All organisms depend on stem cells for their survival. As a result, stem cells may be a prerequisite for the evolution of specific characteristics in organisms that include regeneration, multicellularity and coloniality. Stem cells have attracted the attention of biologists and medical scientists for a long time. These provide materials for regenerative medicine. We review in this paper, the link between modern stem cell research and early studies in ancient organisms. It also outlines details on stem cells in the light of evolution with an emphasis on their regeneration potential, coloniality and multicellularity. The information provided might be of use to molecular biologists, medical scientists and developmental biologists who are engaged in integrated research involving the stem cells.

Key words Developmental biology - early organisms - natural selection - stem cell

Introduction

Evolutionary biologist Theodosius Dobzhansky once wrote 'Nothing in biology makes sense except in the light of evolution'. The term ‘light of evolution’ was used earlier by biologist and statesman of science, Julian Huxley. In fact, the notion light of evolution originally came from Pierre Teilhard de Chardin, who was admired by Dobzhansky. The latter then went on to say that species diversity in planet Earth cannot be explained by the creation fairy tale because of the ecological complexity. The question however is, whether or not the evolution of stem cell started with unicellular organism with its evolutionary linkage? The answer may be hidden behind the properties of the stem cell of self-renewal and differentiation. For example, single cell organisms have unique capabilities not only to renew by self but also perform differentiated role. On the contrary, stem cells in higher organisms have the ability to both self-renew through mitotic cell division and differentiate into a diverse range of specialized cell types.

Fossil evidence indicates the existence of unicellular prokaryotes on Earth over 3.5 billion years ago. One of the foremost multicellular organisms was seaweed that came into existence about 1,300 million years ago. So the existence of stem cells could be traced back to millions of years and these were developed through the process of natural selection. Accordingly, the appearance of stem cells could be viewed as fundamental in the lengthy evolutionary saga. In this review we have highlighted stem cells in the light of evolution. We have outlined details on stem cell multicellularity, regeneration and coloniality potential in the organismic and evolutionary perspectives.
Stem cells and multicellularity

The distribution of stem cells has been recorded throughout the animal and plant kingdoms, and all these organisms are known to be multicellular. However, during the development of multicellular organisms through natural selection, stem cells accompanied the evolutionary process, either in the mode of asexual or sexual reproduction\(^1\). In general, stem cells can be separated by two basic strategies\(^2\). One is asymmetric cell division and the other is stochastic differentiation. In the asymmetric cell division, there are mechanisms that might be described as invariant in which a stem cell gives rise through asymmetric cell division\(^8\) splitting into a stem daughter while the other undergoes differentiation. In the case of stem cell, due to asymmetric cell division, the cells first undergo a divide and produce one daughter like itself that maintains stem cell characteristics while the other programmed to differentiate into a non-stem cell fate leading to the path of differentiation. Such a division can be seen in single cell organisms and invertebrates. Asymmetric cell divisions occur during the development of *Drosophila* and *Caenorhabditis elegans*\(^8\). Similarly, multicellular organisms with a relatively few cell types can go through such splitting. In the multicellular hydra, head and foot can be regenerated in adult from a tiny piece of tissue mass from the body column\(^10\).

Stem cell follows another route of cell division which is ‘stochastic differentiation’. Here the stem cells make a combination of asymmetric divisions and symmetric ones. Finally, they produce either two stem cells (symmetric renewal), or two differentiated cells (symmetric differentiation). Some reports show that in olfactory epithelium\(^11\) and muscle\(^12\) stem cell follows the stochastic differentiation cell division.

**Stem cells and regeneration potential**

Regeneration has received renewed research interest in recent years. Stem cells have been extensively studied due to their regeneration potential. First, this property was shown by histologists in the 19\(^{th}\) century who introduced an abstract term for cells which can specifically repair or regeneration potential. With the discovery of bone marrow cells in the 1950s, the haematopoietic stem cell concepts started to emerge\(^13\). Haematopoietic stem cells are accountable for the steady renewal of the blood cells while mesenchymal stem cells, a group of stromal cells show multilineage segregation ability. Mesenchymal stem cells have been isolated from different multicellular organism like human\(^1\). The process in which a stem cell gives rise to daughter cells with definite probability of being either stem cells or committed progenitors is evident in a vast majority of mammalian self-renewing tissues. Generally, each stem cell division gives rise to a stem and a committed daughter at stable state. However, unevenness can be achieved on a population basis rather than individual cell division basis. Moreover, in some tissues there may be a range of cell behaviour with stem and progenitor cells at opposite ends of a spectrum instead of discrete stem and progenitor populations\(^14-17\). Nevertheless, great variability in the self-renewal process by a stem cell does occur\(^18\).

In simple single cell organisms such as the amoeba for example, a simple cell division is equivalent to reproduction by which a new organism is created more frequently\(^19\). Stem cells of small rodents on the contrary are estimated to replicate about once in four weeks- for cats, it is once in per ten weeks and for higher primates once in 50 wk\(^20\). This is largely due to the intricacy of self-renewal process enhanced by natural selection.

In early animals, single cell carry out several physiological functions while serving as stem cell. A classical example is hydra where single epithelial cells appear to carry out several steady-state physiological functions while serving as stem cell\(^21\). These cells perform both the process of self-renewal and differentiation. Hydra belongs to the exclusively aquatic phylum of Cnidaria- these early branching-animals have survived for millions of years; they do not undergo ageing (senescence) hence biologically eternal. This natural everlasting characteristic can be attributed by the asexual mode of reproduction via budding- it simply requires a tiny tissue of stem cells with continuous self-renewal capability. Stem cell differentiation in the hydra is governed by co-ordinated actions of conserved signaling pathways. Hence, the hydra’s stem cell represents a critical insight of general significance of its biology (*i.e.* cellular senescence, lineage programming and reprogramming, extrinsic signals in fate determination, tissue homeostasis) and the ultimate evolutionary origin\(^22\).

The role of genes in vertebrate regeneration has received great interest among the scientific community and studies have been targeted to cross-examine gene transcription and protein translation during different steps regeneration\(^23-26\). Maki et al\(^23\) reported that the expression of some genes namely *Sox2*, *Klf4*, and *c-myc* have been unregulated when it comes to regenerating potential. These three genes are in fact the
most important induced pluripotent stem cell (iPSC) genes which can be unregulated in regenerating new lens and limb.

The zebrafish fins and Xenopus limbs have also become important models for the study of regeneration. In regenerating zebrafish fins, homologues of genes are related with the pluripotency and expressed to initiate the regeneration process\textsuperscript{27}. Scientists have concluded that blastema cells in zebrafish fins and Xenopus limbs are not completely analogous to induced pluripotent mammalian stem cells but these tend to share some similarities in gene expression\textsuperscript{28}. A study of zebrafish tail fins by Stewart \textit{et al.}\textsuperscript{28} has concluded that histone demethylase is necessary for regeneration by identifying targets of histone methylases and demonstrating histone modifications silence promoters of numerous genes involved in regeneration. The regulatory genes contain bivalent me(3)K4/me(3)K27 H3 histone modifications created by the concerted action of Polycomb (PcG) and Trithorax histone methyltransferases. During the evolutionary process, regeneration appears to be common among the adults of many non-vertebrate organisms. However, among the adult vertebrates, amphibians like the salamanders are unique in a way that they could regenerate limbs\textsuperscript{29,30}. During the larval stage, the developing limb bud is poised of undifferentiated cells while the adult limb is composed of fully differentiated tissues. The limb stem cells appear to help in the regeneration process\textsuperscript{31}. On the other hand, it has been reported that adult human has less potential of regeneration compared to other group of organisms. Nonetheless, some parts such as the skin fingertips, ribs, liver, kidney and heart have the capacity to regenerate and repair themselves to some extent where adult stem cells have been found in very low frequency\textsuperscript{32-34}. It appears that the evolutionary process might have created the less occurrence of adult stem cell in humans.

**Stem cells and coloniality**

Coloniality comprises large congregation of individuals in a place that includes the same species living together with a mutual advantage of self-protection. It is an important question in evolution that how group-living organisms assemble together and how the colonial origin came into existence that harbours over a million individuals breeding and living in proximity. Colonial behaviour itself is an evolutionary ambiguity because individuals pay fitness costs to breed in high densities\textsuperscript{35}. However, the coloniality character has been recorded among some phyla, specifically the tunicates, cnidarians, entoprocta, ectoprocta and bryozoans\textsuperscript{36}. Ascidians are one of the members of chordates that belong to the Tunicata phyla and they offer unique opportunities to investigate the biology of stem cells. At larval stage, the Asciidians have a typical chordate body plan including notochord, dorsal hollow nerve tube and striated musculature. Subsequent to its swimming stage, the larvae settle and undergo extensive metamorphosis\textsuperscript{37}. At that stage chordate characteristics are resorbed and ultimately end up as filter feeding sessile invertebrate mature animal. Due to its small size and rapid development, Ascidian larvae have been used widely as a model to study specification and differentiation events of developmental biology since they exhibit solitary and colonial forms. Solitary ones can reach up to 10 cm while the colonial variety can spread up to several meters\textsuperscript{3}. The colonial ascidians have two developmental pathways to create an identical adult thus unique among chordates with regenerative capability and became an outstanding model for embryonic and stem cells research\textsuperscript{38}.

One peculiar taxon known as Botryllid ascidian has become a model for allore cognition studies because of the allogeneic fusions revealing the evolutionary links between allore cognition, stem cell biology and ecology\textsuperscript{39}. These colonial ascidians live in superficial sea water in all temperate zones around the world\textsuperscript{40}. After hatching from their colony, \textit{Botryllus} tadpoles larvae swim to surface where these attach and undergo metamorphosis resulting in the loss of chordate characteristics (tail, notochord, neural tube, and segmented musculature through the apoptosis)\textsuperscript{41}. At the beginning of bud formation, the vesicle cells are morphologically undifferentiated; these stem cells can self-renew during asexual reproduction. These take part in organogenesis and gonad formation and these occur in clonemates in a vascular fused colony. So the genomes of circulating germline stem cells and somatic stem cells are the same and it is obvious that the individual tadpole is a target of natural selection. Colony fusion offers the opportunity for germline stem cells or somatic stem cells to move from one colony to a genetically distinct colony\textsuperscript{42}.

Another example of coloniality is the hydrozoan colonies. This fauna is a member of the Cnidaria phyla. These colonies consist of multiple polyps connected together by tube like structure and all colonial include some polyps specialized for reproduction. The hydrozoan contains a population of migratory stem
cells and the epithelial cells of the colonies serve as stem cell for some physiological process. Presently, the hydrozoan colonies are point of attraction to study evolution. However, the characteristic of coloniality in different organisms also proves the characteristic of multicellularity.

**Stem cell evolution in branching vertebrates and mammals**

In early branching vertebrates such as fish and amphibians, adult stem cells are within the organ, for example, retinal stem cells are found in the periphery of the retina while the ciliary marginal zone produces new neurons in retina throughout life. In these species, retina grows to keep pace with the enlargement of body. However, among higher vertebrates such as birds and mammals, when they reach adulthood, the retina stops growing so there appears to be no need for such a proliferative area with stem cells. A study suggests that a region similar to the ciliary marginal zone of fish and amphibians exists in the post-natal chick and adult mouse.

In mammals, some evidence supports the properties of stem cell evolution. Due to larger size and longer life, larger mammals require more blood cells. As a matter of fact, the total number of human haematopoietic stem cells (HSCs) is equivalent to the total number of HSCs in cat and mouse. This fact strongly supports that the number of HSCs per animal is conserved in mammals. After injury, active adult stem cells help to renew and regenerate the tissue. It is an essential physiological phenomenon in all mammals. This example shows the evidence of conservation of active adult stem cell in mammals.

**Stem cell and regulatory gene networks**

How does the regulatory gene network perform behind the stem cell in the light of evolution? Various factors such as the microenvironment, signaling events and genetic characteristics are often associated with this property of stem cell. Models like *Clytia hemisphaerica* are available to analyze how stem cell intrinsic factors are integrated with signaling events, and how the microenvironment of ‘stem cell niche’ maintains tissue homeostasis. In the early branching-animal of *Clytia*, for example, nematoblasts occur between ectodermal epithelial cells within the ‘tentacle bulbs’ from where the tentacles grow. The tentacle bulbs are spherical outgrowths of bell margin of medusa. Spatial progression of nematoblast stages along the bulb axis is correlated with differential stem cell marker gene expression. Most of the cells at the base of the tentacle bulb express *Clytia Piwi* homologue gene but not a differentiation marker. In fact, the *Piwi* gene is a widely conserved stem cell marker throughout multicellular eukaryotes, and these cells might be considered as a population of stem cells.

In several invertebrate groups, especially sponges and planarians, tissue plasticity and regeneration capacity based on stem cells are the common characteristics. These organisms harbour a *Piwi* gene, which is a conserved gene for regeneration and plasticity. *Piwi* homologues have been identified from freshwater sponge, *Ephydatia fluviatilis*, as candidate stem cell (archeocyte) markers. Planarian regeneration is based upon totipotent stem cells, the neoblasts. *Piwi*, especially *DjPiwi-1*, has been identified from planarian stem cells during the regeneration process of the stem cell. *DjPiwi-1* gene of planaria is a homologue of *Drosophila piwi*. This gene is a member of the PAZ-*Piwi* gene family and can be used as a marker for stem cell.

Would it be possible for a stem cell gene to change over evolutionary time? Computational tools and development of genomic resources could lend a hand to answer the ultimate evolutionary function of stem cells. In organisms such as the cnidarians, bilaterians and metazoans, *Sox2* is known to date as one of the most conserved stem cell-specific genes. Cnidarians and bilaterians were known to have diverged over 560 million years ago and the discovery of *Sox2* in early branching metazoans suggests that there is a similarity of the regulator gene of stem cell potency that might have present in both groups. The presence of stem cell marker gene like *Sox2* strongly supports the above evidence that appears to be highly conserved. However, *Nanog* or *Oct 3/4* is present in metazoans. On the other hand, there is another example of preserved regulatory gene of a stem cell like *wnt* gene. The *wnt* pathway has been recorded in the preservation of *D. melanogaster* germ, mammalian haematopoietic, gut, and hair follicle stem cells. This *wnt* gene has been conserved in different stem cells during the process of evolution through natural selection.

As a matter of fact, stem cells and their niches evolved in the multicellular organisms and have many features that are conserved between vertebrates and *Drosophila*. One of the well characterized stem cell niches resides at the tip of the *Drosophila* ovary where it regulates germline stem cells. Subsequent types of stem cell, escort stem cells, also reside in this niche and
interact closely with germline stem cells. These escort stem cells division provides one or two squamous cells that wrap each developing germ cell cyst and suggests how niche facilitates co-ordinated activity of these two types of stem cells. Gut stem cells are likely to be controlled by a niche that differs from the germline stem cells or escort stem cells niche in two respects. A niche cell might act as an anchor of the stem cell in position though it is not clear\textsuperscript{60}. Further, the niche appears to repress most gut stem cells in a temporally and spatially regulated manner and most evidence suggests that multicellularism evolved separately. Nonetheless the \textit{piwi} gene in \textit{Drosophila} is more conserved\textsuperscript{61}. It is evident that evolution enveloped systematic regulatory gene network in stem cell for self-renewal process formed by Oct4, Sox2, and Nanog, in particular, controlling embryonic stem (ES) cell pluripotency in mammals\textsuperscript{62} that are also more conserved.

**Conclusions**

Evolutionary theories are based on single gene homologies or cross-kingdom gene transfer assisting convergent evolution through biochemical processess in plants and animals\textsuperscript{63}. Plants have been ignored focus as a resource for stem cell research because single cells from adult plants have the ability to make complete new adult plants\textsuperscript{64-66}. Despite this, plants and animals have some homology, both organisms evolved separately influenced by natural selection. The homology between the \textit{piwi} gene in \textit{Drosophila}, which controls germ line stem cells, and the \textit{ZWILLE} gene in \textit{Arabidopsis}, which controls the stem cells of the shoot meristem has led to the suggestion that “stemness” evolved in a single-celled ancestor, or plants and animals might have shared a multicellular ancestor\textsuperscript{5}. In Metazoans, \textit{Drosophila}, \textit{C. elegans}, and all vertebrates, studies suggest that natural selection otherwise adhering to the principles of Mendel and Hardy-Weinberg and the mechanisms might have been due to fitness selection\textsuperscript{67-70}. However, during the evolutionary process, sequestration of cells in one conspecific animal from another is not the rule; many species tend to exist wherein genotypically distinct cells may intermix within a chimeric entity\textsuperscript{31,72}. The ideas of stem cell-based organogenesis and stem cell-based regeneration are interrelated and developed through the evolutionarily selection process\textsuperscript{62}. Therefore, stem cells are not only the entity of biological organization, accountable for the progress and the regeneration of tissue and organ systems, but also are units in the complex evolutionary process. However, more understanding between the relationships and dynamics of molecular signatures and gene regulatory circuits behind stem cell will lead us to know more about the stem cell in the light of evolution.

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