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All the adult stem cells, where do they all come from? An external source for organ-specific stem cell pools

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Summary Stem cells can self-renew and maintain the ability to differentiate into mature lineages. Whereas the ''stemness'' of embryonic stem cells is not discussed, the primitiveness of a stem cell type within adult organisms is not well determined. Data presently available are either inconclusive or controversial regarding two main topics: maintenance or senescente of the adult stem cell pool; and pluripotentiality of the cells. While programmed senescence or apoptosis following uncorrected mutations represent no problem for mature cells, the maintenance of the stem cell pool itself must be assured. Two different mechanisms can be envisaged for that. In the first mechanism, which is generally accepted, stem cells originate during ontogeny along with the organ which they are responsible for, and remain there during all the lifespan of the organism. Several observations derived from recent reports allow the suggestion of a second mechanism. These observations include: organ-specific stem cells are senescent; adult stem cells circulate in the organism; stem cell niches are essential for the existence and function of stem cells; adult stem cells can present lineage markers; embryo-like, pluripotent stem cells are present in adult organisms, as shown by the development of teratomas, tumors composed of derivatives of the three germ layers; and the fact that the gonads may be a reservoir of embryo-like, pluripotent stem cells in adult organisms. The second mechanism for the maintenance of adult stem cells compartments implies a source external to the organ they belong, consisting of pluripotent, embryo-like cells of unrestricted life span, presenting efficient mechanisms for avoiding or correcting mutations and capable to circulate in the organism. According to this model, primitive stem cells exist in a specific organ in adult organisms. They undergo asymmetrical divisions, which originate one ''true'' stem cell and another one which enters the pool of adult stem cells, circulating through the entire organism. Upon signals liberated by organ-specific niches, this cell becomes activated to express lineage-specific genes, homes to that particular organ and repopulates its stem cell compartment, differentiating thus in what is seen as the organ-specific stem cell. The gonads are the natural candidates for homing the

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primitive stem cells in adult organisms. The model proposed Hin this work for the maintenance of organ-specific stem cell pools from an external source, represented by primitive, embryo-like germinal stem cells present in testes and ovaries, may contribute to the more complete understanding of this complex issue.
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Stem cells

For normal cells in complex organisms, mitosis is accompanied by differentiation. Stem cells are, by definition, the only ones which can self-renew maintaining at the same time the ability to differentiate into mature lineages. Conventionally, two main types of stem cells are recognized. Those isolated from the embryo inner cell masses (embryonic stem or ES cells) or from fetal primordial germ cells (EG cells) are considered totipotent, since they present also the ability to entirely colonize an organism and give rise to all its cell types [1]. Stem cells found in adult organisms are referred to as adult, organ- or tissue-specific stem cells, and are present in most, if not all, adult organs [2]. They are considered pluripotent, since they can originate mature cell types of one or more lineages, but cannot reconstitute the organism as a whole. (Some authors prefer the terms pluripotent and multipotent, respectively, since ES or EG cells cannot produce all of the extraembryonic tissues required for development [3].) Important biological and genetic information has been generated by the study of both types of stem cells, but their application in gene or cell therapy protocols presents marked differences. Embryonic stem cells are more easily obtained and present greater plasticity, but are accompanied by ethical/legal and safety concerns which are not involved in the use of adult stem cells [4].

The intense attention received by stem cells during the past few years has generated a huge amount of new knowledge. Research of ES cells has centered mainly on the development of methods to more efficiently induce differentiation in a variety of cell types. The adult stem cells, on the other hand, have been investigated through many different approaches. One of the most important is related to their identification. Since they are rare and do not present a distinctive morphological profile, alternative methods have been developed. Immunophenotyping is in many cases useful for the enrichment of stem cells, which are generally seen as devoid of lineage markers. Functional characteristics have also shown to be very useful. Mesenchymal stem cells (MSCs), that differentiate into many cell types such as fibroblasts, osteoblasts, chondroblasts, and adipocytes, can be isolated from bone marrow and other organs by adherence to plastic surfaces or extracellular matrix [5]. Stem cells have also been identified by a high level of a specific ABC transport that actively pump the fluorescent dye Hoechst 33328 out; in consequence, they appear as a ''side population'' (SP) when analyzed by flow cytometry [6]. Yet another approach for the identification of stem cells depends on their quiescence. Cells can be marked with a label such as BrdU or green fluorescent protein (GFP) and analyzed weeks or months later, so that slow-cycling, label-retaining cells (LRC) are identified [7].

Renewal of the mature cell compartment is more intense for some tissues, such as the blood or epithelium, but is expected for all tissues and organs. Organ-specific stem cells were shown to undergo asymmetric division, which results in one daughter cell which is committed to differentiate (becoming thus a progenitor cell) and another which remains a stem cell. Both intrinsic and extrinsic signals regulate stem cell fate and some of these regulatory mechanisms, including secreted factors such as the TGFβs and Wnts and pathways mediated by Nanog, Stat3 and Oct4 among other genes, are already known [8,9].

Adult organ-specific stem cell compartments

There is no doubt about the ''stemness'' of embryonic stem cells. To determine the true primitiveness of a stem cell type within adult organisms, however, two characteristics need to be investigated, for which the data presently available are either inconclusive or controversial.

Are adult stem cells immortal?

The first of these characteristics refers to the life span of the cell. Whereas most adult differentiated cells will die either by natural programming (homeostasis) or by injury, the stem cell compartment which replenishes them must persist during the whole life span of the organism. Long-lived cell populations in complex organisms as mammals must however adequately cope with at least two biological phenomena: they must avoid mutation damages and normal senescence mechanisms.

In long-lived organisms, tissues are more susceptible to DNA damage by environmental agents or by

by-products of the normal metabolism, such as reactive oxygen species. *Mutations* can lead to cancer and must thus be corrected, either by gene repair mechanisms or by apoptosis of the cell. To avoid mutations, one of the mechanisms which seem to be used by stem cells is non-random chromosome segregation [10]. The model proposes the segregation of the original (''immortal'') DNA template to the stem cell originated by asymmetric division, whereas the newly synthesized strands (more prone to replication-induced mutation) are passed on to the daughter cells that are committed to differentiation [11].

Senescence, on the other hand, is a physiological property of most cells. Most normal human cells are programmed for a given number of cell divisions (the ''Hayflick limit''), due to progressive telomere shortening [reviewed in 12]. Functional telomerase, a ribonucleoprotein which includes the reverse transcriptase telomerase protein (hTERT) and the telomerase RNA template (hTR), compensates for the loss of telomere repeats by extending the 3′ ends of telomeres. Cells may also bypass the telomere checkpoint by inactivating downstream signaling events (e.g., by loss of p53 function [13]). Immortal cells such as tumor lines and embryonic stem cells present high telomerase levels [14].

Many studies have shown continuous maintenance of stem cells in culture, but direct transposition of these results to the in vivo situation is not possible due to the extremely artificial conditions created by the culture process. On the other hand, accessing the susceptibility of organ-specific stem cells to senescence mechanisms in vivo is a complex task. First of all, the identification of stem cells in most cases is only possible through functional assays, which involve their isolation and in vitro culture. Analyzing whether freshly isolated stem cells present telomerase activity or other mechanisms to avoid senescence is thus very difficult, and controversial results have been reported. Some authors report that adult stem cells do present telomerase activity and surpass the Hayflick limit in vivo [15,16]. Several others, however, show that telomeres shorten during replicative aging in vivo in stem cells from humans and mice, despite detectable levels of telomerase in these cells [17, and references therein].

Are adult stem cells pluripotent?

Differentiation of stem cells into mature lineages is a stepwise process, involving interaction with surrounding cells which compose the *niche*, genetic reprogramming, epigenetic mechanisms and a considerable expansion of cell number [reviewed in 18]. In vitro and in vivo approaches to the question of organ-specific stem cell plasticity have generated controversial results, and it is not yet clear how much of the plasticity observed is due to true transdifferentiation or to cell fusion events [19,20].

Multipotent or pluripotent stem cells have been described in multiple tissues, usually associated with the connective tissue [5,21–23, unpublished observations]. For some of them, the ability to originate tissues from all three layers — endoderm, mesoderm, ectoderm — was observed in vitro and in vivo. It seems thus possible that some types of primitive cells present in adult organisms preserve a high degree of ''stemness'', through mechanisms not yet well understood.

Maintenance of organ-specific stem cell pools

While programmed senescence or apoptosis following uncorrected mutations represent no problem for mature cells, which are then replenished from the stem cell compartment, the maintenance of the stem cell pool itself must be assured. Two different mechanisms can be envisaged for that.

Organ-specific stem cell pools are self-sufficient

In the first mechanism, which is generally accepted (Fig. 1), stem cells originate during ontogeny along with the organ which they are responsible for, and remain there during all the lifespan of the organism. The stem cell compartment in this case is maintained by the generation of new stem cells through asymmetric divisions. This model involves the existence of strict control of mutation damage and absence of senescence in each of the organ-specific stem cell compartments; otherwise, the two consequences would be increased risk of development of diseases such as cancer, and ''exhaustion'' of the stem cell compartments.

Proposed mechanism: organ-specific stem cell pools depend on an external source

Several observations derived from recent reports allow the suggestion of a second mechanism. These observations are summarized below, and only representative references are cited.

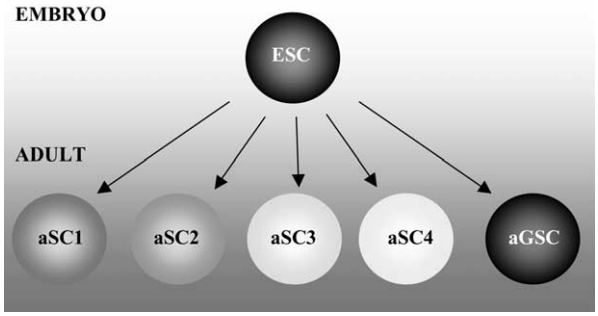

Figure 1 The self-sufficient mechanism. Embryo stem cells (ESC) give origin to all the stem cells present in the adult organism, which are then maintained in organ-specific compartments by asymmetric cell division. Adult stem cells present a distinctive hierarchy. The adult germ stem cells (aGSC), present in the ovary and testis in mammals, maintain totipotency throughout the whole lifespan of the organism. Different types of adult stem cells present variable plasticity — level 1 would be the highest, in this model — and more plastic cells may or may not originate other cell types (not shown).

1. Organ-specific stem cells are senescent. Although still controversial, as described above there is good evidence that most adult, organ-specific stem cell pools such as the hematopoietic stem cells (HSCs) are not maintained during the whole life span of the organism.
2. Adult stem cells circulate in the organism.
   - Organ-specific stem cells migrate through the organism, to sites of pathological insult or following other stimuli; this has been shown for instance for the HSC [24] and for neural stem/progenitor cell [25, and references therein].
   - Adult stem cells of greater plasticity, such as the multipotent adult progenitor cells (MAPCs) and MSCs, have been shown to circulate and colonize other compartments [26,27].
   - Cancer has been shown to be a pathology of the stem cells [28]. Besides proliferation and differentiation, cancer cells present another characteristic — *mobility* — which can be seen as another evidence of the stem cell circulation capacity.
3. The existence and function of stem cells is strictly dependent on the combination of their intrinsic characteristics and their environment, the so-called stem cell niches [reviewed in 18].
   - Bone marrow transplantations show that transplanted HSCs circulate and home into the bone marrow of the host.
   - Studies in *Drosophila* showed that when germinal stem cell niches are experimentally ''emptied'', they can still persist and signal incoming stem cells, supporting ectopic proliferation which maintains some of their stem cell features [29].
4. Adult stem cells can present lineage markers. ''Primed'' HSCs, expressing lineage-affiliated genes such as lysozyme M, which is highly expressed in myelomonocytic cells [30], or other markers (D. Bonnet, personal communication), maintain long-term repopulation potential.
5. Embryo-like, pluripotent stem cells are present in adult organisms.
   - The existence of embryo-like, pluripotent stem cells in adult organisms has been recently suggested by some studies. It was suggested for instance that MAPCs may represent an embryonic stem cell remnant [22]. Young et al. [23] described the isolation of embryonic-like stem cells, called ''postnatal pluripotent epiblastic-like stem cell''.
   - Cancer, as a disease expanding the rare stem cell pool, also shows the existence of pluripotent stem cells able to generate cells from all three layers. Teratoma is a common benign tumor, encountered frequently during the reproductive years but occurring at all ages. Teratomas may develop secondary, high-grade malignant components, including sarcomas and primitive neuroectodermal tumor; metastatic mature teratoma is often present in postchemotherapy surgical specimens of lymph nodes from patients with testicular germ cell tumors [31]. They are composed of derivatives of the three germ layers, indicating the pluripotency of the cell from which they originate. Although its origin has been suggested from blastomeres segregated at an early stage of embryonic development, or from embryonal rests, the most accepted presently is an origin from the primordial germ cell. Teratomas may thus be seen as the abnormal proliferation of ''just'' another stem cell type — only, in this case, a cell which maintains the embryo-like pluripotency.
6. The gonads may be a reservoir of embryo-like, pluripotent stem cells in adult organisms.

- The plasticity of fetal germline stem cells is well known [32], and that of adult cells is beginning to be investigated. In *Drosophila*, for instance, four- and eight-cell germline cystocytes generated in adults were shown to efficiently convert into single stem-like cells, able to develop into functional germline stem cells and support normal fertility [29].
- As it has generally been assumed that in mammals only in adult males these cells persist, whereas most mammalian females lose the capacity for germ-cell renewal during fetal life, little attention has been given to adult GS cells. However, recently Johnson et al. [33] reported the existence of proliferative germ cells that sustain oocyte and follicle production in the postnatal mammalian ovary.
- Stem cells of the germline in the testis, similar to embryonic stem cells, have high telomerase levels [12].

Taken together, these observations suggest the existence of a second mechanism for the maintenance of adult, organ-specific stem cells compartments (Fig. 2), that implies a source which is external to the organ they belong. This source should consist of cells presenting the following characteristics: pluripotency, similar to that of embryonic stem cells; unrestricted life span, so as to be maintained during all the existence of the individual; efficient mechanisms for avoiding or correcting mutations; and the ability to circulate through the organism. The great advantage of such a model over the former one is that in only one cell compartment the usual mechanisms used by the organism for regulating cell growth — senescence and a greater control of cell fate — would be relaxed. According to this model, primitive stem cells (which would be the long-searched for ''true stem cells'') exist in a specific organ in adult organisms. They are probably in small number and undergo asymmetrical divisions, which originate one ''true stem cell'' and another one which loses this status and enters the pool of adult stem cells. These ones periodically circulate through the entire organism, and upon signals liberated by organ-specific niches become activated, express lineage-specific genes (some of them seen as ''lineage markers'') and home to that particular organ, repopulating its stem cell compartment and differentiating thus in what is seen as the organ-specific stem cell. It is very probable that the molecular mechanisms regulating these functions - cell division, circulation, activation, homing - are similar to the pathways already known for the more conventional stem cells. Since this cell compartment has to be more resistant to DNA damage or mutations, special mechanisms must operate, which remain to be determined.

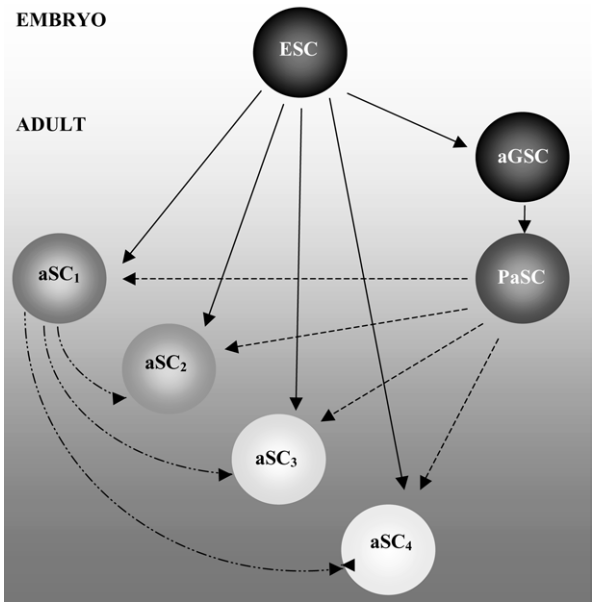

Figure 2 The external source model. Embryo stem cells (ESC) may originate (–—) all the stem cells present in the adult organism. Adult stem cells, however, present a distinctive hierarchy. The adult germ stem cells (aGSC), present in the ovary and testis in mammals, represent level 1 and maintain pluripotency throughout the whole lifespan of the organism. By asymmetric division they can originate pluripotent adult stem cells (PaSC), which represent level 2, circulate in the organism, and may be found in different tissues. These cells can replenish (— — — —) adult, organ-specific stem cell compartments (aSC_1 to aSC_4). Different types of organ-specific stem cells may present variable plasticity, and more plastic cells may originate other cell types (— · — ·).

This model also implies a hierarchy, or a continuum (which has already been suggested, see for instance [16]), for the whole stem cell universe within an adult organism. The embryo-like, pluripotent stem cells would be on the top. MAPCs [26] or other types of pluripotent adult cells [23], which besides being present in many different organs have also been shown to circulate, represent a second level. They do not seem to rank as ''true'', primitive stem cells, since unlike embryonic stem cells they are under strict regulatory growth control [12,23]. Finally, epithelial, neuronal and other types of stem cells represent the third level, for which plasticity is more reduced [20]. For some stem cells, such as the HSC, the question of plasticity and thus the classification is

still controversial. ''Stemness'' levels are inversely correlated to senescence.

The gonads are the natural candidates for homing the primitive stem cells in adult organisms. Male mammals have long been known to present germline stem cells within the testis, and it was recently reported that female mice also contain a population of germline stem cells required to maintain overall follicle numbers during adult life [33]. No studies have yet been conducted to explore the plasticity of the adult germ stem cell pool, but its pluripotency is revealed when the cells undergo mutations or other types of modifications which originate teratomas, composed by cells from all three layers. Actually, the very concept of ''pluripotent embryonic stem cells'' emerged from Leroy Stevens' work with teratomas developing in the testes of mice of strain 129 [reviewed in 34]. Murine strain 129 possibly presents peculiarities related to this primitive stem cell pool, since besides being more susceptible to teratoma formation it is the strain from which embryonic stem cell lines are more easily established [35]. A more detailed comparison of molecular mechanisms operating in this strain as compared to others would possibly provide new insights into the control of the pluripotent stem cell population in adult organisms.

Despite the problems inherent to the cell transplantation systems designed to evaluate stem cell plasticity, this model is amenable to experimental proof. The experiments could be performed by transplanting ovarian fragments, harvested from adult mice transgenic for GFP or LacZ, into the ovarian bursal cavity of wild-type female siblings as described by Johnson et al. [33]. After appropriate intervals the analysis of different stem cell compartments would show whether GFP or lacZ-positive cells, derived from the transplanted germinal stem cells, contributed to renew the stem cell pool. If that is indeed the case, it is very probable that different organ-specific stem cell pools will present different repopulation kinetics. A variation of this protocol could include the induction of chronic lesions or other disease models for which the participation of adult stem cells has been shown [36]. This model is apparently opposed by situations in which agonadism is observed [www.ncbi.nlm.nih.gov/Omim]; true absence of gonadal tissue, however, is difficult to prove, and the case may correspond to that of the thymus, which although considered for a long time to involute with age has now been proven to the contrary to be well maintained in the old age [reviewed in 37]. In situations in which gonads do develop but degenerate or are removed, associated stem cells may be preserved in surrounding tissues.

We believe that a more systemic approach to understand the maintenance of cells, tissues and organs in adult organisms is urgently needed, in order to better understand and more adequately profit of the huge amount of new data on the genetics, biology and applications of stem cells. This systemic approach may explain findings not yet understood, such as the fact that transplanted stem cells not only respond to host factors but also influence the host, in a dynamic reciprocal stem cell—host interaction [38]. This understanding will also increase the potential use of adult as compared to embryonic stem cells in human therapies, avoiding the immunological, legal, ethical and safety problems associated with the latter. The model proposed in this work for the maintenance of organ-specific stem cell pools from an external source, represented by primitive, embryo-like germinal stem cells present in testes and ovaries, may contribute to the more complete understanding of this complex issue.

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