range Wnt gradient in the intestinal stem-cell niche**. *Nature* 2016, **530**:340-343.
The basis of spatially restricted Wnt signaling, key to intestinal homeostasis is revealed in this study.

9. Lopez-Garcia C, Klein AM, Simons BD, Winton DJ: **Intestinal stem cell replacement follows a neutral drift**. *Science* 2010, **330**:822-825.

10. Snippert HJ, van der Flier LG, Sato T, van Es JH, van den Born M, Kroon-Veenboer C, Barker N, Klein AM, van Rheenen J, Simons BD *et al.*: **Intestinal crypt homeostasis results from neutral competition between symmetrically dividing Lgr5 stem cells**. *Cell* 2010, **143**:134-144.

11. Ritsma L, Ellenbroek SI, Zomer A, Snippert HJ, de Sauvage FJ, Simons BD, Clevers H, van Rheenen J: **Intestinal crypt homeostasis revealed at single-stem-cell level by in vivo live imaging**. *Nature* 2014, **507**:362-365.

12. Alcolea MP, Jones PH: **Lineage analysis of epidermal stem cells**. *Cold Spring Harb Perspect Med* 2014, **4**:a015206.

13. Clayton E, Doupe DP, Klein AM, Winton DJ, Simons BD, Jones PH: **A single type of progenitor cell maintains normal epidermis**. *Nature* 2007, **446**:185-189.

14. Doupe DP, Klein AM, Simons BD, Jones PH: **The ordered architecture of murine ear epidermis is maintained by progenitor cells with random fate**. *Dev Cell* 2010, **18**:317-323.

15. Doupe DP, Alcolea MP, Roshan A, Zhang G, Klein AM, Simons BD, Jones PH: **A single progenitor population switches behavior to maintain and repair esophageal epithelium**. *Science* 2012, **337**:1091-1093.

16. Lim X, Tan SH, Koh WL, Chau RM, Yan KS, Kuo CJ, van Amerongen R, Klein AM, Nusse R: **Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling**. *Science* 2013, **342**:1226-1230.

17. Fullgrabe A, Joost S, Are A, Jacob T, Sivan U, Haegebarth A, Linnarsson S, Simons BD, Clevers H, Toftgard R *et al.*: *Dynamics of Lgr6 progenitor cells in the hair follicle, sebaceous gland, and interfollicular epidermis*. Stem Cell Reports; 2015.

18. •• Rompolas P, Mesa KR, Kawaguchi K, Park S, Gonzalez D, Brown S, Boucher J, Klein AM, Greco V: **Spatiotemporal coordination of stem cell commitment during epidermal homeostasis**. *Science* 2016, **352**:1471-1474.
A remarkable technical achievement, this study combines statistical analysis of lineage tracing data, transgenic proliferation assays and intravital imaging to show a single cell type maintains ear and paw epidermis.

19. Mascre G, Dekoninck S, Drogat B, Youssef KK, Brohee S, Sotiropoulou PA, Simons BD, Blanpain C: **Distinct contribution of stem and progenitor cells to epidermal maintenance**. *Nature* 2012, **489**:257-262.

20. Sada A, Jacob F, Leung E, Wang S, White BS, Shalloway D, Tumbar T: **Defining the cellular lineage hierarchy in the interfollicular epidermis of adult skin**. *Nat Cell Biol* 2016, **18**:619-631.

21. Marques-Pereira JP, Leblond CP: **Mitosis and differentiation in the stratified squamous epithelium of the rat esophagus**. *Am J Anat* 1965, **117**:73-87.

22. Rompolas P, Greco V: **Stem cell dynamics in the hair follicle niche**. *Semin Cell Dev Biol* 2014, **25–26**:34-42.

23. Rompolas P, Mesa KR, Greco V: **Spatial organization within a niche as a determinant of stem-cell fate**. *Nature* 2013, **502**:513-518.

24. Hsu YC, Li L, Fuchs E: **Emerging interactions between skin stem cells and their niches**. *Nat Med* 2014, **20**:847-856.

25. Page ME, Lombard P, Ng F, Gottgens B, Jensen KB: **The epidermis comprises autonomous compartments maintained by distinct stem cell populations**. *Cell Stem Cell* 2013, **13**:471-482.

26. Rompolas P, Deschene ER, Zito G, Gonzalez DG, Saotome I, Haberman AM, Greco V: **Live imaging of stem cell and progeny behaviour in physiological hair-follicle regeneration**. *Nature* 2012, **487**:496-499.

27. Goodell MA, Nguyen H, Shroyer N: **Somatic stem cell heterogeneity: diversity in the blood, skin and intestinal stem cell compartments**. *Nat Rev Mol Cell Biol* 2015, **16**:299-309.

28. Oguro H, Ding L, Morrison SJ: **SLAM family markers resolve functionally distinct subpopulations of hematopoietic stem cells and multipotent progenitors**. *Cell Stem Cell* 2013, **13**:102-116.

29. Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, Ema H, Nakauchi H: **Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells**. *Cell* 2013, **154**:1112-1126.

30. •• Wilson NK, Kent DG, Buettner F, Shehata M, Macaulay IC, Calero-Nieto FJ, Sanchez Castillo M, Oedekoven CA, Diamanti E, Schulte R *et al.*: **Combined single-cell functional and gene expression analysis resolves heterogeneity within stem cell populations**. *Cell Stem Cell* 2015, **16**:712-724.
This paper shows the power of the index sorting approach to reveal heterogeneity in what were thought to be single populations.

31. •• Paul F, Arkin Y, Giladi A, Jaitin DA, Kenigsberg E, Keren-Shaul H, Winter D, Lara-Astiaso D, Gury M, Weiner A *et al.*: **Transcriptional heterogeneity and lineage commitment in myeloid progenitors**. *Cell* 2015, **163**:1663-1677.
See annotation to Ref. [30••].

32. •• Notta F, Zandi S, Takayama N, Dobson S, Gan OI, Wilson G, Kaufmann KB, McLeod J, Laurenti E, Dunant CF *et al.*: **Distinct routes of lineage development reshape the human blood hierarchy across ontogeny**. *Science* 2016, **351** aab2116.
See annotation to Ref. [30••].

33. •• Perie L, Duffy KR, Kok L, de Boer RJ, Schumacher TN: **The branching point in erythro-myeloid differentiation**. *Cell* 2015, **163**:1655-1662.
See annotation to Ref. [30••].

34. van Galen P, Kreso A, Mbong N, Kent DG, Fitzmaurice T, Chambers JE, Xie S, Laurenti E, Hermans K, Eppert K *et al.*: **The unfolded protein response governs integrity of the haematopoietic stem-cell pool during stress**. *Nature* 2014, **510**:268-272.

35. •• Sun J, Ramos A, Chapman B, Johnnidis JB, Le L, Ho YJ, Klein A, Hofmann O, Camargo FD: **Clonal dynamics of native haematopoiesis**. *Nature* 2014, **514**:322-327.
This study reveals the dynamics of hematopoietic stem and progenitor cells in homeostasis.

36. Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, Offner S, Dunant CF, Eshkind L, Bockamp E *et al.*: **Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair**. *Cell* 2008, **135**:1118-1129.

37. •• Busch K, Klapproth K, Barile M, Flossdorf M, Holland-Letz T, Schlenner SM, Reth M, Hofer T, Rodewald HR: **Fundamental properties of unperturbed haematopoiesis from stem cells in vivo**. *Nature* 2015, **518**:542-546.
See annotation to Ref. [35••].

38. Tetteh PW, Basak O, Farin HF, Wiebrands K, Kretzschmar K, Begthel H, van den Born M, Korving J, de Sauvage F, van Es JH *et al.*: **Replacement of Lost Lgr5-positive stem cells through plasticity of their enterocyte-lineage daughters**. *Cell Stem Cell* 2016, **18**:203-213.

39. Buczacki SJ, Zecchini HI, Nicholson AM, Russell R, Vermeulen L, Kemp R, Winton DJ: **Intestinal label-retaining cells are secretory precursors expressing Lgr5**. *Nature* 2013, **495**:65-69.

40. van Es JH, Sato T, van de Wetering M, Lyubimova A, Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J *et al.*: **Dll1+ secretory progenitor cells revert to stem cells upon crypt damage**. *Nat Cell Biol* 2012, **14**:1099-1104.

41. Metcalfe C, Kljavin NM, Ybarra R, de Sauvage FJ: **Lgr5+ stem cells are indispensable for radiation-induced intestinal regeneration**. *Cell Stem Cell* 2014, **14**:149-159.

42. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, Morris RJ, Cotsarelis G: **Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis**. *Nat Med* 2005, **11**:1351-1354.

43. Lu CP, Polak L, Rocha AS, Pasolli HA, Chen SC, Sharma N, Blanpain C, Fuchs E: **Identification of stem cell populations in sweat glands and ducts reveals roles in homeostasis and wound repair**. *Cell* 2012, **150**:136-150.

44. Levy V, Lindon C, Harfe BD, Morgan BA: **Distinct stem cell populations regenerate the follicle and interfollicular epidermis**. *Dev Cell* 2005, **9**:855-861.

45. •• Roshan A, Murai K, Fowler J, Simons BD, Nikolaidou-Neokosmidou V, Jones PH: **Human keratinocytes have two interconvertible modes of proliferation**. *Nat Cell Biol* 2016, **18**:145-156.
This study uses live imaging to capture stem cells switching between two modes of behaviour that underpin homeostasis and wound healing.

46. Mossadegh-Keller N, Sarrazin S, Kandalla PK, Espinosa L, Stanley ER, Nutt SL, Moore J, Sieweke MH: **M-CSF instructs myeloid lineage fate in single haematopoietic stem cells**. *Nature* 2013, **497**:239-243.

47. Alexandrov LB, Jones PH, Wedge DC, Sale JE, Campbell PJ, Nik-Zainal S, Stratton MR: **Clock-like mutational processes in human somatic cells**. *Nat Genet* 2015, **47**:1402-1407.

48. Frede J, Adams DJ, Jones PH: **Mutation, clonal fitness and field change in epithelial carcinogenesis**. *J Pathol* 2014, **234**:296-301.

49. Martincorena I, Campbell PJ: **Somatic mutation in cancer and normal cells**. *Science* 2015, **349**:1483-1489.

50. Vermeulen L, Morrissey E, van der Heijden M, Nicholson AM, Sottoriva A, Buczacki S, Kemp R, Tavare S, Winton DJ: **Defining stem cell dynamics in models of intestinal tumor initiation**. *Science* 2013, **342**:995-998.

51. •• Martincorena I, Roshan A, Gerstung M, Ellis P, Van Loo P, McLaren S, Wedge DC, Fullam A, Alexandrov LB, Tubio JM *et al.*: **Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin**. *Science* 2015, **348**:880-886.

A key paper that reveals the extent of somatic mutation in aging normal human epidermis.

52. •• Alcolea MP, Greulich P, Wabik A, Frede J, Simons BD, Jones PH: **Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change**. *Nat Cell Biol* 2014, **16**:615-622.

This study uncovers the cellular mechanisms by which Notch mutant cells colonise and epithelium and shows how oncogenic mutations may collaborate at the level of cell dynamics.

53. •• McKerrell T, Park N, Moreno T, Grove Carolyn S, Ponstingl H, Stephens J, Crawley C, Craig J, Scott Mike A, Hodkinson C *et al.*: **Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis**. *Cell Reports* 2015, **10**:1239-1245.

This study shows how mutant clones come to dominate hematopoiesis as humans age.

54. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, Kennedy JA, Schimmer AD, Schuh AC, Yee KW *et al.*: **Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia**. *Nature* 2014, **506**:328-333.