HYPOTHESIS

Does haemocoelom exclude embryonic stem cells and asexual reproduction in invertebrates?

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The terms embryonic and adult stem cells are explained. Previous studies on identification, description and isolation of the embryonic stem cells in different invertebrate groups are briefly summarized. Most invertebrates, which reproduce asexually have retained the embryonic stem cells in their adult body. A hypothesis is proposed for the possible exclusion of embryonic stem cells and thereby asexual mode of reproduction by the coelom in arthropods and molluscs.

This hypothesis attempts to establish a correlation between the presence of haemocoelom and the absence of embryonic stem cells and the consequent non-occurrence of asexual reproduction in arthropods and molluscs. Most invertebrates reproduce sexually but may switch over to asexual mode of reproduction, when need arises owing to biotic factors, e.g. very high density[1] or abiotic factors, e.g. water quality parameters[2]. The presence of embryonic stem cells is obligatorily required to facilitate the asexual reproduction, as in sponges, cnidarians, turbellarians, clitellates and echinodermates.

Types of stem cells

Based on their differentiation potential, stem cells can be divided into two major types: (i) the embryonic stem cells[3], derived from the inner mass of early blastocytes, as in echinoderms, have retained the capacity to generate all the two/three germinal layers, from which fully developed progeny arises, and (ii) the adult stem cells, hidden deep within the organs and surrounded by millions of ordinary cells in fully developed adult animals, have restricted potential to produce only certain types of cells. The processes of differentiation by embryonic and adult stem cells are known as epimorphosis and morphallaxis respectively[4]. The epimorphosis involves the activation of embryonic stem cells to proliferate, form blastema and differentiate into the regenerated body parts as in *Dugesia tigrina*. The morphallaxis involves the transformation of existing body parts or tissues into newly organized structures without cell proliferation, e.g. *Crepidula plana*.

Investigations since early 1900s on regeneration in triclad turbellarians showed that fully differentiated adult animals harbour unique 'embryonic stem cells'. These cells have retained the capacity for self-regeneration and ability to differentiate into progeny. They are slow-cycling undifferentiated cells and divide asymmetrically into daughter cells, one of which is committed to differentiation and the other retains the capacity of the original stem cells, which can differentiate all the cell types required to generate progeny. Because of their slow cycling, adult stem cells of human with limited potential (to produce certain cell types) can be identified by their prolonged retention of nucleotide analogues like bromodeoxyuridine[5]. This communication points out the need for identification and description of adult and embryonic stem cells in animals bestowed with the capacity for regeneration of a part of the body or an entire organism from 'bits and pieces' of the parental animal.

Animals vary widely in their ability to replace lost body parts through regeneration[6]. The phylogenetic distribution of regenerating ability across animals implies that this capability has been gained and/or lost many times during the chequered history of evolution. Despite the recent surge of interest in adult stem cell research, comparative studies on identification and description of such stem cells in animal groups characterized by different abilities to regenerate the lost parts of body is needed. To date, regeneration studies have focused almost on a few, very distantly related groups such as cnidarians, turbellarians, clitellates and echinodermates.

For reasons yet to be known, arthropods and molluscs, characterized by haemocoelom, have only a minimal capacity to regenerate a stump on the lost part of an appendage, as in arthropods or to regenerate the lost part of the inhalent and exhalent siphons, as in bivalves, but have no capacity to regenerate an entire animal. The deep evolutionary separation between embryonic and adult stem cell model systems and its implication to anatomical differences between them make it nearly impossible to reconstruct, which evolutionary and developmental mechanisms are responsible for such wide differences in the ability of regeneration among these groups. On account of this fact, there is an urgent need for identification and description of tissue/animal regeneration in selected invertebrates, harbouring 'stem cells'.

In sexually reproducing animals, the zygote, a product of fusion of two gametes is developmentally totipotent and has the capacity to generate both (as in sponges and cnidarians) or all three (as in all other higher animal groups) germinal layers and a completely developed progeny. In parthenogenetic animals, the female produces diploid egg, from which completely developed progeny arises. However, in asexually reproducing animals, the equivalent of 'zygotes' namely embryonic stem cells are retained in specialized 'niches' and are capable of producing completely developed progenies.

It is known that adult bone marrow of human contains cells, which can make all types of blood cells. But these stem cells could not be isolated as pure populations, as the techniques for recognizing adult stem cells were developed only after 1980s (ref. 7). As indicated elsewhere, the inconspicuous nature of the stem cells in terms of numbers, size, shape and function make their identification and isolation a herculean task.

These adult stem cells possess an array of proteins on their surface; the surface proteins can be used as 'markers', which characterize individual cell types, i.e. a type of 'molecular marker'. For example, using molecules that recognize and attach the specific surface proteins,

which can be blazed under certain wavelengths of light, a blood stem cell can be distinguished from a mature white blood cell. Unfortunately, not all stem cells can be identified in this way, as 'molecular markers' have not yet been identified for all the stem cell types, which occur in other animal groups, especially the invertebrates. Hence, there is a need for molecular biologists to develop suitable markers to identify the stem cells in adults of different invertebrate animal groups.

Modes of reproduction and regeneration

Asexual mode of reproduction among invertebrates is not homogeneous in its nature, as it proceeds by fragmentation and budding in sponges, cladogenic, blastogenic buddings and strobilation in cnidarians, fission in turbellarians, architomic and paratomic fission in clitellata, and by fission and autotomy in echinodermates. Many scientists have endeavoured to trace the ultimate progenitor cells, from which a complete progeny arose and named those stem cells by different designations namely, archeocytes and thesocytes in sponges, stem interstitial cells and amoebocytes in cnidarians, neoblasts in turbellarians, blastocytes and eleocytes in clitellata, and coelomocytes in echinodermates and indicated that these cells are totipotent/omnipotent or pluripotent/polypotent/multipotent. Of these, the following must be mentioned.

Working on *Oscarella tuberculata,* a homoscleromorph sponge, which shares many morphological, cytological, biochemical and embryological features in common with eumetazoa Ereskovsky and Tokina indicated that this sponge and bilaterians share highly conserved homologies in basic genetic machineries involved in cell differentiation and regulation of development[8]. Thus their research work has provided the first bridge on polarity, axial formation and regulation mechanism of development between the two-layered sponges and the three-layered animals.

In cnidarians, the situation remains a little complicated. The structural cells, i.e. ectodermal plus endodermal cell complexes are responsible for giving the polyp its form and the 'stem cells', i.e. amoebocytes maintained among the structural cells by controlled cell cycle give the polyp its behaviour and sex. The amoebocytes are known to migrate and proliferate at the site of budding. But, Gilchrist[9] showed that the epidermis of a polyp alone is capable of regenerating a complete polyp. Hence it is not clear whether the true stem cells are maintained amidst the structural, i.e. subtentacular cells or interstitial cells. However, heterogeneous asexual modes of reproduction in cnidarians are far more complicated to comprehend a single concept, as has been spectacularly achieved in triclad turbellarians.

The triclads display remarkable power of regeneration and have been the object of numerous researches, especially by the French school led by E. Wolff, who postulated polarity and axial gradient theory. However, the central question concerns the origin of the cells in 'blastema', from which any injured or removed part of the body is reconstructed. Amazingly, it was traced to the free basophilic cells buried in the parenchyma called 'neoblasts' and the theory of neoblasts was proposed as early as in 1889–1901 by Morgan[10,11]. The neoblasts of endodermal origin are regarded as undifferentiated totipotent elements, which remain quiescent from the embryo stage up to the moment at which they participate in formative process. Capable of migrating by means of amoeboid movement, they reach the area in which mutilation has occurred.

Betchaku[12] was the first to obtain selectively a culture of neoblasts. Subsequently, Franquinet[13] and his collaborators[14] developed new culture media, which yielded a large number of neoblasts but still mixed with other cell types. Using the selective adhesive property of the neoblasts to the substrate, they eliminated the other types of cells, which led to the culture of neoblasts with 'high purity'. Thus it was possible as early as in 1985 to have a highly pure culture of neoblasts, i.e. embryonic stem cells, something similar to what has been achieved with molecular markers for the adult stem cells in recent years. Some of these techniques may be handy to zoologists to isolate and culture the embryonic stem cells of other animal groups like the annelids.

It appears that regeneration research in anthozoans, clitellates and echinodermates proceeded in the direction of locating and quantifying the minimum required 'niche' of stem cells to induce successful epimorphosis. Annelids are an excellent group to investigate regeneration abilities in a comparative context. As their bodies are composed of repeated segments, which largely possess the same structures (segmented nerve ganglia and fibres, musculature, gut, blood vessels, nephridia, chaetal bundles and so on), any mutilation made at different axial positions along the body results primarily in the removal of different quantities of a given organ system, rather than the removal of different organs/systems or unique structures and thus facilitates comparisons among the annelid species. The ability to regenerate both anterior and posterior segments is widespread and probably ancestral for the phylum[15]. Some sabellids and lumbriculids are capable of regenerating an entire individual from a single mid-body segment, which indicates that adequate number of embryonic stem cells is retained in every segment[16].

Small and medium sized sea star *Allostichaster insignis* divides throughout the year and the ramets of most individuals regenerates sufficiently to divide again after 6–9 months[17]. In the sea star *Ophiocoma echinata*, a piece of oral disc is necessary to complete regeneration and requires a long duration of two years to completely regenerate the three arms[18] at the energy cost of 0.17 kJ/day[19]. On the other hand, fragments of about 20 cm length are required to regenerate an individual with reproductive capacity in the branching coral *Acropora formosa*[20]. According to the description of Reichensperger, regeneration in *Neocrinus decorus* commences promptly by two types of cells abundant along the nerve cods: the phagocytic amoebocytes and the coelomocytes, filled with rods and granules; they become elongated in shape and assist the process of regeneration[21].

Briefly the epimorphic regeneration occurring in sponges, cnidarians, clitellata and echinodermates originates from totipotent embryonic stem cells according to Borok[5]. Morphallaxic regeneration encountered among arthropods and molluscs originate from multipotent adult stem cells capable of generating the germinal layers/organ specific cell lineages. However the embryonic stem cells appear to be absent in these two animal groups. Arthropods are capable of regenerating undifferentiated mass of tissues on autotomized fraction of appendages[22]. Molluscs have retained multipotent adult

Table 1. Correlation between coelomate type, asexual reproduction and embryonic stem cells in invertebrate groups

Invertebrate group	Coelomate type	Equivalents of embryonic stem cells	Occurrence of asexual reproduction
Sponges	–	Archaeocytes, thesocytes	Yes
Cnidaria	–	Stem interstitial cells, amoebocytes	Yes
Turbellaria	Acoelomate	Neoblasts	Yes
Clitellata	Eucoelomate	Blastocytes, eleocytes	Yes
Echinodermata	Eucoelomate	Coelomocytes	Yes
Arthropoda	Haemocoelomate	Absent ?	No
Mollusca	Haemocoelomate	Absent ?	No
Nematoda	Pseudocoelomate	Absent ?	No
Rotifers	Pseudocoelomate	Absent ?	No
Chaetognatha	Coelomate	Absent ?	No

stem cells capable of regenerating tissues/organs involving mesoderm and ectoderm alone. Many bivalves suffer the 'siphon-nipping', i.e. the removal of the terminal fraction of the siphons by predators. Hodgson[23] estimated the requirement of 92 h time and 0.6 kJ energy to regenerate a 6 mm long siphon representing 20% of total length of the siphon. In *Octopus vulgaris*, O'Dor and Wells[24] recorded the presence of arms with various stages of regeneration. A 'climax' is the case in which organ specific regeneration involving mesoderm and ectoderm has been reported in *C. plana*, which regenerated its head within 14 days following its removal after anaesthetization but the snail failed to regenerate alimentary canal of endodermal origin[25].

The proposed hypothesis

From a careful visual survey through the multivolume series on '*The Invertebrates*' by Hyman, and that on '*Reproductive Biology of Invertebrates*' by K. G. Adiyodi and R. G. Adiyodi, relevant available information on the presence of embryonic stem cells and occurrence of asexual reproduction in major groups of invertebrates was made. For a few minor invertebrate phyla, adequate and reliable information is not yet available. Besides, the internet was surfed using Google.com with the keywords: haemocoelom, asexual reproduction, embryonic stem cells, and invertebrates. From these sources, Table 1 was formulated and the following inferences were made.

- The presence of the equivalents of embryonic stem cells has facilitated the occurrence of asexual reproduction in many major invertebrate groups.
- However, in a couple of minor groups characterized by the presence of pseudocoelom and in the major groups of arthropods and molluscs possessing haemocoelom, asexual reproduction is not known to occur.
- Incidentally, the presence of embryonic stem cells or their equivalents has not so far been recorded in these animal groups.

These inferences lead us to propose a hypothesis, i.e. embryonic stem cells are obligatorily required to facilitate asexual reproduction; pseudocoelom of nematodes and rotifers, and haemocoelom of arthropods and molluscs appear not to have provided the required niche for retaining embryonic stem cells and thereby the non-occurrence of asexual reproduction in these animals. This hypothesis, however is yet to be tested. Incidentally, a rare claim has been made by Vanderspoel[26] on the occurrence of asexual reproduction in a haemocoelomate snail *Clio pyramidata*, which may prove an ideal model to test the hypothesis. Incidentally, it must also be mentioned that despite the presence of embryonic stem cells *Polycelis nigratenuis* has secondarily lost asexual mode of reproduction[27]. Likewise, a large number of polychaetes have secondarily lost the capacity for asexual mode of reproduction[15].

However, sporadic occurrence of sex change from female to male or male to female in sequential hermaphrodites like annelids, e.g. *Sphaerosyllis hermaphrodita*[28], arthropods, e.g. *Clibanarius*[29], molluscs, e.g. *Xylophaga dorsalis*[30] involve dedifferentiation and redifferentiation of organs related to reproductive system. Apparently, all of them appear to have retained multipotent adult stem cells somewhere in the gonad. It is known that the components of reproductive system are of mesodermal origin; however, it is also known that vitellogenin is synthesized in the liver/hepatopancreas/fat bodies of females and transported and deposited in the maturing oocytes of ovary. Hence, the liver of endodermal origin and equivalent organs are 'feminine'. Therefore, all these animals, which change sex from male to female, may also serve as experimental models to test the proposed hypothesis.

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