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REVIEW

Analyzing stem cell dynamics: use of cutting edge genetic approaches in model organisms

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Abstract **Regeneration of many cell types found in adult organs relies upon the presence of relatively small pools of undifferentiated stem cells. Initial studies that attempted to isolate stem cells and propagate them *in vitro* have been complemented by analysis of stem cells in their endogenous tissues where they are subject to a variety of regulatory cues. This has been facilitated by the advent of new methods for lineage tracing and genetic manipulation of stem cells and their associated niche cells. The picture that is emerging is that different stem cell populations utilize diverse processes to ensure maintenance of the stem cell pool accompanied by production of cells committed to regenerate differentiated cells.**

Keywords stem cells, *Drosophila*, mouse, testis, intestine, lineage tracing

Introduction

Stem cells are the key to organ regeneration and tumor growth

The ability to replenish differentiated cells depends on the continued survival and proliferation of their respective stem cell populations. The mechanisms that govern the rate of stem cell division are also crucially important, as they regulate the number of differentiating cells in populations derived from stem cell founders. Stem cells are not only important for regeneration of healthy tissues but also play a key role in pathogenesis. Recent studies have demonstrated that all cells in solid tumors do not play equal roles but a small fraction of cells, the so-called cancer stem cells, contribute to the unlimited growth of the tumor and re-occurrence after tumor resection (Schulenburg et al., 2006; O'Brien et al., 2007; Ricci-Vitiani et al., 2007). Stem cell mitosis results in daughters that may either retain stem cell identity or become committed to differentiation, and in many cases eventual apoptosis. The balance between these choices is crucial; alteration can lead to disastrous consequences, including over-proliferation or loss of the stem cell population. If we are to realize the goals of re-programming tissue differentiation, growing organs for transplantation *in vitro*, regeneration of damaged organs *in vivo* and targeted effective treatments for cancer, it is essential that we understand the molecules and mechanisms that stem cells utilize for renewal and differentiation. The basis for our current knowledge of stem cell dynamics has come from a long history of experiments conducted in both vertebrate and invertebrate tissues that have each provided unique contributions to our overall understanding of stem cell function and regulation.

Identification of stem cells – how do we know they are present?

It has been known for centuries that certain organ systems have the capacity to regenerate, as anyone who has wounded their epidermis or donated blood would understand. In 1909 Alexander Maximow referred to the lymphocyte as a stem cell (Stammzelle) or precursor to the mature cell types found in the different blood cell lineages (Maximow, 1909). Experimental identification of the presence of hematopoietic stem cells did not occur until 1963 when McCulloch and Till isolated cells from mouse bone morrow and transplanted them into irradiated mice. Nodules of proliferating hematopoietic cells occurred in the spleens in direct proportion to the

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numbers of cells that were first injected. By conducting a series of limiting dilution experiments McCulloch and Till were able to estimate the number of regenerative stem cells present in normal bone marrow (Becker et al., 1963). *In vitro* colony forming assays have also been routinely used to determine stem cell numbers but this method is dependent upon availability of culture conditions that support stem cell survival and proliferation (Coulombel, 2004). Simply measuring proliferative potential also provides no indication of the potency of the cells – they may be able to form colonies but can they produce all of the differentiated progeny normally found in the tissue of origin?

Identification of quiescent or label retaining cells

The notion that stem cells were generally quiescent or only underwent cell division infrequently led to the concept that they would maintain nucleotide analogs (e.g., ^{3}H labeled thymidine) for many days or weeks after exposure to the label and hence populations of cells termed "long term label retaining cells" were suggested to mark stem cells in various epithelia. We now understand that stem cell dynamics vary greatly between tissues and hence not all stem cell populations will fall into the long-term label retention class. For example, there are now well characterized examples of rapidly proliferating stem cells in the stomach, small intestine and colon indicating that quiescence is not a universal hallmark of stemness (Clevers, 2013).

Defining stem cell activity by transplantation assays

The gold standard for identification of stem cells is the capacity to reconstitute a tissue and sustain continual tissue renewal for long periods of time. Transplantation assays have commonly been used to assess this. Again, hematopoietic stem cells led the way in development of this assay. Mice subjected to a high enough dose of radiation will die due to complete ablation of hematopoietic stem cells. They can be rescued by transfusion of bone marrow derived stem cells which indicates that these cells have the capacity to produce all of the differentiated cells of the hematopoietic lineages (Kaur et al., 2004). Marking of transplanted cells with lacZ (e.g., using the ROSA-lacZ mouse as a tissue donor) or with fluorescent markers such as RFP (Yui et al., 2012) or GFP, permits observation of donor fate within the host. This technique has facilitated a method for estimating the number of spermatogenic stem cells present within the mouse testis (Oatley and Brinster, 2012). Marked germ cells can be transplanted into the rete testis of mice that are devoid of germ cells (due to genetic background or treatment with a germ cell toxin such as busulfan) and stem cells will home to niches within seminiferous tubules. The number of marked colonies that are seeded by the grafts provide a method to calculate the number of stem cells that were present in the transplanted tissue. Reconstitution assays were not feasible for epithelial tissues for many years as most untransformed epithelial cells undergo anoikis (a specific form of apoptosis) when removed from their underlying basement membrane (Paoli et al., 2013). Recently developed culture conditions have allowed stem cells derived from neuroepithelia and intestinal epithelia to be grown as neurospheres (Reynolds and Weiss, 1992) or organoids (Sato et al., 2009; Sato et al., 2011a, 2011b) respectively, but it is still challenging to transplant these cells into endogenous tissues. This may not be impossible with accessible tissues as it has been shown that introduction of organoids into the lumen of the mouse colon can result in re-seeding of stem cells and generation of functional epithelium into areas of induced tissue damage (Yui et al., 2012) and breast stem cells can be efficiently transplanted into mammary fat pads (Shackleton et al., 2006; Visvader and Stingl, 2014). The ultimate experiment to define the identity and developmental potential of stem cells is to demonstrate the ability of a single isolated cell to repopulate a tissue. Transplantation of single hematopoietic (Osawa et al., 1996), muscle satellite (Sacco et al., 2008) and mammary stem cells (Shackleton et al., 2006) have quite dramatically demonstrated the ability of these cells to generate all cells in the tissue.

***In vivo* lineage tracing assays for analysis of stem cell potential**

Although transplantation studies and culture techniques clearly demonstrate the ability of cells to generate different cell types, they do not demonstrate whether these cells normally produce particular cell types within their endogenous environment. The technique that has revolutionized identification and analysis of epithelial stem cells within tissues is lineage tracing. This technique was originally developed to follow the fate of specific cells in embryos (Conklin, 1905) and relies upon the ability to mark individual cells in a permanent manner such that the mark is transferred to descendants of the marked cell and is maintained as cells differentiate within their endogenous environment. If a stem cell has the ability to reconstitute a tissue it will produce a clone of marked mitotically related daughters that will then differentiate into specific marked mature cell types. By analyzing a tissue at successive time points after cell marking it is possible to determine the time course and pattern of cell differentiation (Kretzschmar and Watt, 2012). Lineage tracing is commonly achieved using mouse models where a hormone-inducible Cre recombinase enzyme is expressed under the control of a putative stem cell population gene promoter. These mice are then crossed with the R26-LacZ or GFP reporter mouse. Upon induction via hormone injection, the Cre enzyme excises a stop codon within the LacZ or GFP gene activating irreversible expression of the marker gene that can be visualized with tissues. The generation of individual clones can be monitored over time providing information on the kinetics of cell growth, production of multiple differ-

entiated cell types and longevity of tissue renewal (Barker et al., 2007).

A very powerful extension of this approach for examining the dynamics of individual stem cell clones over time has been the use of multi-color Cre reporter animals. This approach was originally used to produce "Brainbow" mice with multi-colored neurons (Livet et al., 2007). The use of the R26R-Confetti allele has allowed the behavior of different individual stem cells and their descendants to be followed in numerous tissues including the intestine (Snippert et al., 2010) and the mammary gland (van Amerongen et al., 2012; Rios et al., 2014).

Interestingly, the differentiation capabilities of some stem cell populations have been shown to differ in transplantation versus lineage tracing experiments. Putative stem cells isolated from the bulge region of hair follicles give rise to three cell lineages of the skin in transplantation assays but have a more limited differentiation potential when followed within skin tissue (Morris et al., 2004; Jaks et al., 2008). Similarly, the same mammary stem cell population can display a different potential when transplanted to that observed within the tissue by lineage tracing (van Amerongen et al., 2012). These differences may represent a different potential for cells to differentiate in response to injury which may be mimicked by transplantation assays.

Regulation of stem cell activity by the environment

The survival, proliferation and differentiation of stem cells are regulated by both extrinsic and intrinsic factors. The environment in which a stem cell is located provides molecules that can influence activity of cell surface receptors, adhesion molecules and thereby multiple intracellular signaling pathways. The importance of the hematopoietic stem cell environment was identified by Schofield who coined the term "stem cell niche" (Schofield, 1978). Experimental evidence for the niche hypothesis was not forthcoming until analysis of germline regeneration in *Drosophila* ovaries (Xie and Spradling, 2000) and testes (Kiger et al., 2001; Tulina and Matunis, 2001) identified that somatic support cells acted to maintain germline stem cells in accordance with the niche theory. This concept of a niche has now been examined in many tissues in different species and it is clear that signals from the environment are key modulators of stem cell behavior in many contexts.

Repressors of gene expression play critical roles in maintaining the stem cell state

Many studies have attempted to identify "stem cell factors," proteins that confer pluripotency or "stemness" to undifferentiated cells. The key to maintaining stem cells may not lie with a factor that gives a cell "stemness" but with factors that prevent differentiation. The Jaenisch laboratory demonstrated that the Polycomb complex assists in maintenance of both mouse and human embryonic stem cells by transcriptional repression of genes that play roles in organ development and differentiation (Boyer et al., 2006; Lee et al., 2006). Recent work has suggested that repressors of gene expression play key roles in multiple stem cell populations to prevent expression of differentiation factors. We have shown that the translational repressor Musashi is required for maintenance of *Drosophila* germline stem cells (Siddall et al., 2006; Hime et al., 2007) and that the RNA binding protein, HOW, prevents accumulation of a stem cell differentiation factor (Monk et al., 2010). In the mouse intestinal epithelium Wnt signaling is required for stem cell maintenance and we have recently identified the transcriptional repressor Snai1 as a target of Wnt signaling in these cells (Horvay et al., 2011). The *Drosophila* testis and mouse intestinal epithelial stem cell systems will be further outlined below.

Anaysis of stem cell populations in two organ systems

Drosophila and mouse organs–complementary models of stem cell function

The identification of mechanisms that regulate asymmetric division, daughter cell mitotic amplification and stem cell differentiation have been traditionally difficult to ascertain. The relative complexity of stromal cell – stem cell interactions has limited investigations of these processes. These types of studies have recently benefited greatly from the analysis of genetically tractable systems. For these reasons we have chosen to focus on the *Drosophila* male germ line stem cell niche and the mouse small intestinal stem cell niche as models for studying both intrinsic regulation of stem cell fate and the influence of the surrounding niche (Table 1). Many of the techniques described here would also be applicable to other stem cells systems in *Drosophila* or mice such as those required for regeneration of hematopoietic or neural tissue.

Model 1 – Several conserved stem cell factors have been identified via genetic analysis in the Drosophila testis

Adult *Drosophila* males contain a pair of testes that can be observed as long coiled tubes that are closed at the apical ends and open at the basal ends into seminal vesicles. Each seminal vesicle empties into a common ejaculatory duct, along with a pair of accessory glands that serve to provide components of seminal fluid that are critical for fertility. The testis contains two stem cell populations that maintain the germ cells (germline stem cell, GSC) and somatic (cyst stem cell, CySC) components of the tissue (Fuller, 1993). In contrast to many tissues, the identity of the stem cells, their morphology and physical relationship to surrounding cells are known (Hardy et al., 1979). Each testis contains 8–10 GSCs which lie clustered around a central, somatically-derived hub located at

Table 1 Advantages offered by analysis in the two systems

Mouse intestinal epithelial stem cell niche	1. Mammalian system relevant for modeling human intestinal diseases
	2. Well characterized Cre drivers are available for genetic manipulation
	3. Tissue is accessible to molecules/cells placed in the lumen
	4. Tissue can be grown and manipulated *in vitro* as whole organ culture or as organoids
Drosophila testis stem cell niche	1. A simple, well characterized stem cell niche
	2. Numerous cell specific Gal4 drivers are available for genetic manipulation
	3. *Drosophila* genetics allow complex genetic manipulations
	4. The rapid life cycle of *Drosophila* allows multiple alleles to be introduced into an animal

the apical tip of the testis (Lindsley and Tokuyasu, 1980). GSCs divide with an oriented mitosis such that the mitotic spindle is positioned orthogonally to the plane of adhesion to the hub. This strictly oriented division occurs due to migration of the nascent centrosome to the opposite pole of the cell prior to spindle formation (Yamashita and Fuller, 2005). The daughter cell that maintains contact with the hub retains stem cell identity, while the other daughter becomes committed to differentiation (Spradling et al., 2001). This asymmetric division became a general model for how stem cells maintain the stem cell pool and produce daughters committed to differentiation but strictly oriented divisions may be more the exception than the rule. CySCs also undergo an asymmetric division but utilize a different mechanism – repositioning of anaphase spindles that were originally randomly oriented with respect to the hub (Cheng et al., 2011). As considered in our discussion of mouse intestinal epithelial stem cells, a stochastic model of stem cell differentiation may prevail in many organ systems.

The GSC niche consists of the hub and the CySCs, which are not only stem cells but crucial niche cells for their neighboring GSCs (Leatherman and DiNardo, 2008; Leatherman and Dinardo, 2010). The GSCs are anchored to the hub via adherens junctions (Yamashita et al., 2003) and prevented from differentiating via exposure to short-range signaling proteins of the BMP family, Dpp and Gbb (Shivdasani and Ingham, 2003; Bunt and Hime, 2004; Kawase et al., 2004). The BMP proteins are secreted by the hub and CySCs and stimulate SMAD activity in the GSCs which acts to repress expression of the *bag-of-marbles* (*bam*) gene. BAM protein levels gradually increase in the transit amplifying spermatogonial daughters of the GSCs until a threshold level is reached that triggers differentiation into spermatocytes (Insco et al., 2009). BAM must be excluded from GSCs as ectopic expression of BAM in GSCs results in cell death (Shivdasani and Ingham, 2003). GSC survival also depends upon JAK/STAT signaling in CySCs that is induced via a cytokine-like ligand, UPD, exclusively produced by the hub in *Drosophila* testes (Kiger et al., 2001; Tulina and Matunis, 2001; Leatherman and Dinardo, 2010). The CySCs require JAK/STAT and another hub specific ligand, Hedgehog, in order to self-renew (Amoyel et al., 2013). Apart from undergoing renewal, CySCs produce differentiated cyst cells that no longer have the capacity to divide but enclose the spermatogonial daughters of GSCs and provide signals that regulate spermatogonial mitosis and differentiation (Fuller, 1993). As testes age the number of GSCs in the stem cell pool declines and this has been shown to be due to an age-related decline in UPD expression in the hub cells (Boyle et al., 2007). The notion that aging of the niche directly influences organ senescence has also been described in vertebrate systems. For example, elegant transplantation experiments conducted in mouse testes has shown that although stem cell numbers decrease with age (to a point of resultant infertility) if spermatogenic stem cells from young mice are serially transplanted into young niches they can be maintained well past the point of age-related sterility observed in normal males (Oatley and Brinster, 2012).

Genetic studies in the *Drosophila* testis have identified many molecules that are specifically required in stem cells for maintenance: JAK/STAT pathway members (Kiger et al., 2001; Tulina and Matunis, 2001), BMP pathway (Shivdasani and Ingham, 2003; Bunt and Hime, 2004; Kawase et al., 2004; Schulz et al., 2004), APC (Yamashita and Fuller, 2005), Musashi (Siddall et al., 2006), How (Monk et al., 2010), BHD (Singh et al., 2006); as well as molecules required in the stem cell niche to regulate stem cell division and differentiation: EGFR pathway (Kiger et al., 2000; Tran et al., 2000), BMP pathway (Matunis et al., 1997), Hedgehog/Patched (Amoyel et al., 2013), and the steroid hormone, ecdysone (Li et al., 2014; Qian et al., 2014).

Most of the information that we have gained about the molecules that influence GSC biology have come from either gain or loss of gene function studies within the GSCs or the stem cell niche. The Gal4-UAS bipartite expression system can be used to express protein encoding transgenes or shRNAi molecules in specific cell types within the testis. The Gal4 transcription factor derives from the yeast, *Saccharomyces cerevisiae*, and has no endogenous targets within the *Drosophila* genome. Only when the Gal4 target sequence, Upstream Activating Sequence, along with a miminal promoter sequence is introduced upstream of gene of interest is that gene expressed. This means that the Gal4 driver and the UAS responder can be maintained as separate transgenic lines and ectopic gene expression will only occur in progeny derived from parents carrying each of these transgenes (Brand

and Perrimon, 1993). This makes it possible to study the effects of gene expression that may result in sterility, or lethality, and otherwise prevent maintenance of transgenic lines (Figs. 1A and 1B). Multiple Gal4 driver lines are available that target gene expression to different cell types within the testis (Bunt et al., 2012).

Many of the genes that have been associated with regulation of GSC dynamics are required for development of embryonic or larval tissues and hence loss of function mutations in these genes would result in animal lethality and preclude analysis in the adult testis. Analysis of these genes has benefitted from conditional loss of function techniques that utilize a recombination system derived from *S. cerevisiae*, known as FLP-FRT. The FLP recombinase also has no targets in the *Drosophila* genome but can induce recombination at FRT sequences that have been transgenically engineered at specific locations within *Drosophila* chromosomes. The FLP recombinase is most often expressed from a heat shock (Hsp70) promoter that permits a pulse of temperature increase (from the normal culture temperature of 25° to 37°) to induce FLP expression in a random subset of cells (Xu and Harrison, 1994). Recombination between FRT sequences on homologs will only occur in mitotically active cells (GSCs and their spermatogonial daughters or CySCs). If recombination occurs in a heterozygous animal where a mutation is present on one chromosome and a GFP marker on its homolog then homozygous mutant germ cells can be identified and tracked via an absence of GFP. Heatshock conditions have been defined such that only 1–3 mutant GSCs are produced per testis. This not only allows observation of mutant GSCs in a wildtype tissue background but also allows the fate of their progeny to be lineage traced via following cells that do not express GFP (Fig. 1C).

Many enhancements and modifications have been made to the original Gal4-UAS and FLP-FRT systems including combining them to permit visualization of transgene expression in a specific cell type and simultaneous lineage tracing of its daughters (the G-Trace system) (Evans et al., 2009).

The *Drosophila* testis has provided unique insights into how stem cells regulate survival, division and differentiation and many molecules that have been found to be utilized in this organ have been subsequently studied in other *Drosophila* and vertebrate organs. As an example of a vertebrate organ that has been well studied and has developed unique methods of gene manipulation we will outline dynamics of mouse intestinal epithelial cells.

Model 2 – The intestine is a key model of a stem cell compartment within a regenerative organ

The mouse intestine has been extensively studied as a model of human intestinal development and tumorigenesis. The small intestinal epithelium is organized into crypts and villi surrounded by mesenchyme (Sancho et al., 2003; Clevers, 2013). Intestinal stem cells and proliferating transit amplify-

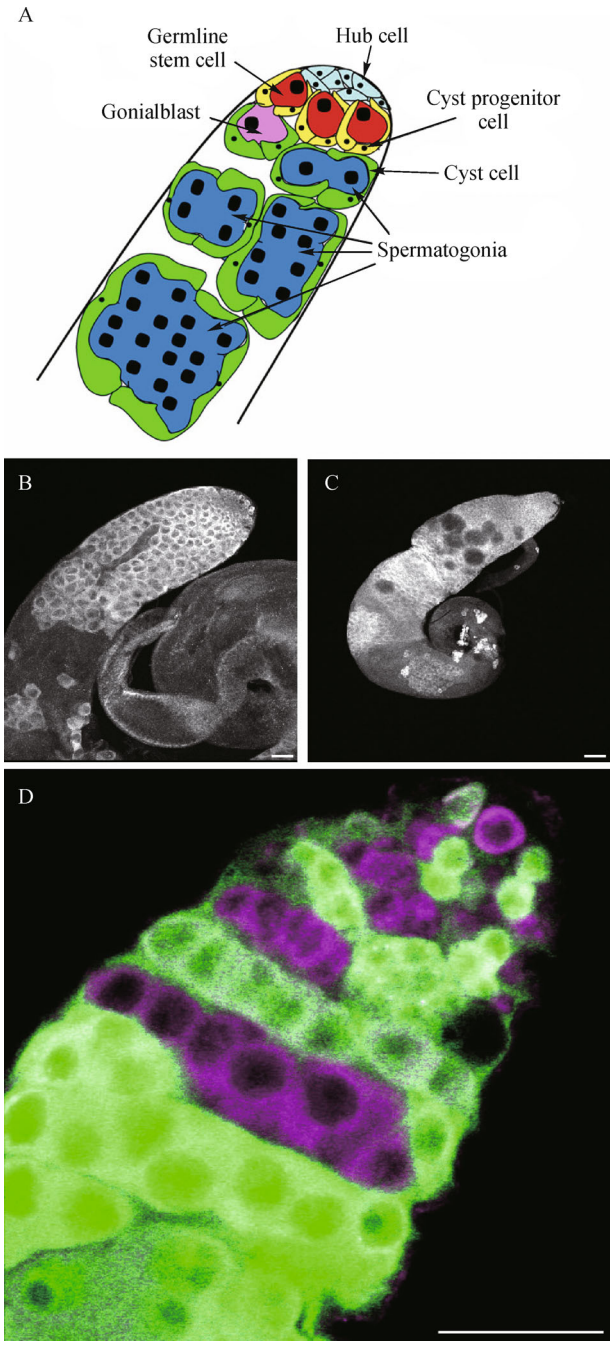

Figure 1 Gene manipulation in *Drosophila* male germline stem cells. (A) Schematic representation of the apical tip of the testis. Hub cells (light blue), Cyst progenitor cells (yellow), Germline stem cells (red), Gonialblast (pink), Spermatogonia (dark blue) and Cyst cells (green) are shown. Adapted from Siddall et al. (2006). (B) Wholemount immunohistochemistry in wild type testis demonstrates that GSCs, mitotic spermatogonia and spermatocytes all express Vasa. (C) A mutant testis where the BMP protein, Dpp, has been ectopically expressed in germ cells using NosGal4×UAS-Dpp. Staining with Vasa demonstrates that spermatogonia fail to differentiate (compared with A). (D) Lineage tracing in a wild type testis via Heatshock-Flp recombinase and an FRT GFP chromosome. A GFP negative stem cell has given rise to cysts of GFP negative spermatogonia and spermatocytes (marked with anti-Vasa, purple). Scale bars are 20 microns.

ing cells reside in crypts and are responsible for regeneration of the entire epithelium every 3–5 days. Transit amplifying cells differentiate and migrate toward the villi where they differentiate into absorptive enterocytes, secretory goblet cells and secretory enteroendocrine cells that reside throughout the crypt-villus axis. Differentiated CD24 + ve Paneth cells, form and occupy the crypt base where they act as niche cells for the adjacent stem cells (Sato et al., 2011b). The colon is similar in overall cellular organization but lacks villi and Paneth cells, although CD24 + ve cells are present and act as a niche (Rothenberg et al., 2012). Gene knockouts have shown a central role for Wnt signaling in maintaining ISCs (de Lau et al., 2007). Knockout studies have also defined important roles for c-Myb (Cheasley et al., 2011), BMP (Haramis et al., 2004; He et al., 2004), Eph/Ephrins (Holmberg et al., 2006) and Notch signaling in regulating ISCs (Fre et al., 2005; van Es et al., 2005). Rapid advances have been made in the identification of stem cell markers and analysis of the kinetics of stem cell behavior using a variety of genetic mouse models. Cheng and Leblond originally described the presence of small, crypt base columnar (CBC) cells localized between the Paneth cells at the base of crypts (Cheng and Leblond, 1974). Lgr5 marks this cell population and was originally identified in a screen designed to detect Wnt target genes in intestinal cells (Barker et al., 2007). Lgr5 encodes a G protein-coupled receptor for the Wnt agonist R-Spondin (Carmon et al., 2012). The generation of Lgr5-EGFP-IRES-CreERT2 mice by introduction of EGFP and a tamoxifen-inducible Cre recombinase (CreERT2) into the Lgr5 gene locus verified expression of Lgr5 in CBC cells by reference to EGFP expression. When crossed to the R26-LacZ Cre reporter strain, lineage tracing experiments can be performed. Following a single dose of tamoxifen LacZ expression can be induced in CBC cells. Over time, ribbons of blue cells are generated containing all epithelial cell types demonstrating that Lgr5 positive CBC cells are multipotent and long lived (Barker et al., 2007) (Fig. 2). Using the R26R-Confetti allele, the stem cell dynamics within intestinal crypts have been examined and no evidence for asymmetric division of CBC cells has been obtained. These studies indicate that Lgr5 positive stem cells divide symmetrically and then undergo a neutral competition process for positioning in the niche (Snippert et al., 2010). Cells that remain in contact with niche Paneth cells retain stemness while those that move away via a stochastic process become committed to a differentiation pathway. Genomic and proteomic analyses of CBC stem cell populations have identified several other markers and key regulators of this population including *Ascl-2*, *Olfm4* (van der Flier et al., 2009), *Smoc2*, *Rnf34* and *Znrf3* (Clevers, 2013). Other studies have shown that slow cycling stem cells labeled by *mTert/Bmi1/Hoxp* are present in the + 4 cell position just above the Paneth cells (Fig. 2) that can replenish the CBC stem cell pool following damage (Sangiorgi and Capecchi, 2008; Montgomery et al., 2011; Tian et al., 2011). However, recent studies have indicated considerable plasticity of cell potential under conditions of damage and subsequent regeneration (Tetteh et al., 2014).

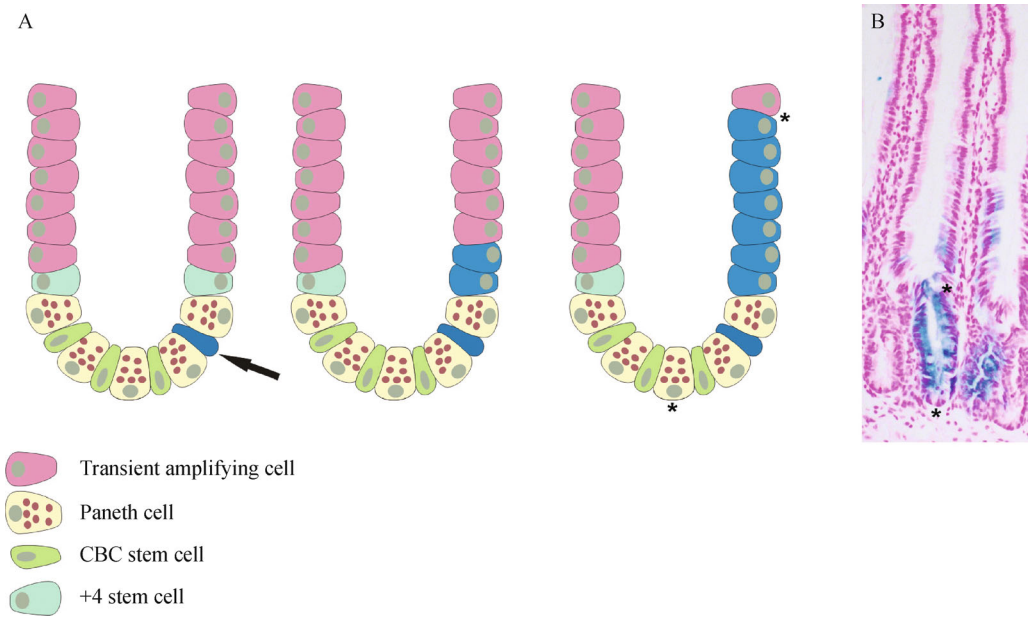

Figure 2 Lineage tracing in the mouse small intestinal epithelium. (A) Schematic of lineage tracing (blue, lacZ positive cells) from the Lgr5 promoter in a CBC stem cell (arrow) following induction of CreER with tamoxifen over a period of several days. (B) Lineage tracing from Lgr5-EGFP-IRES-CreERT2 and ROSA-loxP-STOP-loxP-lacZ. A ribbon of lacZ positive cells (blue) can been seen in a crypt and extending into a villus. The extent of the crypt in the schematic (A) is indicated by asterisks on the micrograph (B). This experiment was originally demonstrated by Barker et al. (2007).

In vitro intestinal culture systems and transplantation assays

The ability to culture intestinal tissue facilitates biological analyses as the tissue is accessible for experimental manipulation and live cell imaging. There are also many potential clinical applications where expansion of stem cell populations *in vitro* could be used to treat degenerative diseases where the intestinal epithelium is damaged. For many years, the only *in vitro* culture systems for the study of intestinal cell biology were epithelial cell lines derived from colorectal tumors that contain many chromosomal abnormalities. Endogenous stem cells are highly dependent on niche factors for survival and when removed from the underlying mesenchyme rapidly undergo apoptosis. There are now several culture systems available where either the mesenchyme is retained or replaced by an environment that recapitulates intrinsic niche factors. These include culture of intact segments of embryonic gut (Abud et al., 2004; Abud et al., 2005), neonatal intestinal culture (Ootani et al., 2009) and adult organoid culture (Sato et al., 2009; Sato et al., 2011a, 2011b). Many of these systems are also amenable to genetic manipulation (Abud et al., 2004; Schwank et al., 2013a, 2013b). Organoids can be established from different regions of the intestinal tract including stomach, small intestine and colon and are maintained in matrigel supplemented with growth factors. The organoids retain the overall organization of the tissue *in vivo* with stem cells, transit amplifying cells and differentiated cell types present. Importantly, organoids have now been established from human tissue and have the potential for establishing models of human disease and to provide tissue for transplantation therapies (Sato and Clevers, 2013). Studies in mice have demonstrated that intestinal organoids can repair regions of epithelial damage in an experimental model of colitis and may prove a viable technique for treatment of patients with conditions such as ulcerative colitis (Yui et al., 2012).

Conclusion

As more studies are conducted in multiple organ systems it is becoming clear that different stem cell populations are regulated via diverse mechanisms. Some systems utilize strict modes of asymmetric division while in others a stochastic process determines if daughter cells maintain a stem cell fate or become committed to differentiate. Even the term “committed” has become problematic as there is now evidence from both *Drosophila* and mouse models that transit-amplifying cells or other differentiated cell types are capable of de-differentiation and re-population of a vacant stem cell niche. Some basic general rules seem to apply to stem cell behavior: stem cells are found in close association with niche cells that utilize intercellular signals to assist in maintenance of the stem cell fate and stem cells interpret these signals to maintain that fate by expression of factors that repress genes involved in developmental processes and differentiation. The cell biological and bioinformatic tools that are now available to allow manipulation and comparison of stem cell systems across phyla will provide information to elaborate the general rules and define the tissue specific functions that regulate maintenance and differentiation of stem cell populations.

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Compliance with ethics guidelines

Gary Hime, Nicole Siddall, Katja Horvay and Helen Abud declare that they have no conflict of interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

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