# What is a stem cell?

**Jonathan M.W.Slack**
**(ORCID ID: slack017@umn.edu)**
**Dept. Biology and Biochemistry,**
**University of Bath,**
**Bath BA2 7AY, UK**

**email: j.m.w.slack@bath.ac.uk**
**No conflicts**

**Article type: Overview**

**Abstract**

The historical roots of the stem cell concept are traced with respect to its usage in embryology and in hematology. The modern consensus definition of stem cells, comprising both pluripotent stem cells in culture and tissue-specific stem cells in vivo, is explained and explored. Methods for identifying stem cells are discussed with respect to cell surface markers, telomerase, label retention and transplantability, and properties of the stem cell niche are explored. The CreER method for identifying stem cells in vivo is explained, as is evidence in favor of a stochastic rather than an obligate asymmetric form of cell division. In conclusion it is found that stem cells do not possess any unique and specific molecular markers; and stem cell behavior depends on the environment of the cell as well as the stem cell's intrinsic qualities. Furthermore the stochastic mode of division implies that stem cell behavior is a property of a cell population not of an individual cell. In this sense, stem cells do not exist in isolation but only as a part of multicellular system.

NOTE: Although this is a new article, some sections of text and some figures have been taken from chapter 1 of "The Science of Stem Cells" by J.M.W.Slack; Wiley (2018). These are reproduced by permission of John Wiley & Sons Inc.

**Graphical/Visual Abstract and Caption: use Figure 3**

**Introduction**

Interest in stem cells continues to accelerate, as may be seen from the exponential increase in the number of papers on the topic over the last four decades (Fig.1). Because of this rise in interest, many biomedical scientists have felt that their chances of funding would be improved if they were seen to be working on stem cells, and this has led to the dilution of what is meant by the term "stem cell" to a level that becomes unhelpful. Sometimes virtually any cell that divides is called a stem cell. Of course the meaning of a term depends on how it is defined and if all cells that divide are to be called stem cells then this might be considered a legitimate usage. However definitions cease to be useful if they become all-embracing. To remain useful they need to accord with reality as much as possible, or, as philosophers say, correspond to a "natural kind" [1].

There are two natural kinds of stem cell: the pluripotent stem cell growing in culture, otherwise known as embryonic stem cells (ESC) or induced pluripotent stem cells (iPSC); and the tissue-specific stem cells which maintain the turnover of certain tissue types in mammals and other animals. Each of these has its own historic background in terms of nomenclature, in the course of which the stem cell concept was created and refined. In the end these two entities do have enough in common to share the same name, but they also have many differences. Here I shall look briefly at the history of these two traditions and then enquire how well various methods used today measure up in terms of detecting stem cells. Finally I shall review evidence indicating that stem cells do not exist outside their environmental context and that stem cell behavior is an emergent property of a multicellular system rather than of a single cell.

## Formulation of stem cell concepts

Early uses of the term "stem cell" are reviewed by Maehle [2]. The German equivalent, Stammzelle, was first used by Haekel in an evolutionary sense to represent the most primitive unicellular organisms from which all others had evolved. Later he applied the term to the fertilized egg, as the cell of origin of all cells in a single organism. Boveri later applied the term to the cell lineage in the nematode *Ascaris*, that underwent repeated divisions generating offspring of different fate before becoming the germ line. Following these early works, the term "stem cell" is barely mentioned in the first half of the twentieth century. Wilson, following Boveri, uses it to indicate the cells of nematode embryos undergoing reiterated divisions before becoming the germ line (Fig.2). However, "stem cell" does not figure in most embryological monographs written up to 1980, for example those by Spemann, Kumé and Dan, or Davidson [3-5]. One exception is the development of the leech. Here a number of cells called teloblasts undergo repeated divisions to generate segmentally reiterated progenitors and these were sometimes called stem cells [6]. In conclusion, the embryological tradition made little use of the term stem cell, and where it was used it was associated most clearly with reiterated division to produce more committed descendants.

The historical background to embryonic stem cells is reviewed by Solter [7]. It arises more from cancer biology than from direct studies on embryos. Tumors called teratomas or teratocarcinomas had been known since antiquity and were unusual in containing a variety of structures and tissue types [8]. They often occur in the gonads and by the early 20th century they were considered to be formed from germ cells by a deranged version of embryonic development. In 1954 Stevens discovered that mice of strain 129 spontaneously developed testicular teratomas, albeit at low

frequency. The tumors could be transplanted to other immunocompatible mice, and could be cultivated in vitro. Subsequently it was found that the LT strain showed an elevated number of ovarian teratomas. The embryonic nature of these tumors became well established when it was found that they could be initiated by transplanting fetal genital ridges or whole mouse embryos to extragonadal sites. Cell lines grown in vitro from the tumors became known as teratocarcinoma or embryonal carcinoma (EC) cells. A single cell from a teratoma transplanted to a new host could initiate another tumor contained a wide range of structures and tissue types [9]. Even in the 1950s, the idea of cancer stem cells existed [10] and the initiating cells of the teratomas were sometimes called stem cells, although were more usually referred to as embryonal carcinoma (EC) cells. It was found that in rare cases, EC cells could become integrated into early stage embryos of mice and that the cells were able to contribute to multiple tissues of the resulting adult mouse [11]. It was thus apparent that the EC cells were pluripotent and that their malignant quality could, at least on occasion, be suppressed by the environment of the early embryo.

An important technical advance rendering the in vitro culture of EC cells much more reproducible was the introduction of feeder layers of irradiated fibroblasts which secreted factors supporting the growth of the EC cells. Using feeder cultures or conditioned medium the growth of pluripotent cells directly from mouse blastocysts was achieved by Evans and Kaufman and, independently, by Martin [12, 13]. These cells were able to grow without limit in culture. They could differentiate into embryoid bodies in vitro, and could form teratomas when grafted into immunocompatible animals. They could participate in mouse embryo development when injected into blastocysts, and even generate germ line chimeras capable of producing mice in the next generation, carrying the genes of the ES cells [14]. In the original papers Evans and Kaufman did not use the term stem cell although Martin did. The cells they discovered, or rather created, became generally known as embryonic stem (ES) cells. In this case the principal characteristic underlying the name is the pluripotent behavior of the cells.

The most robust lineage of the concept of stem cell resides in the literature on hematopoiesis (formerly spelled hemopoiesis). The idea that all cells of the blood derived from a single type of cell in the bone marrow is usually attributed to the Russian histologist Maximow (also spelled Maximov or Maksimov) [15] although the German hematologist Pappenheim may have been the first to call this hypothetical cell type a Stammzelle [2]. The term "stem cell" was often used thereafter in works on hematology and histology to indicate blood precursor cells in the bone marrow [16, 17]. In the mid-twentieth century interest in the stem cell concept re-emerged in the context of radiation biology combined with the study of hematopoiesis. According to Maximow's unitary theory, all blood cells (myeloid and lymphoid) arose from a common precursor, later called the 'hemocytoblast" and then the "hematopoietic stem cell" (HSC). But this theory was not universally accepted and until recent decades many considered that lymphoid cells had a different origin. Since the evidence at the time depended simply on histological study of normal marrow or on observations of hematopoiesis in various pathological situations, it was not possible to distinguish between the unitary and other theories. This had to await the development of techniques for the genetic marking of cells in vivo.

It had been discovered during wartime research on atomic weapons that a graft of bone marrow could rescue irradiated animals from otherwise certain death. In 1956 it was shown using visible chromosome markers that the active ingredient of the marrow was cells, rather than some

sort of hormone [18]. In 1961 Till & McCulloch found colonies of hematopoietic cells in the spleens of lethally irradiated mice that had received bone marrow grafts [19]. These colonies contained erythroid, granuloid and megakaryoid cells and it was soon shown that they could generate further colonies on retransplantation into another irradiated host. The colonies were found to be clones using chromosomal markers [20]. In the original paper the authors do not themselves mention stem cells, and cautiously call the initiating cells "colony forming units-spleen" (CFU-S). However from the time of their discovery the CFU-S were generally considered to be hematopoietic stem cells. Later it became apparent that they were what we should now call multipotent progenitors rather than true stem cells, for two reasons: first they do lose potency on retransplantation considerably faster than normal marrow [21, 22]; secondly they did not form lymphocytes and by this time it was becoming accepted that the "true" endogenous HSC generated lymphoid as well as myeloid cells [23].

An influential paper by Cairns [24] assumed that stem cells persist for the entire life of the organism and investigated the mechanism whereby such long lived cells might evade or reduce the number of somatic mutations that could initiate cancers. Lajtha (pronounced "Loitha") [25] summarized the state of affairs as perceived by radiation biologists/hematopoiesis specialists/leukemia specialists and came up with the conclusion that hematopoietic stem cells survive at least as long as the whole organism, and that they are slower dividing than their progeny (the transit-amplifying cells). At about the same time Schofield [26] proposed that the true stem cells occupied a fixed number of niches in the bone marrow and that the immediate progeny of a stem cell could remain a stem cell so long as it occupied a niche. By 1983, Potten could edit a comprehensive collection of reviews which extended the stem cell concept from hematology to embrace also comparable cells in epithelial tissues, tumors, and lower organisms [27]. The first chapter is by Lajtha recapitulating his earlier paper and this volume does mark the point at which the definition of stem cells had acquired its modern form. Although this volume is still useful today, a surprising absence from it is the embryonic stem cell, indicating that this tradition had yet to impinge on the world of the radiation biologists.

During the historic development of the stem cell concept, the terms totipotency, pluripotency and multipotency were extensively used, sometimes without much discrimination. But they have now acquired different meanings from one another [28]. There is a reasonable consensus that "totipotent" means able to form any cell in the body plus the trophectoderm of the placenta. "Pluripotent" means able to form any cell in the body. "Multipotent" means able to form more than one cell type. According to these definitions, the only totipotent cell type is the fertilized egg ~~itself~~and its early cleavage products, while pluripotent stem cells comprise the ES and iPS cells. The stem cells in the adult body that maintain tissue turnover are normally multipotent although there is one notable exception in the shape of the spermatogonia of the testis, which can produce only sperm.

## Modern definition of stem cell

Different individuals may cling to different usages of words, but the modern definition of stem cells is very much that set out by Lajtha [25]. This commands a reasonable consensus [1, 29-31] although not everybody may agree with all aspects. Although derived from the hematological tradition, it

effectively embraces also the ideas from embryological tradition featuring reiterated divisions, and from the ES cell tradition emphasizing pluripotency.

- Stem cells reproduce themselves.
- Stem cells generate progeny destined to differentiate into functional cell types.
- Stem cells persist for a long time.
- Stem cell behavior is regulated by the immediate environment (the niche)

This is shown diagrammatically in Fig.3. The first two items on the list indicate the key abilities of self-renewal and the generation of differentiated progeny. "Destined to differentiate" means that cell division may continue for a while before differentiation, but not indefinitely. Cells that proliferate for a limited number of cycles before differentiation are called progenitor cells or transit amplifying cells. The third item on the list means that if the stem cell population is one of those that exist in tissue culture then it should be capable of indefinite growth; while if it is part of an organism it should be very long lasting, normally persisting for the whole life of the organism. It is this criterion of indefinite lifespan that really distinguishes stem cells from progenitor cells. The fourth characteristic indicates that all stem cells exist in a specific micro-environment that controls their program of division and differentiation. This may seem at first sight only to apply to stem cells within the body and not to those grown in vitro, but, in order to get them to grow, the cells in vitro are always provided with specialized medium ingredients that, in effect, mimic the components normally provided in the niche. It is important to note that this definition involves not just intrinsic properties of stem cells but also properties that depend on aspects of their environment such as the lifespan of the animal, the nature of the niche, or the composition of the culture medium. So to understand stem cell behavior, the characteristics of the environment are just as important as the intrinsic properties of the stem cells themselves.

The above definition is of value in indicating the special characteristics of stem cell behavior, but is also helpful in indicating what is not stem cell behavior. The overall classification of cell behavior in terms of proliferation is due to the long term work of Leblond based on studies of mitoses and of radiolabeling with tritiated thymidine [32, 33]. Leblond showed that some cell types, which he called "static", are completely postmitotic. They include multinucleate muscle fibers and neurons which are mostly formed in embryonic life and do not divide thereafter. Some cell types, which he called "expanding", continue to divide in the postnatal growth period and during adult life they may divide occasionally and are able to repair damage. The "expanding" types comprise connective tissues and many epithelial tissues including the liver, kidney and pancreas. Finally there are the renewal tissues in which there is continuous cell production and loss throughout life, fed by a population of stem cells. Renewal tissues include the epidermis, intestinal epithelium, hematopoietic system and spermatogonia. Evidently the cells classified by Leblond as static or expanding are not stem cells. But the fact that a specific cell type is not a stem cell does not mean that there are no stem cells in the tissue to which this cell type belongs. For example, there are some neural stem cells in some parts of the CNS [34], and there are numerous satellite cells in the skeletal muscle which can form new muscle fibers following damage [35]. However, several recent studies have confirmed the lack of stem cells in the normal liver or pancreas [36, 37].

A rather surprising consequence of adopting the consensus definition of stem cells is that the cells of the early mammalian embryo that are the precursors of ES cells, namely the cells of the inner

cell mass and the epiblast, are not themselves stem cells. This is because their pluripotency is very short lived and they become committed to various pathways of differentiation within 1-2 days. The definition indicates that stem cells, with their indefinite lifespan, may arise from progenitor cells, which have a finite lifespan in a particular state of commitment. The origin of stem cell populations from progenitor cells does occur repeatedly in normal development with respect to the formation of the various types of tissue-specific stem cell.

Despite the reasonable consensus underpinning the definition presented here, a common term still found in the literature is "stem/progenitor cell". This is a singularly unhelpful designation as it conflates two entirely different cell behaviors, and it would be best avoided.

## Types of stem cell

So what cell types do correspond to stem cells under this definition? As indicated above, real stem cells comprise two fundamentally different types: the pluripotent stem cells that exist only in vitro, and tissue-specific stem cells that exist in vivo in the postnatal organism.

Pluripotent stem cells comprise embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) (Fig.4). ES cells are cultivated from the inner cell mass of early embryos. A subclass consists of the SCNT lines where the embryo was created by somatic cell nuclear transfer(SCNT) into an oocyte and the resulting embryos are used to establish the cell lines [38]. iPS cells are created by expressing key pluripotency-associated transcription factors in differentiated cell types such as fibroblasts, leukocytes or epithelial cells [39, 40]. The best quality iPS cell lines are very similar to ES cells and the best mouse lines can form germ line chimeras when injected in mouse blastocysts, and can even form a complete mouse embryo using the technique of tetraploid complementation [41, 42]. Genomic analysis of human pluripotent cell lines shows that the SCNT-derived cells are close to ES cells in epigenetic status while iPS cells may diverge more [43].

Pluripotent stem cells may exist in one of two distinct states named "naive" and "primed" corresponding ~~roughly~~ respectively to cells of the ~~early epiblast~~inner cell mass or of the egg cylinder epiblast of mouse embryos [44]. Naive cells have both X-chromosomes active in females, can form chimeras in mouse blastocysts, and maintain pluripotency in response to leukemia inhibitory factor (LIF). Primed cells have undergone X-inactivation, do not form chimeras, and maintain pluripotency in response to fibroblast growth factor (FGF). Both types also share a number of properties: they can be propagated without limit in vitro, and, under appropriate culture conditions, they are able to give rise to a wide variety of cell types, perhaps all the cell types in the normal organism except for the trophectoderm of the placenta. Both will form embryoid bodies in vitro and establish teratomas when grafted to immunocompatible hosts.

The other principal class of stem cell comprises the tissue-specific stem cell populations which exist within the adult body and generate progeny to repopulate the tissue in question. Well studied examples include those of the hematopoietic system, the epidermis, the intestinal epithelium and the spermatogonia of the testis. Under normal circumstances, tissue specific stem cells do not produce cells characteristic of other tissue types. All types of tissue specific stem cell reside within a niche, which provides an environment suitable for continued function of the stem cells. This often, but not always, secretes Wnt factors to maintain stem cell proliferation [45].

There are also some types of stem cell that do not undergo continuous division, but seem to be kept in reserve to deal with tissue regeneration when required. A good example is the muscle satellite cell, which is normally quiescent but able to be mobilized to divide and fuse to form new myofibers following injury [35]. A more contentious example is the periportal cell of the liver that may, under experimental circumstances where hepatocyte division is prevented, generate new hepatocytes to repopulate the liver [46]. This type of stem cell behavior is sometimes called facultative.

By definition, stem cells must produce differentiated progeny, but how many differentiated cell types does each sort of stem cell actually produce? The answer is very variable and depends on the tissue concerned. In the intestine, stem cells produce absorptive, goblet, tuft and Paneth cells, together with several types of enteroendocrine cells. In the bone marrow, the hematopoietic stem cells produce all the cell types of the blood and immune system. At the other end of the scale, the spermatogonia of the testis produce only sperm. Epidermal stem cells are often said to produce only keratinocytes, but they can also form a type of neuroendocrine cell called the Merkel cell, responsible for touch sensitivity. The examples of both the intestine and the epidermis indicate that neuroendocrine cells can arise from epithelial stem cells which are quite separate from the central or peripheral nervous systems. But the formation of neuroendocrine cells in these tissues is not indicative of a wider potency of stem cells enabling cell types of other tissue to be formed.

## Stem cell markers

How can you discover whether a cell is a stem cell? Or, more accurately, how can you tell if a cell exhibits stem cell behavior? Early attempts using RNA profiling to discover a "stemness" signature characteristic of all stem cells proved unsuccessful [30, 47, 48]. However, very often a cell is said to be a stem cell because it expresses one or more specific gene products associated with stem cells. In fact, there is no molecular marker that identifies all stem cells and excludes all non-stem cells. Those components required for general cell metabolism and cell division are certainly found in all stem cells, but they are also found in many other cell populations as well.

Pluripotent stem cells (ESC and iPSC) express an important network of transcription factors which are necessary for maintenance of the pluripotent state [49]. A key member of the pluripotency group is the POU-domain transcription factor OCT4 (also known as OCT3 and POU5F1). The presence of OCT4 is certainly necessary for the properties of pluripotent stem cells. However, conditional knockout of the *Oct4* gene indicates that it is not required for the maintenance of any tissue specific stem cell population in the mouse [50]. So OCT4 cannot be considered to be a general stem cell marker. Claims of discovery of OCT4-expressing cells in mouse or human, other than germ cells, have been made, but are complicated by the existence of cross-reacting antigens and pseudogenes [51].

### Telomerase

A component that might be expected to be found in all stem cells is the telomerase complex [52]. At the end of each chromosome is a structure called the telomere, made up in vertebrate animals of many repeats of the simple sequence TTAGGG. Because of the nature of DNA replication, the double helix cannot be copied right up to the end, so a part of the telomere is lost in each cell cycle. After enough cycles, the erosion of the chromosome ends activates a system which senses DNA double stranded breaks and causes death of the cell. This process is an important reason for the

limited survival time of most primary tissue culture cell lines, which undergo senescence after a certain number of population doublings in vitro. Obviously there must be a mechanism for repairing telomeres in vivo, and this is provided by the telomerase complex, of which the most important components are an RNA-dependent DNA polymerase called TERT, and an RNA called TERC which contains the template CCCTAA for the telomere sequence in the DNA [53]. High levels of telomerase are found in germ cells, ensuring the survival of full length chromosomes for the next generation. Telomerase is also upregulated in permanent ("transformed") tissue culture cell lines and in most cancers. However most types of somatic cell have little or no telomerase. Tissue specific stem cells do contain some telomerase; generally enough to maintain cell division for a normal lifetime, but not enough to fully reverse the erosion of the telomeres. In situations such as repeated transplantation of hematopoietic stem cells from one mouse to another there is an upper limit to the number of possible transplants and this is determined at least partly by telomere erosion [54]. The presence of telomerase can be considered to be a stem cell marker, although it is not specific, being also found in permanent tissue culture lines, early embryos and most cancers.

**Other possible stem cell markers**

The cell surface glycoprotein CD34 is found on human hematopoietic stem cells (HSCs) and can be used to enrich them from bone marrow by fluorescence-activated cell sorting (FACS) [55]. It has been considered by some to be a more general stem cell marker [56]. However it is also found on non-stem cell types, such as capillary endothelial cells, and it is unclear whether it is actually necessary for the stem cell behavior of the HSC. In fact, since it is not found on mouse HSC, which are generally similar in behavior to human HSC, it is probable that it is not necessary. CD34 is not found on human embryonic stem cells or on most epithelial stem cell types, indicating that it is not a generic stem cell marker.

A molecular marker which is known to be required for stem cell function is LGR5 [57]. This is an accessory receptor for the Wnt family of signaling molecules and is found on stem cells in the intestine, hair follicle, mammary gland and stomach [58, 59]. These types of stem cell all depend on Wnt signaling from their environment for continued cell division, so the presence of the LGR5 is really necessary. However it is not found on other types of stem cell so cannot be considered to be a universal marker.

An interesting type of marker is that offered by dye exclusion, in particular exclusion of Hoechst 33342. This is a bisbenzimide dye, excited by UV light to emit a blue fluorescence. It is widely used as a DNA-binding reagent, but it is also actively pumped out of some cell types. If a subgroup of cells has lost more dye than the rest of the population then it appears in flow cytometry as a cluster of cells showing less blue fluorescence than average. This is called a side population [60]. The side population is enriched for stem cells in some situations, especially in murine bone marrow where it provides a similar degree of enrichment of hematopoietic stem cells using fluorescence activated cell sorting (FACS) as does a suitable panel of cell surface markers [61]. The dye exclusion property is due to the activity of cell membrane transporter molecules including the P-Glycoprotein (MDR1) and transporters of the ABC class. Dye exclusion is indicative of an increased capacity for export of all hydrophobic small molecules, many of which are toxic to cells. Although useful to the

investigator, it is unlikely that this capacity is really important for stem cell behavior. For example, mouse embryonic stem cells show dye exclusion while human ones do not [62].

In summary, there is probably no single gene product which is found in all stem cells and not in any non-stem cell. Many so-called stem cell markers are not necessary for stem cell behavior. Of those gene products which are necessary for stem cell behavior, some, such as the cell division machinery and telomerase, are found in stem cells and in some non-stem cells. Others, such as OCT4 or LGR5, are found in some, but not all, types of stem cell.

**Label-retention**

In his article of 1979, Lajtha expressed the view that hematopoietic stem cells were slow dividing compared to their transit cell progeny [25]. He considered that a long period between divisions would allow the cells to undergo full DNA repair and thus minimize the effects of somatic mutations which might otherwise cause cancer. Since then, it has become a widely held view that label retention is associated with stem cell character [63].

The reason that label retention correlates with slow division is the following. When a cell population is exposed to a DNA precursor, such as the nucleoside bromodeoxyuridine (BrdU), which is metabolized by cells in the same way as thymidine, all cells undergoing DNA synthesis will incorporated it into their DNA and so become labeled [64]. The BrdU in the cell nuclei can be detected by immunostaining. After the BrdU supply is withdrawn, so long as cell division is continuing, then the level of BrdU in the DNA will halve with every subsequent S phase and become undetectable to immunostaining after about 5-6 divisions. If a cell divides slower than average, it will retain detectable BrdU for longer. In Fig.5 is shown an image of a hematopoietic stem cell (HSC) visualized with an antibody to the cell surface marker CD150. It retains a DNA precursor label (EdU) from a pulse given 30 days previously. Likewise, muscle satellite cells, cells of the hair follicle bulge and of the limbus of the eye are characteristically label retaining [65]. This relatively quiescent behavior is considered necessary to maintain regenerative function of some types of stem cell over a lifetime. If the mechanisms of quiescence are disturbed in mice by knocking out key components, then hematopoietic stem cells or muscle satellite cell populations have been shown to become exhausted during the lifetime of the animal because they are dividing too much [63].

However, label retention is by no means universal among stem cells. For example, it is not shown by intestinal or epidermal stem cells. It is also, of course, not shown by the pluripotent stem cells (ES or iPS cells) which undergo rapid division in culture. Moreover there are many other cell populations that divide slowly, especially differentiated cells that undergo occasional division. So label retention cannot be considered as a universal stem cell marker.

## The niche

The concept of a stem cell niche arose in the 1970s to explain the fact that the spleen colony-forming cells from the bone marrow had a lesser differentiation potency than hematopoietic stem cells in vivo [26]. The idea is that stem cells require continuous exposure to signals from surrounding cells in order to maintain their stem cell behavior. This was first proved experimentally by Spradling, using the fruit fly *Drosophila* [66, 67, 68].

In the *Drosophila* ovary there are germline stem cells (Fig.6). These lie in contact with somatic cells called cap cells, which secrete a TGFβ-like molecule called Decapentaplegic (Dpp). Dpp maintains the stem cells in mitosis. But as they divide, some of their progeny become displaced from contact with the cap cells, and are then exposed to less Dpp. The fall in Dpp signal lifts a repression on the oocyte maturation process and enables them to differentiate to cystoblasts. These undergo a fixed differentiation program, dividing four times to generate a post-mitotic complex of one oocyte and 15 supporting nurse cells. This illustrates the behavior of a niche very nicely. The stem cells continue to divide so long as they are in contact with the niche, and they differentiate when they are no longer in contact. If a stem cell is removed experimentally, its position may be taken by a cystoblast which would normally have differentiated, but because of its renewed occupancy of the niche it remains a dividing stem cell.

~~In the *Drosophila* ovary there are female germ cells called cystoblasts (Fig.6). These lie in contact with somatic cells called cap cells, which secrete a TGFβ-like molecule called Decapentaplegic (Dpp). Dpp maintains the cystoblasts in mitosis. But as they divide, some of the cystoblast progeny become displaced from contact with the cap cells, and are then exposed to less Dpp. The fall in Dpp signal lifts a repression on the oocyte maturation process and enables the cystoblast to differentiate. It then undergoes a fixed differentiation program, dividing four times to generate a post-mitotic complex of one oocyte and 15 supporting nurse cells. This illustrates the behavior of a niche very nicely. The cystoblasts continue to divide so long as they are in contact with the niche, and they differentiate when they are no longer in contact. If a cystoblast is removed experimentally, its position may be taken by a progeny cell which would normally have differentiated, but because of its renewed occupancy of the niche it remains a dividing cystoblast.~~

Probably all the stem cells types in the mammalian body exist within specific niches like this which control their behavior. For example the intestinal stem cells lie adjacent to Paneth cells which supply WNT [69], and spermatogonial stem cells lie adjacent to Sertoli cells that supply them with Glial Derived Neurotrophic Factor (GDNF) [70]. In both cases the signaling molecules are needed to maintain the stem cells in mitosis, and removal from the niche brings an end to cell division unless the factors are provided experimentally. In the bone marrow, there has been considerable controversy about the exact nature of the niche [71], but hematopoietic stem cells are often found adjacent to blood vessels, as shown in Fig.5. The WNT signaling system is needed for continued function of a variety of stem cells: at least those of the intestine, stomach, epidermis, hair follicles and mammary gland [45]. But it is probably not possible to say that "niche=WNT" as there are clearly other factors also at work and the role of WNT in relation to the HSC and spermatogonial stem cells is still unclear.

## Transplantation and in vitro culture

Should transplantability be part of the definition of the stem cell? Perhaps surprisingly, since transplantation has been so important to work on the hematopoietic stem cell, it did not form part of the definition of Lajtha [25]. There are two major difficulties with the idea. First, cells may be transplanted to immunocompatible hosts, and continue to function, even when they are not stem cells. Examples are grafts of hepatocytes [72] or of islet cells [73], but there are many others. Secondly, the nature of cells may change on transplantation because there are the stresses of dissociation, isolation and injection, followed by effects of the host environment. In hematopoietic

transplantations, hosts are usually heavily irradiated or treated with cytotoxic drugs, and this can also have effects on cell behavior. It is becoming clear that the behavior of HSC following transplantation is not the same as in undisturbed homeostatic hematopoiesis [74]. Following transplantation only a few HSC clones dominate the regenerated blood, whereas in normal homeostasis the number of clones is much larger, and the survival time of some multipotent progenitors is much longer.

A type of stem cell defined almost entirely by transplantation is the so-called cancer stem cell. These are subsets of cells from tumors, isolated using various stem cell markers, which will generate tumors in immunodeficient hosts following grafting, under conditions where the majority of cells from the same tumor do not. The concept of cancer stem cells has been around for many decades [9, 10] although it has been recently revived in popularity. Some cancers certainly do contain stem cells, like their parent tissues [75]. But others do not [76], and some doubt has been cast on the usefulness of the concept by the non-specificity of many of the markers used for cancer stem cell isolation, and by the strong effects of the host genetic constitution on which cells will or will not successfully transplant [77].

Cell properties can change on transplantation but they can do so even more drastically during in vitro culture. Tissue culture media are normally formulated to achieve rapid cell growth, whereas most cells in vivo are slowly dividing or quiescent. For this reason it is not surprising that cells showing stem cell behavior may be grown in culture even when their in vivo progenitors did not show stem cell behavior. The most obvious example is the embryonic stem cell itself. In vitro, ES cells are stem cells as they can proliferate without limit, and can generate a wide range of differentiated progeny. But in vivo the parent cells rapidly acquire more specialized forms of commitment, depending on the morphogen gradients present in the early embryo. There are other examples. For example, neurospheres are small cell clumps that grow in suspension. They contain both undifferentiated cells together with neurons and glia. When dissociated and replated, a small proportion of the cells generate new neurospheres. Although there are some real neural stem cells in the mammalian brain [34], neurospheres can be cultivated from any part of the CNS, whether it contains stem cells or not [78].

There are numerous cell lines described as "stem cells" that have very uncertain connections to real stem cells in vivo. Most notably there are the "stem cells" which are now marketed for human therapy by various companies [79]. Many of these are mesenchymal stem cells (MSCs). MSCs do exist in the bone marrow and are probably precursors of bone [80]. The in vitro cell lines are often supposed to have wide plasticity and to be able to form many cell types after transplantation. However, following a vogue for such reports around the year 2000 it is now considered that such abilities were seriously overstated [81] and that any beneficial effects they might have following transplantation are due to effects on inflammation, angiogenesis and immune modulation [82]. Most would not be considered stem cells by the criteria used here.

## In vivo lineage labeling

Without doubt the most reliable method for establishing the existence of stem cell behavior in vivo is genetic lineage labeling. This was pioneered in *Drosophila*. An early study of the ovary with genetic mosaics showed that there must be more than one germ line stem cell in each ovariole [83].

Later, using the FRT system with a heat shock to induce labeled clones, Margolis and Spradling found that both germ line and follicle cell clones were labeled [84]. This work showed that a label in a stem cell would persist indefinitely and that each ovariole contained 2-3 germ cell stem cells and about 2 somatic stem cells. In the mouse ~~using~~ the CreER system has been the most widely used for stem cell labeling [85, 86]. ~~So far it has only been widely used in mice, but t~~The availability of CRISPR-Cas9 technology will soon make it available for other organisms as well as the mouse. The principle is to use a DNA recombinase enzyme (Cre) to impart a permanent genetic label to a cell type in vivo that has a particular promoter highly active. The label is subsequently heritable on cell division and is unaffected by any differentiation events occurring in the progeny cells. A modification of the Cre recombinase (CreER) which is activatable by estrogen-like hormones enables the labeling to be initiated at a specific time. A low dose of tamoxifen causes the labeling of just a few cells, and if their progeny remain well separated this can enable clonal analysis in vivo. Once it has been labeled, a stem cell will produce a sector of labeled tissue in which all its dividing and differentiated progeny carry the label. The labeled sector will grow initially as cells divide and mature and will eventually reach a steady state in which addition of new labelled cells is balanced by the removal of dead ones. This pattern should then remain unchanged in the long term. By contrast, labeling a transit amplifying cell will produce only a transient patch of labeled cells which will disappear as they mature and die. Examples from the intestine and from the epidermis are shown in Figs.7 and 8. This method has been used to visualize stem cells in the intestine, stomach, epidermis, hair follicle, mammary gland and testis. It is, of course, dependent on the specificity of the promoter used to drive expression of the *CreER* gene and great care needs to be taken to characterize this fully.

It is often thought that all stem cells must undergo asymmetric divisions, with one daughter being a stem cell and the other destined to differentiate [87]. This does sometimes occur, but it is also common for stem cells to have a less rigid program of cell division with some divisions producing two stem cells, some two progenitor cells, and some producing one of each. This is called the stochastic model [88]. Maintenance of a steady state requires that the stem cell number remains constant, although there may be occasions where it needs expanding, such as during normal growth of the organism or following injury. Study of the early divisions following lineage labeling in both the intestine [89] and the epidermis [90] and testis [91] have indicated that they do follow the stochastic rather than the obligatory asymmetric type of division.

The stochastic model gives different predictions from the obligate asymmetric division model about the nature of the labeled stem cell clones visualized by the CreER system. In the situation of the obligatory asymmetric division, labeled stem cell clones each comprise one stem cell plus all their descendants. When the steady state has been reached, the labeled clones should persist for life and stay the same size. However in the stochastic model, clones may be lost if their stem cell divides to form two transit amplifying cells. They may also increase in size if their stem cell divides to form two stem cells. This situation has been modeled mathematically and it predicts that the number of labeled clones should steadily decline with time while the size of clones should become progressively more disparate, with the average size increasing. This means that the proportion of the tissue occupied by the labeled clones remains roughly constant but the number of labeled clones becomes progressively fewer and their size more varied (Fig.8). This behavior is precisely what is observed when lineage labeling data are analyzed quantitatively, at least for the epidermis, spermatogonia and intestinal epithelium. In particular there is a property called “scaling

behavior" which means that the frequency distribution of labeled clone sizes, divided by the average clone size, stays the same over time. Under such circumstances, which may turn out to be the norm for mammalian stem cell systems, the key stem cell properties of self-renewal, persistence and differentiation are still maintained, but they exist at a cell population level rather than as the properties of a single cell. If the stochastic model is indeed correct, it does imply the existence of some mechanism that can control the number of stem cells and prevent them from either dying out or growing like a tumor to overwhelm the organism. This might be a property of the niche, as in the *Drosophila* ovary where, ifstem cells move too far from the source of Dpp, they become differentiating cystoblasts. Alternatively there might be some kind of density-dependent control exterted by the stem cells themselves. In any case this is a topic for future research.

**Conclusion**

The above discussion indicates that stem cells carry no universal molecular marker, that they are not all quiescent, that they are not necessarily the same as transplantable cells, and even that they may not be definable at the individual cell level at all but only at a population level. None of this should be a surprise. Various other authors who have examined the concept have also concluded that stem cell behavior is a system property of a tissue, not a property of individual cells [30, 31, 48, 92].

Of course, what is considered to be a stem cell or not to be a stem cell all depends on the definition employed. The definition given here comprises four properties which do make up a consensus view of stem cells acceptable to most scientists today. However, some will disagree with one or another element of the set. It is always possible to change the definition and thereby change what counts as a stem cell and what does not. For example, there are some cells at the periphery of the developing mammalian kidney that are sometimes called stem cells because they reproduce themselves and generate new kidney tubules [93]. But these cells only persist for a few cell cycles during fetal development so do not satisfy the third criterion given here. They could be counted as stem cells only if this property were abandoned. As another example, some will insist that a stem cell must be able to give rise to more than one type of differentiated progeny, in which case the spermatogonia have to be removed from the list of tissue specific stem cells since they produce only one type of differentiated cell: the sperm.

Perhaps because of the high profile of stem cell research generally, some attention has been given to the stem cell concept by philosophers of science. In a recent book, Laplane considers the various proposed attributes of stem cells and classifies these as categorical, dispositional, relational and system-based. A categorical property is essential and intrinsic, for example the presence of OCT4 in pluripotent stem cells. A dispositional property is a property revealed under particular conditions. For example intestinal stem cells need a Wnt signal from their niche in order to proliferate, so this property depends not just on the nature of the stem cell, but also on something else. A relational property is said to exist if it depends entirely on something else: for instance a hypothetical niche which would make any cell whatever that occupied it into a stem cell. Finally a systemic property belongs, in this context, not to individual cells but to the whole system, for example a tumor in which cells might acquire or lose stem cell behavior in specific circumstances. Laplane concludes that stem cells do comprise a "natural kind" (i.e. a real thing, out there, not just a figment of our imagination), but that they require a complex definition with a general part "stem

cells are the cells from which tissues are developed and maintained", and a set of specific parts which in effect list the various properties discussed above. Apparently philosophers will accept that a natural kind may be defined by a set of attributes which are both categorical and dispositional, and of which not all need apply in every instance.

Debates such as these may cause momentary panic: how can something as familiar as the stem cell become so intangible when examined critically? The contribution of philosophers may be considered negative if it just makes familiar entities disappear. However the philosophical view is worthwhile if it makes us examine the concepts critically. In the case of stem cell biology what emerges from a critical evaluation is that we should think not about stem cells as such but about *stem-type behaviors* that may be shown by various cell populations in specific circumstances. Defining stem cells is slippery and difficult, but defining stem cell behavior is relatively easy. In order to manipulate it in practical situations we need to understand the complete context.

In the popular media and even in some medical circles, stem cells are presented as miracle cells that can do anything. When administered to a patient with some serious disease they will rebuild the damaged tissues and make the patient young again. Alas, in reality there are no such cells. But there are certainly cells that exhibit stem cell behavior and the future of regenerative medicine will undoubtedly be built on a good scientific understanding of their properties.

**Figures**

Figure 1. Mentions of "stem cell" in the abstracts of all papers in the Web of Science Core Collection. The totals are shown in 5 yearly intervals and rise from 372 in 1977-82 to 71545 in 2012-16.

Figure 2. A portion of Fig. 135 from "*The Cell in Development and Heredity*" by E.B.Wilson (1925). This shows the term "stem cell" being used to describe early divisions of embryo blastomeres leading to the germ line.

Figure 3. A consensus diagram showing stem cell behavior. The stem cell exists in a specific niche which enables it to undergo self-renewing divisions. It also generates ~~a variety of~~ differentiated cells via a population of committed but still dividing transit-amplifying cells. Not all stem cell types generate multiple types of differentiated cell.

Figure 4. Different kinds of pluripotent stem cells. (a). Mouse ESC. (b). Mouse iPSC. (c). Human iPSC induced to a naive pluripotent state with chemical inhibitors. (d). Human ESC. (e). Human iPSC. (f ). Mouse EpiSC. Note the flatter colony morphology for (d)–(f ). (From: Robinton, D.A. and Daley, G.Q. (2012) The promise of induced pluripotent stem cells in research and therapy. Nature 481, 295–305. Reproduced with the permission of Nature Publishing Group.)

Figure 5. Hematopoietic stem cell, identified by staining with an antibody to CD150 (red), and also labeled with EdU (white) from a pulse given 30 days previously. The green color shows pericytes expressing nestin-GFP surrounding a small arteriole. (Kunisaki, Y., et al. (2013) Arteriolar niches maintain haematopoietic stem cell quiescence. Nature 502, 637–643. Reproduced with the permission of Nature Publishing Group.)

Figure 6. The stem cell niche in the *Drosophila* ovary. ~~Cystoblasts are f~~Female germ cell stem cells ~~that~~ require continued contact with cap cells to remain stem cells. Once they lose contact with cap cells they differentiate into a cyst of one oocyte and 15 nurse cells.

Figure 7. Descendants of stem cells in the mouse intestine visualized by the CreER method. The stem cells express a protein, LGR5 whose promoter is used for labeling. (a). The mice were labeled 1 day previously, in (b) 5 days previously, and in (c) 60 days previously. The initial label is in the LGR5 positive cells themselves (arrows); subsequently, ribbons of descendant cells up the crypts and villi become labeled. (Originally from: Barker, N. et al. (2007) Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449, 1003–1007. Reproduced with the permission of Nature Publishing Group.

Figure 8. Lineage tracing of epidermal stem cells in mouse skin. The mice express Axin2-CreER with an R26R type reporter expressing GFP (green) following Cre mediated recombination. The blue color is DAPI stain for cell nuclei, and the red is immunostaining for Dickkopf3, a Wnt inhibitor present in the superficial layers. Tamoxifen was given on postnatal day 21 and the images show the situation at 1 day and 2 months thereafter. (a) 1 day, a few basal layer cells are labeled. (b) 2 months, clones of labeled cells are visible leading from the basal layer to the surface. (From: Lim, X., Tan, S.H., Koh, W.L.C., et al. (2013) Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. Science 342, 1226–1230. American Association for the Advancement of Science.)

Figure 9. Stochastic stem cell model. (a) The four types of stem cell division, producing two, one or no daughter stem cells. (b) Disappearance of labeled clone, and doubling of size of labeled clone. (c) Consequent tendency of labeled clones to become fewer but larger with time.

**References**


1. Laplane L. *Cancer Stem Cells. Philosophy and Therapies.* Cambridge, Mass.: Harvard University Press; 2016.

2. Maehle A-H. Ambiguous cells: the emergence of the stem cell concept in the nineteenth and twentieth centuries*. Notes and Records of the Royal Society* 2011.

3. Spemann H. *Embryonic development and induction*. NY: Garland. Reprinted 1967 Hafner NY; reprinted 1988

4. Kumé M, Dan K. Invertebrate Embryology*. Republished 1988 by Garland Publishing Inc., New York* 1957.

5. Davidson EH. Gene Activity in Early Development*. 3rd edition New York: Academic Press* 1986.

6. Shankland M. Leech segmentation: A molecular perspective*. Bioessays* 1994, 16:801-808.

7. Solter D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research*. Nature Reviews Genetics* 2006, 319.

8. Bulic-Jakus F, Katusic Bojanac A, Juric-Lekic G, Vlahovic M, Sincic N. Teratoma: from spontaneous tumors to the pluripotency/malignancy assay*. Wiley Interdisciplinary Reviews: Developmental Biology* 2016, 5:186-209.

9. Kleinsmith LJ, Pierce GB. Multipotentiality of Single Embryonal Carcinoma Cells*. Cancer Research* 1964, 24:1544-1551.

10. Makino S. Further evidence favoring the concept of the stem cell in ascites tumors of rats*. Annals of the New York Academy of Sciences* 1956, 63:818-830.

11. Brinster RL. The effect of cells transferred into the mouse blastocyst on subsequent development*. The Journal of Experimental Medicine* 1974, 140:1049-1056.

12. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos*. Nature* 1981, 292:154-156.

13. Martin GR. Isolation of a pluripotent cell-line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem-cells*. Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* 1981, 78:7634-7638.

14. Evans M. Discovering pluripotency: 30 years of mouse embryonic stem cells*. Nature Reviews. Molecular Cell Biology.* 2011, 12:680-686.

15. Novik AA, Ionova TI, Gorodokin G, Smolyaninov A, Afanasyev BV. The Maximow 1909 centenary: A reappraisal. *. Cellular Therapy and Transplantation* 2009, 1:31-34.

16. Cowdry EV, ed. *Special cytology. The form and functions of the cell in health and disease*. Vol. 2. New York: P.B.Hoeber Inc.; 1932.

17. Maximow AA, Bloom W. *A Textbook of Histology. 4th edn.* Philadelphia: W.B.Saunders; 1942.

18. Ford CE, Hamerton JL, Barnes DWH, Loutit JF. Cytological identification of radiation chimaeras. *Nature* 1956, 177:452-454.

19. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiation Research* 1961, 14:213-222.

20. Becker AJ, McCulloch EA, Till JE. Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 1963, 197:452-454.

21. Harrison DE. Normal Production of Erythrocytes by Mouse Marrow Continuous for 73 Months. *Proc. Nat. Acad. Sci. USA* 1973, 70:3184-3188.

22. Harrison DE, Astle CM. Loss of stem cell repopulating ability upon transplantation. Effects of donor age, cell number, and transplantation procedure. *The Journal of Experimental Medicine* 1982, 156:1767-1779.

23. Abramson S, Miller R, Phillips R. The identification in adult bone marrow of pluripotent and restricted stem cells of the myeloid and lymphoid systems. *The Journal of Experimental Medicine* 1977, 145:1567-1579.

24. Cairns J. Mutation, selection and the natural history of cancer. *Nature* 1975, 255:197-200.

25. Lajtha LG. Stem cell concepts. *Differentiation* 1979, 14:23-34.

26. Schofield R. The relationship between the spleen colony-forming cell and the haematopoietic stem cell. A hypothesis. *Blood Cells* 1978, 4:7-25.

27. Potten CS, ed. *Stem Cells. Their identification and characterisation*. Edinburgh: Churchill Livingstone; 1983.

28. Smith A. A glossary for stem-cell biology. *Nature* 2006, 441:1060.

29. Alison MR, Poulsom R, Forbes S, Wright NA. An introduction to stem cells. *The Journal of Pathology* 2002, 197:419-423.

30. Robert JS. Model systems in stem cell biology. *Bioessays* 2004, 26:1005–1012.

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

32. Leblond CP. Classification of cell populations on the basis of their proliferative behaviour. *Natl.Cancer Inst.Monog.* 1964, 14:119-150.

33. Leblond CP. Life history of renewing cells. *Am. J. Anatomy* 1981, 160:113-158.

34. Bond Allison M, Ming G-l, Song H. Adult Mammalian Neural Stem Cells and Neurogenesis: Five Decades Later. *Cell Stem Cell* 2015, 17:385-395.

35. Brack Andrew S, Rando Thomas A. Tissue-Specific Stem Cells: Lessons from the Skeletal Muscle Satellite Cell. *Cell Stem Cell* 2012, 10:504-514.

36. Yanger K, Knigin D, Zong Y, Maggs L, Gu G, Akiyama H, Pikarsky E, Stanger Ben Z. Adult Hepatocytes Are Generated by Self-Duplication Rather than Stem Cell Differentiation. *Cell Stem Cell* 2014, 15:340-349.

37. Teta M, Rankin MM, Long SY, Stein GM, Kushner JA. Growth and Regeneration of Adult [beta] Cells Does Not Involve Specialized Progenitors. *Developmental Cell* 2007, 12:817-826.

38. Tachibana M, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee H-S, Sritanaudomchai H, et al. Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer. *Cell* 2013, 153:1228-1238.

39. González F, Boué S, Belmonte JCI. Methods for making induced pluripotent stem cells: reprogramming à la carte. *Nat Rev Genet* 2011, 12:231-242.

40. González F, Huangfu D. Mechanisms underlying the formation of induced pluripotent stem cells. *Wiley Interdisciplinary Reviews: Developmental Biology* 2016, 5:39-65.

41. Boland MJ, Hazen JL, Nazor KL, Rodriguez AR, Gifford W, Martin G, Kupriyanov S, Baldwin KK. Adult mice generated from induced pluripotent stem cells. *Nature* 2009, 461:91-94.

42. Zhao X-y, Li W, Lv Z, Liu L, Tong M, Hai T, Hao J, Guo C-l, Ma Q-w, Wang L, et al. iPS cells produce viable mice through tetraploid complementation. *Nature* 2009, 461:86-90.

43. Ma H, Morey R, O'Neil RC, He Y, Daughtry B, Schultz MD, Hariharan M, Nery JR, Castanon R, Sabatini K, et al. Abnormalities in human pluripotent cells due to reprogramming mechanisms. *Nature* 2014, 511:177-183.

44. Nichols J, Smith A. Naive and Primed Pluripotent States. *Cell Stem Cell* 2009, 4:487-492.

45. Clevers H, Loh KM, Nusse R. An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control. *Science* 2014, 346.

46. Yanger K, Stanger BZ. Facultative stem cells in liver and pancreas: Fact and fancy. *Developmental Dynamics* 2011, 240:521-529.

47. Ramalo-Santos M, Yoon S, Matsuzaki Y, Mulligan RC, Melton DA. "Stemness": transcriptional profiling of embryonic and adult stem cells. *Science* 2002, 298:597-600.

48. Leychkis Y, Munzer SR, Richardson JL. What is stemness? *Studies in History and Philosophy of Biological and Biomedical Sciences* 2009, 40:312-320.

49. Dunn S-J, Martello G, Yordanov B, Emmott S, Smith AG. Defining an essential transcription factor program for naïve pluripotency. *Science* 2014, 344:1156-1160.

50. Lengner CJ, Camargo FD, Hochedlinger K, Welstead GG, Zaidi S, Gokhale S, Scholer HR, Tomilin A, Jaenisch R. Oct4 expression is not required for mouse somatic stem cell self-renewal. *Cell Stem Cell* 2007, 1:403-415.

51. Liedtke S, Enczmann J, Waclawczyk S, Wernet P, Kogler G. Oct4 and its pseudogenes confuse stem cell research. *Cell Stem Cell* 2007, 1:364-366.

52. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 2005, 6:611-622.

53. Bekaert S, Derradji H, Baatout S. Telomere biology in mammalian germ cells and during development. *Developmental Biology* 2004, 274:15-30.

54. Flores I, Blasco MA. The role of telomeres and telomerase in stem cell aging. *Febs Letters* 2010, 584:3826-3830.

55. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proceedings of the National Academy of Sciences* 1992, 89:2804-2808.

56. Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise Review: Evidence for CD34 as a Common Marker for Diverse Progenitors. *STEM CELLS* 2014, 32:1380-1389.

57. van der Flier LG, Clevers H. Stem Cells, Self-Renewal, and Differentiation in the Intestinal Epithelium. *Annual Review of Physiology* 2009, 71:241-260.

58. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, Sato T, Stange DE, Begthel H, van den Born M, et al. Lgr5+ve Stem Cells Drive Self-Renewal in the Stomach and Build Long-Lived Gastric Units In Vitro. *Cell Stem Cell* 2010, 6:25-36.

59. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, Barker N, van de Wetering M, van den Born M, Begthel H, Vries RG, et al. Lgr6 Marks Stem Cells in the Hair Follicle That Generate All Cell Lineages of the Skin. *Science* 2010, 327:1385-1389.

60. Challen GA, Little MH. A Side Order of Stem Cells: The SP Phenotype. *Stem Cells* 2006, 24:3-12.

61. Goodell MA, Brose K, Paradis G, Conner AS, Mulligan RC. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. *The Journal of Experimental Medicine* 1996, 183:1797-1806.

62. Zeng H, Park JW, Guo M, Lin G, Crandall L, Compton T, Wang X, Li X-J, Chen F-P, Xu R-H. Lack of ABCG2 Expression and Side Population Properties in Human Pluripotent Stem Cells. *Stem Cells* 2009, 27:2435-2445.

63. Rumman M, Dhawan J, Kassem M. Concise Review: Quiescence in Adult Stem Cells: Biological Significance and Relevance to Tissue Regeneration. *STEM CELLS* 2015, 33:2903-2912.

64. Goodlad RA. Quantification of epithelial cell proliferation, cell dynamics, and cell kinetics in vivo. *Wiley Interdisciplinary Reviews: Developmental Biology* 2017, 6:e274.

65. Fuchs E, Horsley V. Ferreting out stem cells from their niches. *Nat Cell Biol* 2011, 13:513-518.

66. Xie T, Spradling AC. decapentaplegic is essential for the maintenance and division of germline stem cells in the Drosophila ovary. *Cell* 1998, 94:251-260.

67. Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. *Science* 2000, 290:328-330.

68. Losick Vicki P, Morris Lucy X, Fox Donald T, Spradling A. Drosophila Stem Cell Niches: A Decade of Discovery Suggests a Unified View of Stem Cell Regulation. *Developmental Cell* 2011, 21:159-171.

69. Farin HF, Jordens I, Mosa MH, Basak O, Korving J, Tauriello DVF, 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.

70. Yoshida S. From cyst to tubule: innovations in vertebrate spermatogenesis. *Wiley Interdisciplinary Reviews: Developmental Biology* 2016, 5:119-131.

71. Hoggatt J, Kfoury Y, Scadden DT. Hematopoietic Stem Cell Niche in Health and Disease. *Annual Review of Pathology: Mechanisms of Disease* 2016, 11:555-581.

72. Cantz T, Sharma AD, Ott M. Cell therapies for hereditary metabolic liver diseases—concepts, clinical results, and future developments. *Stem cells* 2015, 33:1055-1062.

73. Bruni A, Gala-Lopez B, Pepper AR, Abualhassan NS, Shapiro AMJ. Islet cell transplantation for the treatment of type 1 diabetes: recent advances and future challenges. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy* 2014, 7:211-223.

74. Busch K, Klapproth K, Barile M, Flossdorf M, Holland-Letz T, Schlenner SM, Reth M, Hofer T, Rodewald H-R. Fundamental properties of unperturbed haematopoiesis from stem cells in vivo. *Nature* 2015, 518:542-546.

75. Melo FdSe, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, Hung J, Anderson JE, Storm EE, Modrusan Z, Koeppen H, et al. A distinct role for Lgr5+ stem cells in primary and metastatic colon cancer. *Nature* 2017, 543:676-680.

76. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. *Nature* 2008, 456:593-U533.

77. Clevers H. The cancer stem cell: premises, promises and challenges. *Nature Medicine* 2011, 17:313-319.

78. Reynolds BA, Rietze RL. Neural stem cells and neurospheres—re-evaluating the relationship. *Nature Methods* 2005, 2:333-336.

79. Turner L, Knoepfler P. Selling Stem Cells in the USA: Assessing the Direct-to-Consumer Industry. *Cell Stem Cell* 2016, 19:154-157.

80. Park D, Spencer Joel A, Koh Bong I, Kobayashi T, Fujisaki J, Clemens Thomas L, Lin Charles P, Kronenberg Henry M, Scadden David T. Endogenous bone marrow MSCs are dynamic, fate-restricted participants in bone maintenance and regeneration. *Cell Stem Cell* 2012, 10:259-272.

81. Wagers AJ, Weissman IL. Plasticity of adult stem cells. *Cell* 2004, 116:639-648.

82. Nombela-Arrieta C, Ritz J, Silberstein LE. The elusive nature and function of mesenchymal stem cells. *Nat Rev Mol Cell Biol* 2011, 12:126-131.

83. Schüpbach T, Wieschaus E, Nöthiger R. A study of the female germ line in mosaics of Drosophila. *Wilhelm Roux's archives of developmental biology* 1978, 184:41-56.

84. Margolis J, Spradling A. Identification and behavior of epithelial stem-cells in the Drosophila ovary. *Development* 1995, 121:3797-3807.

85. Hayashi S, McMahon AP. Efficient recombination in diverse tissues by a tamoxifen-inducible form of cre: a tool for temporally regulated gene activation/inactivation in the mouse. *Developmental Biology* 2002, 244:305-318.

86. Hsu Y-C. Theory and Practice of Lineage Tracing. *STEM CELLS* 2015, 33:3197-3204.

87. Knoblich JA. Mechanisms of Asymmetric Stem Cell Division. *Cell* 2008, 132:583-597.

88. Klein AM, Simons BD. Universal patterns of stem cell fate in cycling adult tissues. *Development* 2011, 138:3103-3111.

89. 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.

90. 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.

91. Klein AM, Nakagawa T, Ichikawa R, Yoshida S, Simons BD. Mouse Germ Line Stem Cells Undergo Rapid and Stochastic Turnover. *Cell Stem Cell* 2010, 7:214-224.

92. Lander AD. The 'stem cell' concept: is it holding us back? *Journal of Biology* 2009, 8:70.

93. Kobayashi A, Valerius MT, Mugford JW, Carroll TJ, Self M, Oliver G, McMahon AP. Six2 Defines and Regulates a Multipotent Self-Renewing Nephron Progenitor Population throughout Mammalian Kidney Development. *Cell Stem Cell* 2008, 3:169-181.