(Re)defining stem cells

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Summary
Stem-cell nomenclature is in a muddle! So-called stem cells may be self-renewing or emergent, oligopotent (uni- and multipotent) or pluri- and totipotent, cells with perpetual embryonic features or cells that have changed irreversibly. Ambiguity probably seeped into stem cells from common usage, flukes in biology's history beginning with Weismann's divide between germ and soma and Haeckel's biogenic law and ending with contemporary issues over the therapeutic efficacy of adult versus embryonic cells. Confusion centers on tissue dynamics, whether stem cells are properly members of emerging or steady-state populations. Clarity might yet be achieved by codifying differences between cells in emergent populations, including embryonic stem and embryonic germ (ES and EG) cells in tissue culture as opposed to self-renewing (SR) cells in steady-state populations.
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Introduction

Remarkably, one can rarely be confident that one knows what is meant by "stem cell" when encountering it in the literature[1,2] Stem cells have "long been regarded as undifferentiated cells capable of proliferation, self-renewal, production of a large number of differentiated progeny, and regeneration of tissues",[3] even if such stem cells elude detection. As early as 1979, Christopher Potten pointed out that "stem cells cannot be reliably morphologically identified and their study is restricted to various functional tests".[4] In 1990, Markus Loeffler joined Potten in placing stem cells at the center of a biological uncertainty principle: "Here, we find ourselves in a circular situation: in order to answer the question whether a cell is a stem cell we have to alter its circumstances and in doing so inevitably lose the original cell."[5]

Today, some stem cells, such as the gonocytes of neonatal rat testis[6] are defined morphologically, but morphology provides only tantalizing hints for identifying other stem cells. The goal of identifying stem cells with molecular markers has also proven elusive even with the aid of fluorescent markers and green fluorescent protein (GFP), flow cytometry via the fluorescence-activated cell sorter (FACS), the exclusion of the DNA-binding dye, Hoechst 33342,[7] cDNA subtraction, microarray techniques and in situ hybridization.[8] Ironically, an extensive cDNA microarray analysis shows that six human stem-cell lines "are, overall, similar to each other and express a unique molecular signature of 92 genes",[9] while "there are only *six* genes shared between the sets identified by [two groups of researchers, and members of a] . . . third group were able to identify only *one* gene that appeared on all three lists of genes for 'sameness'!"[10] Massive efforts to identify key regulatory gene candidates and stem-cell-enriched genes, involving hundreds of antigens expressed in stem cells, have been useful only in "enrichment, rather than purification protocols".[3]

Patterns of cell division also fail to identify stem cells unambiguously. "For readers who are not stem-cell biologists, it is pertinent that stem cells [retain] . . . the continued capacity to proliferate during adult life (unlike mammalian primordial germ cells . . .)",[11] even if mitotically arrested primordial germ cells have been considered stem cells since the term was first coined in the 19th Century (see below), and many stem cells divide slowly compared to other proliferative cells.[12]

Stem-cell potency—the cell's breadth of competence for differentiation—is even more ambiguous as a criterion for stemness. Hematologists, immunologists and others have applied a criterion of oligopotency to stem cells—potency stretching from unipotency (one cell type) to multipotency (several related cell types) but not beyond lineage-specific cell types. In contrast, one commentator insists that, "[c]ells that are *unipotent* [committed to differentiate into one type of cell], though sometimes referred to as stem cells, should not be so described".[10] Stem cells (even "multipotent adult progenitor cells or MAPCs"[13]) are thus required to exhibit pluripotentiality—the capacity to differentiate across cell lines representing the three embryonic germ layers, ectoderm, mesoderm and endoderm.

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Abbreviations: EC, embryonal carcinoma; ES, embryonic stem; EG, embryonic germ; HSC, hematopoietic stem cells; ICM, inner cell mass, MAPCs, multipotent adult progenitor cells; PGC, primordial germ cell; SR, self-renewing; SSC, spermatogonial stem cells; TA, transit amplifying.

Deconstructing and reconstructing stem cells

Where does the confusion come from? Several possibilities are readily suggested.

Lexical latitude

Imprecision probably seeped into the "stem" of stem cells from conflicting definitions prevailing in common usage. As a verb,

"to stem" is defined as (1) to staunch or to hinder, obstruct or stop something, such as blood from flowing as opposed (2) to originate, drive, make headway against or be the cause of something. The adjective extends ambiguity to "solidly built or substantial".

Thus, biologists understandably apply "stem" to anything from the tip of a branch to its base and to everything giving rise to an array of differentiated tissue or to subdivisions of organ systems. Taxonomists use "stem" for the stock or main ancestral line that gives rise to a branch on a family tree, and systematists use "stem" for a hypothetical, fundamental or primitive node that branches into members of a clade. Anatomists find stems at the beginning, middle and end of structures (e.g. the brain stem connecting the spinal cord to the forebrain), and developmental biologists identify stem cells in both undifferentiated and differentiated tissue.

Eddies in biology's history

Biology's tortuous history undoubtedly contributed to the dilemma of stemness. Indeed, "stem cells" underwent several metamorphoses since their coinage.

The original stem cell: the origins of unique clonal lineages

In the late 19th Century, "egg rollers" around the world followed the lineage of blastomeres by squinting through mounted magnifying glasses while gently rotating glass tubes containing nearly transparent invertebrate embryos. The work was helped by the discovery that the chromosomes of some blastomeres in nematodes and arthropods underwent distinct fracture and diminution, while the chromosomes of other blastomeres retained whole chromosomes. Theodore Boveri proposed that the blastomeres retaining whole chromosomes formed a "stem line" leading to primordial germ cells (PGC), while the blastomeres exhibiting chromosomal fission formed various "somatoblast lines" leading to germ layers and embryonic rudiments.[14]

But it is E.B. Wilson who is credited with introducing "stem cell"[15] in the first edition of *The Cell* as a synonym for mitotically quiescent PGCs formed in the pin worm *Ascaris* and the water flea *Cyclops* (Fig. 1).[16] In his definitive 1925 edition,[17] Wilson added several dipterans and "higher invertebrates" to his list of organisms with PGCs irrespective of chromosomal change and enlarged "stem cell" to include the progenitors of oogonia and spermatogonia generally.

The notion of "stemness" quickly spread from PGCs to blastomeres at the beginning of unique clonal lineages. For example, in spirally cleaving nematodes, such as *Caenorhabditis elegans*, the intestinal "founder cell" ("embryonic blast") arising at the third cleavage[18] became known as a "stem cell", and other synonyms for stem cells were coined: "teloblasts" in clitellates, annelids[19] and mollusks,[20] somatic "set-aside" cells in echinoderms[21] and other marine invertebrate embryos, larvae and nymphs, and the "imaginal disks" in endopterygote (holometabolous) insects.[22] In the case of vertebrates, large numbers of cells were typically

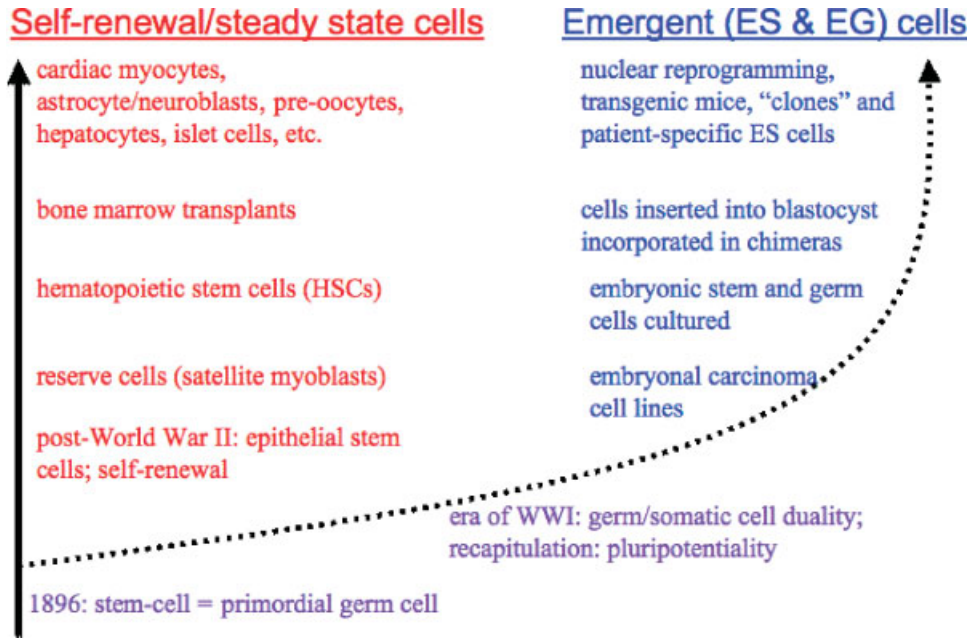

Figure 1. Which stem cell?

produced before discrete germ layers were formed, but in fish[23] and amphibians, larval structures were sometimes traceable to individual blastomeres.[24,25]

Germ layers and organ rudiments in eutherian mammals do not seem to arise by clonal lineages from stem cells. The inner cell mass (ICM) is initially partitioned from the morula's (or trophoblast's) blastomeres by horizontal (periclinal or paratangential) cell division.[26] The ICM forms a unilaminar epiblast, and "[i]n the mouse, germ cell competence is induced at embryonic day 6.5 in proximal epiblast cells by signals emanating from extraembryonic ectoderm".[27] The epiblast delaminates a hypoblast while forming a bilaminar embryonal plate, and PGCs are found in the proximal epiblast. Polyclonal endoderm and mesoderm are formed by the ingression of de-epithelialized primitive groove cells, and PGCs migrate to the posterior endoderm where they can be found 8 days post coitus.[28]

Stem cells' first metamorphosis: undifferentiated/pluripotent

In the era prior to World War I, the distinction between PGCs and somatoblast lines echoed in the chasm left by August Weismann between the germ and soma of an organism.[29] Weismann's germ consisted of primitive, undifferentiated single cells conveyed linearly between generations with the help of the soma; the soma consisted of everything in a multicellular organism other than the germ. Weismann proposed that germ cells were primitive and immortal, while somatic cells surrendered immortality in exchange for differentiation achieved through evolution for the enhancement of reproduction. Ernst Haeckel proceeded to place the dualism at the fulcrum of evolution. His theory of recapitulation or the biogenic law—ontogeny epitomizes phylogeny—twisted the germ line into the "stem" of the entire organism.

Some embryologists continued to think of embryonic cells as being much the same as other cells, albeit functioning in the development of germ layers, organ rudiments and ultimately adult organisms. Other embryologists, followers of Haeckelian recapitulation advanced the notion of a somatic fountainhead of undifferentiated stem cells at the beginning of development. Their view of stem cells found its way to the present in the form of recalcitrant pluripotent cells retained by adult tissues.

Historically, the dualism degenerated into a contest over "which came first?" In the preponderance of metazoans, germline cells separated from the soma via epigenetic mechanisms and not via the determinism implied by chromosomal fission.[30,31] But the argument was not resolved by data. The biogenic law had become the central issue of evolutionary theory and, not unlike contemporary squabbles over evolution, the implications were broader than the subject, extending to religion, to eugenics and to notions of superior races.

By dint of persistent distortion and obsessive argument, recapitulation won the hearts and minds of many biologists.[32,33] As a result, a developmental germ cell was promoted to the role of originator of multicellular animals much as the primitive protozoans occupied the place of metazoan ancestors. This germ cell became the model for a pluripotent stem cell that would give rise to all the somatic tissues of growing and developing embryos, larvae and adults!

Stem cells' second metamorphosis: self-renewal/steady state

In the post-World War I years of the first half of the 20th Century, arguments over recapitulation lapsed in favor of research on the inheritance of traits via genes, the induction of compound organs and organ systems via organizers, and the elaboration of morphogenic patterns via physiological gradients.[34,35] Stem cells fell into disuse, but attitudes toward them changed again with the tragic beginning of the atomic age in World War II. The arrival of radiation sickness revived interest in stem cells capable of restoring tissue, while the introduction of radioactive markers made it convenient to trace cell lineages.

Consequently, a self-renewing (SR) stem cell was proposed, capable of maintaining adult tissue in the steady state through asymmetric cell division. Studies on tissue dynamics soon supported the existence of this SR stem cell in differentiated epithelia, blood and ultimately pathological tissue.[36–38] In adults, stem cells were credited with the ability to maintain differentiated tissues and regenerate them without loss of function.

In choanoflagellate colonies, nonflagellar stem cells divided "without requiring any reduction in the number of flagellar cells that provide propulsive and feeding currents" (i.e. the basal body of a flagellum was not shared with the centriole of a mitotic apparatus)[39] In more complex metazoans, the somatic stem cell played its role when cell division conflicted with function (e.g. stem cells supplied neuroblasts when neurons could not afford to sacrifice half their synapses for the sake of cell division). Indeed, without stem cells bridging the gap between division and differentiation, the evolution of multicellular complexity might have been stymied at the start.

Other adult cells seem related to adult stem cells. Although morphologically undifferentiated, some cells seem functionally differentiated for repair. These cells, known as reserve cells, included muscle satellite cells (quiescent myoblasts) in skeletal muscle,[40] astrocytes in the brain[41–43] and quiescent spermatogonia in seminiferous tubules.[44,45]

Another stem-cell-like population comprises a cache of morphologically differentiated and functional cells capable of participating in regeneration following traumatic tissue loss caused by poisoning (peri-biliary cells) or ablation (hepatocytes[46,47] and pancreatic islet cells[48,49]). Finally, some cells seem capable of repair and wound healing but not morphological regeneration (e.g. endothelial cells, fibroblasts, osteocytes, and possibly cardiac myocytes[50,51]).

Stem cells' third metamorphosis: emergent stem cells, ES & EG cells in tissue culture

But research in biology was poised to change again, and even more dramatically. In the wake of the discovery in 1953 of Watson-Crick base pairing in deoxyribonucleic acid (DNA), genetics rose to the top of biology's research agenda, ultimately spawning the biotechnology industry.

Stem-cell research was not far behind. The new stem cell was the child of tissue culture, but the midwife assisting the birth was cancer research. The first new stem cells were embryonal carcinoma (EC) cells, and cell lines originating in tumors presumably of gonadal origin were maintained through passage in vitro or in vivo.[52,53] Some EC cell lines consisted of immortally transformed cells that divided and did not differentiate, while others were mortal blast cells that differentiated and ceased dividing. EC lines generally formed embryocarcinomas (teratocarcinomas) when injected into tolerant mice, but some EC cells could also differentiate into somatic tissue following introduction into blastocysts.[54–56]

The research game was also played in the opposite direction: Tissue culture cells were isolated from blastocysts. Resembling EC cells, but obtained from the inner cell mass (ICM), these tissue-culture cells were christened "embryonic (or embryonal) stem" (ES) cells or "primitive stem cells".[57,58] And like EC cells, various lines of ES cells had the emergent properties of embryonic cells. Maintained in complex media including the cytokine leukemia inhibitory factor (LIF) and/or feeder layers of fibroblasts,[59,60] ES cells in tissue culture expressed the intrinsic transcription factor known as Oct4[61] and, ideally, maintained a normal karyotype, remained proliferative, rounded (rodent) or flattened (human and primate), and formed spherical (rodent) or fasciculate (human) colonies. Above all, ES cells exhibited pluripotentiality following introduction into blastocysts or "tweaking" in vitro.[62,63] ES cells differentiated into cells of all the embryonic germ layers, including trophectoderm capable of synthesizing human chorionic gonadotropin, embryoid bodies and cells expressing markers for neural precursor cells and rhythmically contracting cardiac muscle.[64]

Still another type of tissue-culture stem cell was isolated from later blastocysts and gonadal ridges of mice[65–67] and men.[68,69] Christened "embryonic germ" (EG) cells (also germline stem cells [GSCs]), the new cells mimicked ES cells. EG cells exhibited pluripotentiality when cultured with the usual ingredients, forming teratocarcinomas upon injection into tolerant mice (e.g. nude mice) and parts of chimeras upon introduction to blastocysts.

Of course, ES and EG cells are not embryonic or germ cells as such unless they are "tweaked" into it.[27,70–73] ES and EG cells are artifacts of tissue culture. "In fact, ES cells do not exist as such in embryos; they arise after being cultured"[74] when continuously propagated and their population allowed to expand indefinitely without differentiation in vitro. Moreover, germ cells do not have a clonal lineage in normal animals, since "no exclusive germ cell lineage existed in the preimplantation embryo".[75]

Interest in stem cells rapidly tilted toward ES and EG cells for a host of reasons not the least of which was the possibility of treating chronic and acute diseases, their sequelae and the effects of trauma.[76] Furthermore, hundreds of murine cell lines became available for injection into blastocysts and incorporation into embryos, including germ cells. ES and EG cells thus became the starting point for transgenic and knockout mice, for the technology of nuclear transfer (NT), cloning if not the creation of patient-specific ES cells from blastocysts following somatic cell nuclear transfer (SCNT).[77]

The dominance of ES and EG cells in stem-cell research has not diminished, but it has been challenged by adult stem cells from steady-state tissues and hybrid cells containing the nuclei of both ES and adult cells.[78] Indeed, some stem cells from adults seem to express embryo-like stem-cell properties.[79–83] and MAPCs derived from bone marrow mesenchyme exhibit pluripotency following transplantation to blastocysts.[13]

Pluripotent adult stem cells are thought to be ethically desirable, since they circumvent the moral conundrum posed by destroying blastocysts. But claims made for the pluripotency and transdifferentiation of adult murine stem cells are now greeted with skepticism, since the putative changes in the direction of stem-cell differentiation may be experimental artifacts such as consequences of cell fusion.[84–86] Moreover, presumptive HSC do not exhibit pluripotency following transplantation to blastocysts, although the progeny of the HSC may exhibit some reprogramming of gene expression.[87]

Confounding models of tissue dynamics

The historical mixture of stem-cell concepts boils down to two conflicting stem-cell types: steady-state and emergent stem cells. These, in turn, represent parameters in two different models of tissue dynamics: homeostasis or autopoiesis[88] as opposed to expansion and growth.[89]

Steady-state tissue dynamics

Many tissues are kept in the steady state through the action of self-renewing (SR) stem cells (also called "stereotypic", "actual" and "functional stem cells"[90]) that undergo asymmetric division, giving rise to a new stem cell (or "daughter" cell) and to a transit amplifying (TA) cell (also known as "proliferating precursor" and "progenitor cell"). (TA and its alternatives are "usually used interchangeably, but some [investigators] use progenitor cell to refer to a cell with greater developmental potential than a precursor cell".[91]) In steady-state cell populations, only the new SR stem cell remains in the stem-cell population, while the TA cell leaves the population, generally giving rise via further cell division to a large, clonal

lineage that, in turn, becomes a non-proliferative differentiating or maturing population, refreshing tissues and organs with replacement cells.[5] Similarly, in tracheophytes, a meristem consists of a proliferative module of small SR stem cells near the tip of a stem or root giving rise to initiating cells and hence derivative cells that differentiate as plant tissues.

SR stem cells in adult tissues, thus, maintain their own population while providing the cells that ultimately sustain the integrity of tissues, organs and hence the organism for the duration of an adult lifetime or until the steady state is disrupted. Ordinarily, cellular addition balances cellular loss, but the balance of proliferation and differentiation of the clonal lineage is presumably adjusted to accommodate circumstances such as moderate loss and wound healing. Regeneration of adult tissue would seem to take place when morphogenesis accompanies adjustments in TA proliferation and differentiation.

The fate of cells produced by asymmetric division may be decided stochastically (when 0, 1 or 2 cells are SR cells following division, but, on average, one turns out to be an SR stem cell)[92,93] or determinately (when one SR stem cell is always produced). Whether stochastic or determinate, cells may be influenced by any of a number of intracellular and extracellular mechanisms. For example, products of the cell-autonomous promoter (e.g. *piwi*[94]) may mediate gene expression; self-feedback, autocrine and paracrine influences may trigger intrinsic and extrinsic signaling and lateral inhibition; hormones, growth factors, cytokines and short-range cell-to-cell signaling pathways[95] may release intracellular cascades (e.g. Notch, Hedgehog and Wnt[96–98]). Hormones from remote sources (e.g. follicle-stimulating hormone [spermatogonia]; erythropoietin [erythropoietic stem cells]), positional cues residing in chemical gradients in extracellular space or a cell's resting position in the stroma may also influence, if not determine the choice of pathway.[99] Possibly, the new SR stem cell retains template strands of DNA, while the TA cell acquires the newly replicated strands.[12] ''Stem-cell niches'', ''anchors'' or ''focal sites'' may determine the fate of stem cells and the path of differentiation open to TA cells. Adhesion may influence the cell's decision to divide in the first place as well as the cell's commitment to differentiate one way or another.[100]

The asymmetric division of stem-cell residents of steady-state adult tissues represents a small proportion of the cell division occurring within these tissues.[4,5] The proliferation of TA cells represents the lion's share of cell division, but division may be distributed over lineages giving rise to different cell types. For example, in intestinal glands, TA cells differentiate into enteroendocrinocytes, exocrinocytes, goblet cells and intestinal absorptive cells.[12,93] HSC in situ seem to give rise to stem cells for T- and B-type lymphocytes, erythrocytes and two myeloid stem-cell lines, osteoclasts and ''clean-up cells'' such as hepatic fixed macrophage, alveolar macrophages, macrophage-monocyte cells in epidermis, various antigen-processing and -presenting cells of the mucosa-associated lymphoid tissue and dendritic (microglia) cells in brain.[8,101]

Emergent tissue dynamics

Development is a one-way street. Coupled to growth in embryos, larvae, organ rudiments and regeneration blastemas, development is progressive, irreversible change if nothing else, although ''negligible senescence'' or continuous growth without apparent aging may be available to some organisms.[102]

The stem cells of emergent tissues may not be programmed so much as they are programmable, and they may not be restricted so much as they choose among alternative pathways of differentiation. Pluripotential ES and EG cells are thought to acquire potency by reading instructions from stem-cell niches and switching their determination between the genetically stable alternatives otherwise available via the cells' developmental history.[103] The newly instructed cell undergoes symmetric division, leading to clonal expansion of committed cell lineages. Pluripotentiality gives way to differentiation, and cells are funneled into cell types.

Strictly speaking, emergent tissues are transient, and cells in emergent populations do not exhibit self-renewal. Initially, symmetric divisions produce a growing population of cells, and later emergent cell populations disappear entirely (with the exception of cancers, tissues in ever-growing, non-senescing organisms, and cells transferred successively in vivo or in vitro). Thus, the zygote, blastomeres, founder cells, telomeres, germ layers and organ rudiments are only temporary parts of developing organisms. Of course, these developing populations are normally replaced by various sorts of steady-state populations, and clonal expansion of developing tissues is replaced by SR stem cells and lineages of TA and differentiating cells.

Conclusions

The definition of stem cells cannot ''bear a certain fuzziness without any significant side-effects . . . [and] proceed unfettered ... in the absence of a consensus''.[10] On the contrary, ''reality grows to precisely the same extent as the work done to become sensitive to differences''.[104]

Presently, the long history of confusion over concepts of stemness boils down to two categories of stem cells: steady-state and emergent. The question is, what, if any, is the relationship between these ''stem cells''? The category of emergent stem cells comprises transient players in embryos and other developing tissues (founder cells, telomeres, germ layers and neural crest cells) and ES and EG cells in tissue culture exhibiting pluripotentiality and possibly morphogenesis in vitro and in vivo. Steady-state stem cells, on the other hand, are self-renewing and exhibit oligopotentiality in support of homeostasis but are typically difficult to raise in tissue culture.

It may turn out that a single class of "stem cell" can accommodate cells in the two categories. Ever since Weismann, the embryo has been seen as constantly expressing new genetic potential even if some of that potential now seems to depend on epigenetic silencing. Tissue culture may very well reverse silencing, turning a cell into a "jack of all trades" in vitro and in vivo. The steady state may then turn out to limit "cell types capable of extensive self-maintenance (self renewal) *in spite of* physiological or accidental removal or loss of cells from the population"?[105] But these premises might be premature.

How are asymmetric division, self-renewal and the steady state related to symmetric division, expansion and development? If the fate of SR stem cells following asymmetric division is indeterminate and decided stochastically, the problem of moving between development and steady-state population dynamics would seem one of signaling mechanisms that direct cells along paths of differentiation and integrate cell populations. But if the fate of an SR cell is determinate and nonnegotiable, then emergent dynamics would seem categorically different from steady-state dynamics. SR stem cells might coexist with or accumulate and ultimately replace emergent stem cells, but the transition between populations dominated by emergent and steady-state stem cells would be normally irreversible, since, were it reversible, the consequences would be catastrophic rather than salubrious.

Ultimately, answers to a few questions will determine how emergent and steady-state stem cells are related. (1) Does a stem cell change through its history, or is an SR stem cell in an adult an embryonic holdover "retained throughout life to participate in regeneration and repair"?[106](2) Are the features of SR stem cells in steady-state tissues inherited from stem cells in emergent tissues or dictated by stem-cell niches? (3) Are stem cells, like germ cells, "reprogrammable" and capable of expanding their potential for differentiating or is the potential of stem cells confined by history and the circumstance of their differentiation? (4) Are putative stem cells isolated from embryos and raised in tissue culture—so-called ES and EG cells—capable of skipping (or treading lightly) over the history that ordinarily directs adult stem cells, or must emergent stem cells experience the imprint of history before becoming steady-state stem cells?

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