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Aging and longevity in the simplest animals and the quest for immortality

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Abstract

Here we review the examples of great longevity and potential immortality in the earliest animal types and contrast and compare these to humans and other higher animals. We start by discussing aging in single-celled organisms such as yeast and ciliates, and the idea of the immortal cell clone. Then we describe how these cell clones could become organized into colonies of different cell types that lead to multicellular animal life. We survey aging and longevity in all of the basal metazoan groups including ctenophores (comb jellies), sponges, placozoans, cnidarians (hydras, jellyfish, corals and sea anemones) and myxozoans. Then we move to the simplest bilaterian animals (with a head, three body cell layers, and bilateral symmetry), the two phyla of flatworms. A key determinant of longevity and immortality in most of these simple animals is the large numbers of pluripotent stem cells that underlie the remarkable abilities of these animals to regenerate and rejuvenate themselves. Finally, we discuss briefly the evolution of the higher bilaterians and how longevity was reduced and immortality lost due to attainment of greater body complexity and cell cycle strategies that protect these complex organisms from developing tumors. We also briefly consider how the evolution of multiple aging-related mechanisms/pathways hinders our ability to understand and modify the aging process in higher organisms.

Keywords

Metazoa; Bilateria; Cnidaria; planaria; flatworms; hydra; sponge; regeneration; rejuvenation; stem cell; pluripotent; neoblast

1. Introduction

The human lifespan is fairly long compared to that of many other animals, but is nevertheless limited with most people living about 80 years and rare individuals reaching 100 years. Yet there are some animals, plants and fungi that can live for several hundred or

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even several thousand years, and often show negligible senescence. What are the mechanisms underlying great longevity and can we apply such knowledge to enhance the health and longevity of humans? The longest living animals are also among the simplest ones – the ones that are called basal metazoans, a group that includes sponges, corals, jellyfish, comb jellies, hydras, and sea anemones. All of the more advanced animals including humans are bilaterians, and the simplest of these are the flatworms (Table 1). Basal metazoans typically maintain many pluripotent stem cells that are capable of differentiating into all types of cells in the body (Table 2); this gives these animals incredible abilities to grow, regress, regrow and regenerate their bodies as needed (Table 3; Rando, 2006; Pearson and Sánchez Alvarado, 2008; Tanaka and Reddien, 2011; Rink, 2013; Solana, 2013). They can become in some cases potentially immortal (Rando, 2006). However, during the evolution of more complex animal body forms, these abilities were reduced or lost, apparently in an effort to produce complex body structures for sophisticated functions while still avoiding the production of destructive tumors (Rando, 2006; Pearson and Sánchez Alvarado, 2008; Popov, 2012). Nevertheless, there is no direct correlation of increased body complexity with reduced lifespan. For example, among the bilaterians, some vertebrates such as tortoises and whales can live more than 200 years and some clams live over 500 years, while the adult roundworm, *Caenorhabditis elegans*, although only a little more complex in design than flatworms, lacks somatic stem cells and lives only a couple of weeks (Rando, 2006; Pearson and Sánchez Alvarado, 2008; Popov, 2012; Petralia et al., 2014; Butler et al., 2013; Rink, 2013; Treaster et al., 2013; Aitlhadj and Stürzenbaum, 2014). The evolution of animals with greater complexity included the development of mechanisms for limiting lifespan and senescence. In this review, we concentrate on aging and associated phenomena in the basal metazoans and flatworms. We will start by briefly reviewing basic mechanisms of aging. Next we will describe the one-celled organisms that evolved before multicellular animal life and look at what strategies for lifespan and aging were retained in the first animals. Then we will examine these strategies in each of the groups of simple animals; and finally we will consider in general terms how all of this changed in the evolution of the higher Bilateria including those with long lifespans like humans as well as those with short lifespans like *C. elegans*.

2. Mechanisms of aging overview

In this review, we will discuss the extensive literature that reveals a fascinating feature of aging throughout animal evolution, that is, a gradual decline in the abundance of totipotent/pluripotent stem cells. Since these cells maintain the potential to give rise to any cell type that makes up the organism, their reduction or absence in more complex animals means that most fully differentiated adult cells in complex animals will age and die, eventually leading to the death of the animal, therefore preventing the possibility of immortality.

Aging of the fully differentiated adult cells results from a combination of mechanisms (Fig. 1; López-Otin et al., 2013). We will describe several of these mechanisms throughout this article. In this section, we briefly discuss five problems, which are known to play prominent roles in aging. We refer the reader to López-Otin et al., 2013 for a comprehensive review of aging mechanisms.

First, the genomic DNA and epigenetic problem. Throughout the lifespan, it is inevitable for a cell to encounter a range of challenges and insults from the surrounding environment and within the cell. These insults cause lesions to DNA molecules (Hoeijmakers 2009). The examples of such DNA lesions include point mutation, double-strand break, translocation, and telomere shortening (Hoeijmakers 2009; López-Otin et al., 2013). In order to fix these DNA lesions, cells are equipped with highly elaborate and dedicated DNA repair machineries (Lord and Ashworth, 2012; Yang et al., 2013). Extensive evidence has suggested that increased DNA lesions, and or, weakened DNA repair systems directly contribute to the aging process (Lord and Ashworth, 2012; Baker et al., 2013; López-Otin et al., 2013; Yang et al., 2014). In addition to DNA molecules themselves, epigenetic factors are also critical to aging processes. Among these epigenetic factors, histone modifications seem to play particularly important and widespread roles in aging (Han and Brunet, 2012; Kanfi et al., 2012; Kawahara et al., 2009; Schellenberg et al., 2011). For example, the reduction of NAD-dependent SIRT6 deacetylase (a member of the sirtuin family) significantly shortens the lifespan in mice; conversely, forced overexpression of this enzyme in mice enhances longevity (Kanfi et al., 2012; Kawahara et al., 2009). The roles of histone modifications in regeneration and stem cell biology have been studied in the planarian *Schmidtea mediterranea* (Robb and Sánchez Alvarado, 2014).

Second, the protein quality control problem. Once proteins are synthesized, they undergo careful folding, which is an uncompromising requirement for functionally competent proteins and for healthy cells (Balch et al., 2008). Accordingly, cells ranging from unicellular organisms to humans all have developed tightly-regulated systems that execute the proper folding (Brodsky, 2014), and also promptly remove those proteins that are unfolded or misfolded (Jarosz et al., 2010). The maintenance or disruption of such proteostasis directly impacts cellular aging (Koga et al., 2011). The importance of proteostasis is further illustrated by the characteristic pathology - aggregation of misfolded proteins - found in several age-related diseases, such as Alzheimer's disease and Parkinson's disease (Fig. 2; Valastyan and Lindquist, 2014).

Third, the organelle problem. Changes during aging are not restricted to DNA and proteins, but also affect, and are affected by cellular organelles. In the case of the nucleus, the lamina - one of the nuclear envelope components - has been implicated in the aging process. For example, mutations in genes encoding components of the nuclear lamina have been found to be the cause of several premature aging syndromes (Eriksson et al., 2003; Cabanillas et al., 2011). Studies of normal aging cells have demonstrated that lower levels of, or defective lamin A or B subtypes accelerate the aging process (Schffidi and Misteli, 2008; Shimi et al., 2011). For this reason, nuclear lamina has been used as a biomarker of aging (Ragnauth et al., 2010; Freund et al., 2012). A recent study of healthy centenarians has shown that nuclear accumulation of prelamin A contributes to longevity by preventing stress-induced DNA damage (Lattanzi et al., 2014), further supporting the critical role of nuclear lamina in maintaining healthy aging. Another class of organelle that is associated with cellular aging is mitochondria (Trifunovic and Larsson, 2008; Lanza and Nair, 2010). Mitochondrial DNA is particularly susceptible to age-dependent mutations, at least in part, due to the DNA repair systems that are less powerful than those used for genomic DNA (Linnane et al., 1989; Vermulst et al., 2008; Wallace, 2010). Moreover, since mitochondria provide a major level

of regulation to a range of intracellular processes such as converting energy substrates into ATP, reactive oxygen species (ROS) metabolism, calcium signaling, and iron homeostasis (Mattson, 2008; Mallikarjun et al., 2014), it is likely that the dysfunction of mitochondria has major impacts on the molecular processes that contribute to normal aging and age-related diseases.

Fourth, the signaling pathway problem. A number of signaling pathways have been shown to play key roles in cellular aging. Prominent among such age-regulating pathways is the insulin/IGF-1 signaling pathway (Tatar et al., 2003; Guarente and Picard, 2005). The insulin/IGF-1 pathway is important because it regulates energy metabolism, food intake and stress responses (Mattson et al., 2004). The involvement of the insulin/IGF-I pathway in aging has been documented in a variety of organisms. In hydras, a protein that bears resemblance to mammalian insulin receptor is expressed, and cells expressing such receptors undergo active proliferation in response to mammalian insulin (Steele et al., 1996). In *Drosophila*, an insulin receptor substrate protein CHICO affects cell growth and the loss of this protein extends lifespan significantly (Clancy et al., 2001). In *C. elegans*, extensive evidence suggests that the insulin/IGF-1 signaling events directly influence longevity (for example, see Kenyon et al., 1993). And in mice, it has been found that proteins that transduce insulin/IGF-1-like signaling have direct roles in determining lifespan (Migliaccio et al., 1999; Holzenberger et al., 2003). Together, these studies suggest a remarkable conserved role for the insulin/IGF-1 pathway in longevity.

Last, the oxidative stress problem. Cells generate reactive oxygen species (ROS) during mitochondrial respiration and by the activity of various oxygenases; these include superoxide anion radical, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite (Lambert and Brand, 2009). Such ROS can damage proteins, nucleic acids and cell membranes (lipid peroxidation). However, cells have evolved multiple mechanisms to prevent the formation of, or remove, ROS including the superoxide dismutases (SOD1 and SOD2), glutathione peroxidases, catalase, and glutathione. Such ROS-related mechanisms have been described in hydras and other cnidarians where they are involved in growth, development and reproduction (Blackstone, 2003; Habetha and Bosch, 2005); and ROS are involved in injury-induced cell death and activation of regeneration in *Hydra*, as well as in *Drosophila* and zebrafish (reviewed in Vriza et al., 2014). In essentially all species that age there occurs increased accumulation of oxidatively damage molecules, and reducing this oxidative damage can extend lifespan in experimental animals such as yeast and *C. elegans* (for review see Labuschagne and Brenkman, 2013; Merksamer et al., 2013).

Interestingly, however, when cells and organisms are subjected to mild intermittent oxidative stress, they become resistant to more severe stress as a result of adaptive responses that bolster their antioxidant defense systems (Arumugam et al., 2006). Such adaptive stress response mechanisms are highly conserved and may be largely responsible for the extension of lifespan by energy restriction and exercise (Mattson, 2012).

3. Aging in individual cells – general considerations

Aging studies usually involve research on clonal organisms at the population level, whether referring to a colony of single-celled organisms or an individual multicellular organism (groups of cells that arose asexually from a single cell). Aging can also be studied at the individual cell level, either single-celled organisms or individual cells in a multicellular organism. Single-celled organisms usually divide in a few hours or days. Each daughter cell can begin a potentially immortal cell clone. Even daughter cells of prokaryotes such as bacteria are believed to be potentially immortal (Ackermann et al., 2003); laboratory colonies have been maintained for over 25 years and more than 50,000 generations (Wiser et al., 2013). However, in some single-celled organisms, the division is uneven. The bacterium, *Caulobacter crescentus*, begins as a motile swarmer and then attaches to a substrate with a stalk (Fig. 3A; Ackermann et al., 2003). Cell division is asymmetric: the larger stalked “mother” producing a smaller, free-swimming daughter swarmer. Over time, the stalked mother cell shows an accelerating decrease in reproductive rate, indicating that it is undergoing senescence.

The best-known example of asymmetric division associated with cell aging is found in budding yeast. In the common budding yeast, *Saccharomyces cerevisiae*, a mother cell produces a smaller daughter cell as a bud that separates from the mother (Fig. 3B); the mother cell can only go through a specific number of divisions, depending on the strain, and then dies (reviewed by Osiewacz, 2002; Gershon and Gershon, 2000). Typically, budding yeast have a cell cycle of ~2 hours and have 20–30 generations (of budding). Aging yeast cells show changes reminiscent of human aging: cell size increases and the cell’s surface wrinkles as loss of turgor develops. Moreover, bud scars increase; generation (cell cycle) time increases; vacuole size increases and its acidity decreases; protein synthesis, ribosome activity and polysome recruitment decrease; and nucleolar fragmentation appears (Jazwinski, 2001; Clay and Barral, 2013). In addition, there is increased formation of extrachromosomal ribosomal DNA circles, which are linked to genomic instability; these are preferentially retained in the mother cell (Clay and Barral, 2013). Mother cells also selectively retain those mitochondria with more oxidizing redox potential and higher superoxide levels; thus the mother retains the lower-functioning mitochondria that ultimately help cause age-associated decline in cellular fitness (Jazwinski, 2002; McFaline-Figueroa et al, 2011; although as the mother gets older, her daughters inherit progressively lower functioning mitochondria from her).

Stated simply, mother cells retain all the ‘bad stuff’ to assure that their daughters can have a fresh start in life. In contrast to budding yeast, fission yeast such as *Schizosaccharomyces pombe* divide symmetrically, with both daughter cells relatively equal so that each daughter can give rise to a potentially immortal cell clone (Coelho et al., 2013; Moseley, 2013). However, under stress induced by heat or oxidation, large protein aggregates form and the major aggregates are segregated into one of the daughters during cell division (Fig. 3C). Like the mother cell of budding yeast, the cell with the big protein aggregates tends to get old and die (Coelho et al., 2013; Moseley, 2013).

To our knowledge, there are no cases reported of individual single-celled organisms living for years without dividing, although documenting this would be difficult. The exception though may be organisms that are revived after being in stasis for long periods. The ciliate protozoan, *Euplotes neapolitanus*, can survive encystment, induced by sudden starvation, for up to 6 months (Wichterman, 1964). Goodey (1915) was able to revive protozoa from soil samples that were nearly 50 years old. But the record is probably ~34,000 years for bacteria (Schubert et al., 2010). Similarly, for cells within a multicellular organism, the record may be ~32,000 years for regeneration of fertile plants from seeds of *Silene stenophylla* found in fruit tissue buried in Siberian permafrost (Yashina et al., 2012). Viruses also have been revived from Siberian permafrost after ~30,000 years (Legendre et al., 2014).

In vertebrates, some somatic mitotic cells live and function in the G_0 phase of the cell cycle for many months. Such cells may exhibit a high quality of maintenance of proteome homeostasis (proteostasis), thus minimizing the accumulation of damaged or misfolded proteins (Vilchez et al., 2014; also Treaster et al., 2013). Postmitotic cells such as neurons can survive and function normally for the entire life of the animal, in some cases (e.g., fish, tortoises, whales) for over 200 years. Interestingly, the cellular niche can control the lifespan of these neurons; for example, embryonic neuron precursors from short-lived mice (~18 month life span) that are transplanted into rats (~30 month life span) live twice as long (Magrassi et al., 2013). The neurons arising from the transplanted neuronal precursors (Purkinje cells) remain smaller than the corresponding rat neurons but retain normal polarity and orientation with age; they also show a similar age-dependent loss of dendritic spines with age as seen in the rat neurons. While these long-lived neurons function much as they do in their youth, there are numerous changes that occur with normal aging, especially at the synapses between these neurons, a topic covered in a recent review (Petralia et al., 2014).

4. Aging in ciliate protozoa – clonal immortality, regeneration, and dedifferentiation/differentiation contribute to longevity

The protozoa are one-celled, eukaryotic organisms, usually heterotrophic, that often show active movements like metazoan animals. Generally, the term is not commonly used in the recent literature, and instead, the organisms are defined only as belonging to particular phyla of protists (protists are eukaryotes that are not fungi, plants or animals). Here we are concerned mainly with aging in ciliates, which include many of the most well-known and studied protozoa, such as *Paramecium* and *Tetrahymena* (we will discuss another group, the choanoflagellates, in the section on Porifera). We want to develop here the main premise of this review, as noted in the Introduction: that immortal clonal lines of protozoa evolved into the first metazoans (multicellular animals) where they continued as large populations of totipotent/pluripotent stem cells; this gave these basal animals both great regenerative powers and potentially very long lives. Subsequent evolution resulted in animals with greater complexity and fewer and less multipotent stem cells, and thus reduced regenerative powers and shortened lifespan.

Aging varies among protozoa both in the lifespan of individual cells and in that of clonal cultures. Most protozoa reproduce asexually by dividing into two equivalent daughter cells,

but some reproduce by asymmetric budding. As described above for budding yeast, budding protozoa are mother cells that produce a juvenile daughter cell. Some, such as the sessile suctorian ciliates, *Acineta tuberosa* (Bardele, 1970) and *Tokophrya infusionum* (Rudzinska, 1961; Millecchia and Rudzinska, 1970; Karakashian et al., 1984), have endogenous budding, a process that looks superficially like vertebrate live birth (Fig. 3D). The mother cell carries the embryo in a brood sac that may be derived from the excretory vacuolar system of the mother cell. She gives birth by forcibly extruding her daughter through the birth pore; the daughter later goes through a metamorphosis, changing from the motile juvenile form to the sessile adult form. Mother cells of *Tokophrya infusionum* live for about 10 days (up to 37 days) and show distinctive signs of progressive senescence. In addition, daughters produced by older mothers tend to show greater variability in life span and in the number of daughters that they produce. Old mother cells display numerous signs of senescence including: enlarged size and sometimes an irregular outline; fewer feeding tentacles that also are less efficient at capturing prey; slower feeding; lower reproduction rate and finally a loss of reproduction; fewer and more peripheral mitochondria; accumulation of lipid-like pigment granules; large oval or circular areas filled with large, dense particles (possibly accumulating solid waste products; Rudzinska, 1961). Diet also affects the lifespan of the mother: those fed little food with intermittent starvation live twice as long as those fed regularly (Rudzinska, 1952). Very overfed individuals can turn into giants (180 μm versus 35 μm) that stop reproducing and die young (Rudzinska, 1951).

Typically, protozoans divide into two daughter cells of the same size. The daughter cells initially have a juvenile structure while they are reorganizing. In the large, complex ciliate *Bursaria*, dedifferentiation of the gullet occurs prior to normal cell division and later the two daughter cells differentiate, reforming adult structures (Lund, 1917). Interestingly, a similar sequence occurs if the organism is cut into two pieces with a scalpel: dedifferentiation precedes differentiation; the process of encystment is also similar (Lund, 1917). Thus, the morphogenetic processes of asexual reproduction, regeneration, and encystment in protozoa may be various manifestations of the same event. In the same sense, the somatic mechanisms of aging and regeneration in simple multicellular animals (basal metazoans) may be related to each other (Sánchez Alvarado, 2000). As mentioned above, discussions of aging in most protozoa, where cell division is symmetrical, concerns the aging of the clonal cultures and not the individual organisms, since cells typically divide every few hours or days (e.g., Hungate, 1942; Wichterman, 1964). We can draw direct analogies between this clonal aging in protozoa and the aging process in multicellular animals (Nanney, 1974). We will consider clonal aging in protozoa in the same way that we consider its involvement in the immortality of totipotent/pluripotent somatic stem cells; we will discuss the implications of this for aging in animals in later sections of this article.

Seemingly immortal lines of cultured protozoans are well known. Woodruff (1943) noted that a culture of *Paramecium aurelia* that was started in 1907 was still viable after 36 years and ~21,800 generations. Similarly, a culture of an amiconucleate strain of *Tetrahymena pyriformis* started in 1923, was still viable 51 years later (although there were some inconsistencies in the strains reported in the literature; Nanney, 1974). Note that ciliate protozoa typically have both large polyploid macronuclei for somatic (vegetative) growth and small diploid micronuclei that maintain the germline for sexual reproduction (and there

can be several different sexes). But generally, these immortal lines of protozoa lose most or all of their ability to produce sexual offspring after a certain number of asexual cell divisions (they may fail to complete sexual encounters or if they do, the offspring may die; Nanney, 1974; Nanney and Simon, 1999). In some protozoa this clonal aging may produce structural abnormalities; in *Euplotes minuta*, the number of dorsal ciliary rows decreases gradually after about 500 fissions (i.e., beginning with an individual formed from a sexual encounter, called conjugation), and the clonal culture terminates at ~700 fissions; Nanney, 1974).

In summary, protozoa can possess several characteristics relevant to aging, including asexual clonal immortality, regeneration, and the ability to cycle between dedifferentiation and differentiation; these features likely predisposed some of them to evolve into the first multicellular animals, as we shall discuss in the following sections.

5. Aging in multicellular organisms – general comments

As noted above, most aging studies look at clonal collections of cells derived from a single cell; this includes multicellular organisms typically derived from a single (usually fertilized) egg cell. In many cases, these cell clones appear to be immortal, showing no signs of aging over many years, as we will discuss in more detail in subsequent sections. We will concentrate in this review on aging in multicellular animals with a simple body design, especially looking at the role of totipotent cells and the nature of immortality, and compare this to aging in humans as representatives of more complex animal body plans.

But other multicellular organisms also show this propensity for immortality. In the multicellular fungi, there are some cases of senescence occurring after a short lifetime of about 25 days, but most multicellular fungi appear to be immortal, continuously growing by tip elongation of their filamentous cells, called hyphae (Osiewacz, 2002). These can form into a largely underground structure called a mycelium (a thallus) that can cover many acres, and individual organisms may live for thousands of years (>1,500 years for an individual of *Armillaria bulbosa*; Smith et al., 1992; Casselman, 2007). Plants grow by cell division of stem cell areas called meristems so that even though, periodically, parts of the plant such as the leaves may show senescence and death, the plant as a whole can live on for many years (Heyman et al., 2014). In several cases, a single clonal organism, in which an extensive group of above ground trees originates from a single mass of underground roots or rhizomes, has been shown to be living for many thousands of years (Wherry, 1972; Munné-Bosch, 2008). For example, Wherry (1972) describes a Box-huckleberry that may be 13,000 years old, and other studies have claimed even greater ages, although some of these claims may be excessive (Mock et al., 2008). Nevertheless, even single individual trees can reach great ages – about 5,000 years for the bristlecone pine (Munné-Bosch, 2008).

6. Aging in basal metazoans – among three lesser groups of basal metazoans, tissue reorganization and regeneration affect longevity

Five main groups constitute the simplest multicellular animals or metazoans, including the Placozoa, Porifera (sponges), Ctenophora, Cnidaria, and Myxozoa; the simplest metazoans evolved from groups of single-celled protozoans (Evans et al., 2010; Schnitzler et al., 2012;

Ryan et al., 2013). The simplicity of their multicellularity can be demonstrated readily by breaking them up into individual cells in the laboratory; when this has been done to some placozoans, sponges and cnidarians, the group of cells often can reorganize themselves back into the whole animal, as will be discussed below. Many also can rejuvenate their bodies, as we shall discuss. Placozoans and Porifera (sponges) are the simplest; they show definite differentiation into various cell types with specialized functions, but lack a definitive nervous system. Ctenophores (comb jellies) and Cnidaria (hydras, corals, jellyfish) are a little more complex, having a basic nervous system, and for many cnidarians, simple eyes. Placozoa and Cnidaria may have evolved from ancestors that evolved into the Bilateria - more complex animals with bilateral symmetry, including insects, clams, humans, etc. Bilateria are triploblastic animals, the embryo showing endoderm, mesoderm and ectoderm, and show a basic bilateral symmetry (lost to some extent in adult echinoderms). The simplest major groups within the Bilateria are the flatworms (Platyhelminthes and Acoelomorpha) and they are the first known kinds of animals to evolve a true brain.

Little is known about aging in three of these groups – ctenophores, placozoans, and myxozoans, and they will be covered here only briefly. Myxozoans are small parasites of fish and aquatic invertebrates. They originally were considered protozoa but then phylogenetic analyses showed that they are metazoans, either related to Cnidaria or more likely, to Bilateria (Evans et al., 2010). Typically, host animals are infected by spores containing one or more sporoblast cells and one or more polar capsules. The polar capsules contain a filament that anchors the spore to the host tissue, and resemble the nematocysts (stinging cells) of cnidarians. The spore-producing stage may be a multicellular, often ameboid plasmodium, containing generative cells within the multinucleate plasmodium, although some kinds form into a closed sac or a worm-like organism (Lom and Dyková, 2006). The latter form (Buddenbrockia) has four longitudinal muscle bands reminiscent of nematodes, and appears to be a triploblastic animal like a bilaterian (Okamura et al., 2002). Little is written about aging of Myxozoa, probably because they are all parasitic and tied to the life cycle of their hosts. Typical infections of fish develop over several months. This may be preceded by an initial infection in an invertebrate such as the tubifex worm, and that also can last several months (El-Matbouli et al., 1992).

Ctenophores are an enigmatic group; they may have evolved from ancestors prior to the ones that evolved into Porifera and Placozoa, and may have a nervous system unrelated to that of cnidarians and Bilateria (Schnitzler et al., 2012; Pennisi, 2013; Ryan et al., 2013). The ctenophore, *Pleurobrachia pileus*, has been reared from eggs and maintained in culture for up to 250 days (Greve, 1970). Ctenophores often have very delicate bodies but can regenerate parts that are damaged (Greve, 1970). *P. pileus* tentacles undergo a continuous regeneration process since they are injured or destroyed easily during feeding. Stem cells are found in restricted areas within the tentacle roots as well as in other main parts of the body and contain genes involved in germline determination and maintenance such as *Piwi* and *Vasa*, as described in subsequent sections (Alié et al., 2011).

Placozoans are simple plate-like animals up to 1–2 mm in diameter with an upper and lower epithelium and some cells in between. They typically divide by binary fission every 2–3 days, but also can reproduce asexually by other means, producing smaller daughter animals

via floating swimmers that bud off of the dorsal surface, as well as by non-floating spheres or by extension of a long stolon from the dorsal surface (sexual reproduction also occurs; Thiemann and Ruthman, 1990, 1991). Most cell types in the placozoans retain the ability to divide, and totipotent cells may occur along the margins of the upper and lower epithelia (Thiemann and Ruthman, 1991; Jakob et al., 2004). The body remains very malleable; impaled on a pin, these animals simply flow around the obstruction (Thiemann and Ruthman, 1991).

Animals that are disaggregated experimentally will reaggregate and in some cases can reform into a viable animal again (we will discuss this more for sponges and cnidarians; Ruthman and Terwelp, 1979). There is little discussion in the literature about aging in placozoans. However, placozoans from old cultures may go through a degenerative phase where they can form into nearly spherical moribund structures (Grell and Benwitz, 1971; reviewed in Thiemann and Ruthman, 1990).

6a. Aging in sponges –their totipotent/pluripotent somatic stem cells, perhaps representing an evolution from choanoflagellate ancestral protozoa, control their great longevity

Sponges (Porifera) may represent the simplest stage of metazoan evolution, and show very close relationships to certain protozoans, especially the choanoflagellates, which appear to be a sister group to all metazoans (Paps et al., 2013). Some choanoflagellates form colonies that resemble clusters of the main filtering cell of sponges, the collar cells or choanocytes (Fig. 4). Structural complexity may be an obstacle to cell-line immortality, and so one might expect the simplest animals to be longest living also. We already have discussed the idea that protozoa and other single-celled organisms can form into potentially immortal cell clones, but it is technically difficult to find evidence of great age in such cell clones. In contrast, sponges can be aged fairly accurately based on the structure of their skeletal elements called spicules, and these studies indeed have indicated that sponges are the longest-living animals. Thus, Jochum et al. (2012) provide substantial evidence that an individual of the deep-sea sponge, *Monorhaphis chuni*, that forms giant spicules up to 3 m long, is about 11,000 years old. Other studies have estimated that various sponges could be hundreds or thousands of years old.

Sponges then offer a clear connection between potentially ageless lines of single-celled organisms and the earliest and simplest multicellular animals that are equally ageless. This evolutionary sequence would begin with solitary choanoflagellate protozoa (Carr et al., 2008; King et al., 2008; Dayel et al., 2011; Funayama, 2013). As discussed above for other protozoa, during cell division, choanoflagellates regress to a simpler morphology. In this case, they lose their flagellum just prior to cell division and the daughter cells show other juvenile features (Karpov and Leadbetter, 1997; Leadbetter, 2008a). Next in evolution would be the colonial choanoflagellates (Leadbetter, 2008a; Dayel et al., 2011; Maharbiz, 2012). One colonial choanoflagellate, *Salpingoeca rosetta*, can differentiate into at least 5 different cell types including 2 that form into different kinds of colonies; cells in colonies are connected by fine intercellular bridges, bear filopodia, and sit in a shared extracellular matrix (Dayel et al., 2011). In comparison, sponges have 5–10 different cell types organized

into a multicellular animal (Funayama, 2013). The evolutionary transition from colonial choanoflagellates to sponges presumably involved intermediate forms that started to form different cell types to specialize in different functions (Fig. 4). Early on there would be a stage where some of the cells retained their ability to proliferate while others became nonproliferative. The proliferative cells in the sponges and their ancestors then would represent the immortal cell clones that evolved from similar immortal cell clones of protozoa (Funayama, 2013). Sponges contain two kinds of proliferative cells that are considered totipotent/pluripotent somatic stem cells, i.e., cells from which all other cell types may be derived by differentiation and division. These include the choanocytes, which look like choanoflagellate protozoans, and are the main filter-feeding cell of the sponge, and archeocytes, which are ameboid cells that migrate through the central matrix of the sponge, called the mesohyl (Funayama, 2013). Archeocytes are the best-studied stem cells, but in some sponges, choanocytes can dedifferentiate and become wandering ameboid cells that are probably archeocytes, and choanocytes may be the main type of totipotent/pluripotent stem cell in some kinds of sponges. Both types express genes involved in germline determination and maintenance such as Piwi and others (Funayama et al., 2010; Funayama, 2013). There are no separate germ cells in sponges and it is these 2 cell types that form gametes for sexual reproduction. They also are involved in the various forms of asexual reproduction seen in sponges, such as gemmule formation, budding, and stolon formation. Also, under environmental stress, some sponges can degenerate, withdrawing their cells into a central mass of ameboid cells that are mainly archeocytes; the sponge then regenerates under more favorable conditions (Bisbee et al., 1989; Shostak, 2008). In addition, like placozoans and some cnidarians, sponges can be dissociated into cells that then can aggregate and reconstitute themselves into a whole sponge again (reviewed in Funayama, 2013).

So what might explain both the great longevity and seeming immortality of sponges (and other basal metazoans as described later here) as well as their great regenerative powers? Based on a great deal of evidence, as summarized briefly above, we can speculate that an ancestor similar to the living colonial choanoflagellates evolved into true multicellular animals with an organized structure made up of several different cell types with different functions, and supported by a siliceous or calcareous skeleton (interestingly, some living choanoflagellates produce siliceous “house” structures called lorica; King, 2004; King et al., 2008; Leadbetter, 2008b; Dayel et al., 2011; Funayama, 2013). The choanoflagellates would become the choanocytes of these animals, and would essentially remain as continuous ageless clones; but instead of the daughter cells living on their own, they become cells of the growing sponge ancestor. These choanocyte stem cells would produce all the other types of somatic cells of the sponge ancestor. But as these animals evolved into larger species, some choanocytes would dedifferentiate to form wandering stem cells, i.e., the archeocytes, that could disperse readily through the animal and be more available for replacement of the various somatic cell types throughout the body (Funayama et al., 2010; Funayama, 2013). The living sponge thus retains its agelessness through the continuous proliferation of its stem cells that are the equivalent of a colony of ageless protozoans.

6b. Aging in cnidarians – their abilities to reorganize, rejuvenate and regenerate can produce great longevity and potential immortality

Among the basal metazoans, the Cnidaria and Ctenophora are the most advanced, possessing a simple nervous system, sense organs (eyes in Cnidaria and statocysts in both), and muscles; in contrast, definitive examples of these structures are lacking in the simplest metazoans, the Porifera and Placozoa. Thus, cnidarians and ctenophores represent an intermediate stage of body complexity between poriferans and placozoans, and the Bilateria, which includes all of the other major phyla of animals. Interestingly, they are also intermediate in their maximum lifespans. As described above, there is good evidence that some individual sponges live for more than 10,000 years. In contrast, some individuals in the major groups of Bilateria have lifespans up to about 500 years, and often with negligible senescence (e.g., >500 years for some marine clams, 50–100 years for lobsters, and over 200 years for some vertebrates; Finch, 2009; Petralia et al., 2014; Treaster et al., 2013). Among the Cnidaria, individual living corals, which are formed by a colony of polyps (an elongate structure with a mouth surrounded by tentacles at the top) that are joined together by a continuous sheet of living tissue (the coenosarc), can be more than 4,000 years old (Roark et al, 2009). Perhaps this indicates a reciprocal relationship between maximum ages and body complexity in the evolution of animal aging. Of course it is difficult to study changes in aging of a long-lived creature in the laboratory; but among cnidarians, some sea anemones have been observed to live in captivity for more than 80 years, without obvious signs of aging, budding continuously; apparently, they died of neglect, so they probably would have lived longer if cared for properly (reviewed in Comfort, 1956). Ashworth and Annandale (1904) described these latter sea anemones when they were about 50 years old, and noted that they were a little more sensitive to cold and dark compared to young mature sea anemones, and their sexual reproductive fertility seemed to have decreased; however, it was not clear if these differences were due to different rearing conditions. Nevertheless, senescence and death from old age do occur in some Cnidaria. In particular, the individual polyps in corals or hydrozoans typically have a short life. For example, polyps of the colonial hydroid, *Campanularia flexuosa* live for 4–8 days at 25°C; ATP content decreases during their lifespan, yet they seem to catch and eat food normally until they die (Strehler and Crowell, 1961). *Acropora cytherea* corals show growth anomalies in the older, central portions of large colonies; these anomalies are associated with reduced productivity and dysfunctional gametogenesis (Irikawa et al., 2011). However, in both of these cases, it is the older polyps within a clonal colony that are dying, and the colony, which is really a single organism with continuous cytoplasm, lives on. But the entire colony of the branching coral, *Stylophora pistillata*, dies after a few years, and shows decreases in reproduction and growth about 6 months earlier (Rinkevich and Loya, 1986).

Above we discussed the idea of agelessness or immortality in protozoa and sponges. Similarly many cnidarians are likely immortal. This was best illustrated in a study by Martínez (1998) in which individuals of the common polyp-type cnidarian, *Hydra vulgaris*, were followed for 4 years; during the study period, animals showed no signs of age-related mortality and there were no definitive changes in reproductive rates for either asexual (budding) or sexual reproduction (animals grew testes or eggs normally throughout the study, but were kept isolated). Analyses of reproduction and mortality rates led to

predictions that these animals indeed were immortal. One mortality trajectory of *Hydra magnipapillata*, based on a laboratory study lasting several months (Schaible et al., 2011), estimated that 5% of adults would be alive after 1400 years (Jones et al., 2014). Hydras have a long trunk with a foot or basal disc at one end and a head with mouth surrounded by tentacles at the other end (Fig. 3G). The cells of the main body column divide continuously and these replace all of the non-dividing, differentiated cells in the hydra within 20 days; thus the hydra's body is renewed continuously - at least 60 times over the 4 years of the Martínez study (Martínez, 1998; Watanabe et al., 2009; Tanaka and Reddien, 2011; Gold and Jacobs, 2013). Hydras typically maintain a steady adult size; most excess cells are formed into buds that develop into daughter hydras that separate from their mother (Fig. 3G); the remaining excess cells are sloughed off of the ends of the hydra. Cell divisions involve 3 cell types; epithelial cells of the 2 body wall layers (ectoderm and endoderm) divide about every 3–4 days, while small interstitial cells (I-cells) from the ectodermal layer divide about once a day to form several specialized cell types: neurons, nematocysts (stinging cells), secretory cells, and gametes. These I-cells then are considered the multipotent stem cells of the hydra. The rejuvenating (regenerative) powers of hydras are amazing. Dissociated hydra cells can reaggregate and form a new hydra (Gierer et al., 1972; Bosch, 2007), as noted also in the previous sections for some sponges and placozoans. Shimizu et al. (1993) showed that a tiny piece of tissue cut from the trunk of a hydra (*Hydra magnipapillata*) will reorganize into a 0.2 mm sphere (the animal is about 7 mm long) with only about 270–300 cells, and then finally into a complete mini-hydra less than a mm long. Many factors are probably involved that help guide the cells to reorganize into the hydra shape. For example, Wnt signaling, important in axial patterning in the vertebrate embryo, induces head formation in the hydra (Lengfeld et al., 2009; Watanabe et al., 2009). Regeneration of hydra into a new animal is mainly due to a reorganization of existing cells rather than via cellular proliferation; this is called morphallaxis (Bosch, 2007). Thus, the hydra may attain immortality; essentially it keeps itself young by having a highly malleable and renewable body and by replacing its cells continuously. But not all hydras may be immortal. Yoshida et al. (2006) examined aging in 3 species of hydra kept at 10°C to induce and maintain the sexual phase. They found that one species, *Hydra magnipapillata*, that still reproduced asexually by budding (apparently along with sexual reproduction) at this temperature did not seem to age during the experimental period. But the other two hydra species (mainly *Hydra oligactis*) showed signs of aging and died off in a few months. The aging hydra showed declines in food capture, contractile movements, and reproduction; tentacles became shorter progressively and there was general atrophy of the body after 60 days. This possible correlation of senescence with sexual reproduction has been noted for some other cnidarians (Gold and Jacobs, 2013) and we already have mentioned this phenomenon briefly for protozoa above. Yoshida et al. (2006) noted that the I-cells of *H. oligactis* started producing mainly germ cells for sexual reproduction, and decreased their production of nerve cells, nematocytes, and gland cells; this explains the decline in food capture as the animals got older. The authors also found changes in gene expression during degeneration and found genes in these hydras that are associated with the aging process of other animals, such as members of the insulin/IGF-I signaling pathway and sir2.

In contrast to the hydra, which is among the simplest of cnidarians, most other kinds of cnidarians have complex body plans and life cycles, with thus many variations in aging and regeneration phenomena. We can only cover some highlights of this variety in this review. Hydras require 3 kinds of cells to continuously regrow their bodies, but in contrast, the marine colonial hydroid, *Hydractinia echinata*, has I-cells that are true totipotent/pluripotent migratory stem cells, similar to the arrangement that we described for the sponges (Müller et al., 2004; Künzel et al., 2010). In *Hydractinia echinata*, Polynem (Pln), a POU domain transcription factor gene, is a key regulator of these stem cells, as are similar POU genes in mammalian embryos (Millane et al., 2011; Gold and Jacobs, 2013). These stem cells also have various known cnidarian stem cell markers including germline markers, Nanos, Vasa and Piwi, similar to what we described for sponge stem cells. The presence of true stem cells are not definitive in many other cnidarians; in some cases, interstitial cells or amebocytes have been identified as stem cells, but more study of their function is needed (Gold and Jacobs, 2013). Isolated striated muscle cells from the medusa (jellyfish) stage of *Podocoryne* can dedifferentiate into a stem cell in the laboratory, and when combined with endoderm cells, can make all of the cell types and form a new, partial medusa structure that can feed, grow, produce gametes, and live for months (Schmid et al., 1982; Gold and Jacobs, 2013). Thus, cnidarians may retain a broad ability to create stem cells from various cell types, to regenerate and rejuvenate their bodies; this is similar to what we described above for the stem cell functions of choanocytes and archeocytes in sponges.

The most amazing case of body rejuvenation among cnidaria and possibly among all animals is seen in the life cycle of *Turritopsis nutricula* (Fig. 5; Piraino et al., 1996). Like many cnidarians, this animal goes through a complex metamorphosis; sexually mature medusae produce mobile larvae (planula stage) that eventually settle to form a colonial hydroid (polyp) stage, which grows and produces new medusae asexually. In many other Cnidaria with this life style, medusae eventually die after reproducing sexually one or more times. But in *Turritopsis nutricula*, sexually mature medusae, if stressed in the laboratory, will reverse metamorphose back to the colonial polyp stage that can feed and bud off new medusae. This is analogous to a butterfly changing back into a caterpillar! Here then may be an example of an animal that can achieve immortality by regularly cycling between a complex mature adult stage and a simpler larval stage.

7. Aging in flatworms – longevity depends on asexual fission, controlled shrinkage during starvation, and regeneration and involves abundant totipotent/pluripotent neoblast stem cells

Planaria and other flatworms are very important model animals for studying stem cells and regeneration (e.g., Liu et al., 2013; Sikes and Newmark, 2013; Umesono et al., 2013). Flatworms are probably the most primitive of the Bilateria; all are bilaterally symmetrical, but unlike most higher Bilateria (earthworms, clams, insects, humans, etc.), they lack a body cavity (acoelomate). They make up 2 phyla including: 1) Platyhelminthes, which includes planaria and other free-living flatworms of marine, freshwater, and land environments, as well as two large groups of parasites, the trematodes or flukes, and the tapeworms; and 2) the Acoelomorpha (a phylum or subphylum recently separated from Platyhelminthes) that

includes the acoel flatworms, which have no gut tract. The acoel flatworms may be close to the basal group of the Bilateria, representing the transition between the basal metazoans such as placozoans, sponges and cnidarians, and the more advanced Bilateria such as planaria, insects, humans, etc.

Aging in planaria and other flatworms is not well understood. Lifetimes of both individuals and clonal colonies in the laboratory have been variable, probably due both to species and culture differences; a number of studies indicate that many kinds of flatworms can live for at least several years, maybe in the range of mice and rats (Balázs and Burg, 1962; Haranghy and Balázs, 1964; Mouton et al., 2010).

Individuals of *Dugesia tigrina*, collected in the wild, were still alive in captivity after 7 years and produced egg capsules annually (Goldsmith, 1942). Parasitic flatworms such as flukes and tapeworms live in a protected environment in their hosts, and may have even longer lifespans; records of 25 to more than 35 years have been noted (reviewed in Comfort, 1956). Christopherson (1924) reports that a scientist infected with *Schistosoma* flukes passed live eggs in his urine for at least 28 years (the life cycle requires an intermediate host), after which he stopped monitoring their passage; eggs were absent when checked after a total of 42 years.

Mouton et al. (2010) found that the free-living flatworm, *Macrostomum lignano*, ages gradually with an average lifespan of 205 days (maximum is 2.4 years). Signs of old age in these animals include body deformities, grooves in the head region, and liquid-filled cysts, as well as disintegration of gonads in some animals (Fig. 1). The size of cysts in this animal does not increase with age; they seem to form as aging animals lose their ability for efficient wound healing, so cyst size may be more dependent on the size of the initial wound rather than animal age (Crucke, 2010). In contrast to *M. lignano*, *Schmidtea polychroa* does not show signs of metabolic aging, at least not over a three-year experimental period (Mouton et al., 2011). After an initial growth phase of five months (sexual maturation by two months), *S. polychroa* undergoes alternating phases of regression and growth. Those few animals that did die during the experiment first showed intense regression and sometimes deformities, prior to death. In addition to these examples, asexual strains have been maintained from several species, including *Stenostomum tenuicaudatum*, *Schmidtea mediterranea* and species of *Dugesia* (Nuttymcombe and Waters, 1938; Baguña and Romero, 1981, Newmark and Sánchez Alvarado, 2002; Isaeva et al., 2005 [colony maintained for 40 years]; Ishizuka et al., 2007; Rink, 2013), and these are considered to be immortal lines (Newmark and Sánchez Alvarado, 2002, Saló, 2006, Nuttymcombe and Waters, 1938; Tan et al., 2012), as we have described in previous sections for examples of exclusive asexual reproduction in Protozoa and Cnidaria. Furthermore, individual flatworms may be potentially immortal, as described above for *S. polychroa* (Mouton et al., 2011) and also for *S. mediterranea* (Tan et al., 2012). Interestingly, the asexual and sexual strains of *S. mediterranea* differ genetically by a chromosome translocation: animals with the translocation reproduce only asexually by transverse fission and those without it reproduce only sexually (as hermaphrodites; Baguña et al., 1999; Newmark and Sánchez Alvarado, 2002, Zayas et al., 2005, Saló, 2006). In the wild, there may be different populations of sexual and asexual planarians (Baguña et al., 1999).

Generally, flatworms can employ three mechanisms to avoid aging: asexual reproduction via fission, controlled shrinkage during starvation, and regeneration from body fragments (Newmark and Sánchez Alvarado, 2002; Saló, 2006; Mouton et al., 2011); all of these are believed to involve a rejuvenation of the animal, as we have discussed in previous sections for other organisms. During fission, the posterior, tail end splits off somewhere in the last two-thirds of the body and both parts regenerate into whole animals (Fig. 3F). Probably the most elaborate fission mechanism is found in *Stenostomum incaudatum* (Sonneborn, 1930; Nuttycombe and Waters, 1938; Newmark and Sánchez Alvarado, 2002). The posterior end of the body starts to differentiate into a miniature worm (called a zooid) and its head and brain start to become distinctive prior to separation from the anterior, typically longer end (Fig. 3E). Other zooids may start to grow before the end zooid separates and chains as long as nine zooids may occur. In a four month study, 100% of the anterior ends died, while only 8.5% of the posterior ends died; this phenomenon of differential aging in uneven divisions has been discussed in previous sections for budding yeast and for the protozoan, *Tokophrya infusum*; basically the larger “mother” division of the organism grows old and dies eventually, after producing a number of offspring. Dying *Stenostomum incaudatum* worms stop growing and decrease in size and often show various abnormalities. The most elaborate fission methods are found in *Convolutriloba* acoels, which in different species produce small juveniles from the posterior end by: 1) transverse fission; 2) transverse fission followed by longitudinal fission of the juvenile into two individuals; or 3) reversed-polarity budding where the two juveniles bud from the posterior end of the mother, developing their heads pointed away from the mother (Sikes and Bely, 2008).

Many flatworms respond to starvation by decreasing in size significantly, while generally maintaining body proportions and integrity (Baguña and Romero, 1981; Newmark and Sánchez Alvarado, 2002; Saló, 2006). In a few months, the worm can decrease its length 5–10x or more. Its cell number decreases proportionally; for example, a 7 mm *Dugesia tigrina* with about 520,000 cells can change into a miniature individual, 2 mm long with 60,000 cells, yet proportions and distribution of the different cell types show little change overall, and are similar to those of a juvenile individual. Thus, the starving worm appears to return to its juvenile state (Baguña and Romero, 1981). Many flatworms adjust their size by periods of growth and shrinkage to adapt to food availability and other environmental factors (Baguña and Romero, 1981; Rink, 2013).

Planarians are famous for their capacity to regenerate. For example, Montgomery and Coward (1974) found that a fragment as small as 0.08 mm³ of an asexual *Dugesia dorotocephala* (7–12 mm) can regenerate completely. This fantastic ability to regenerate depends on the presence of large numbers of pluripotent stem cells called neoblasts (Reddien and Sánchez Alvarado, 2004; Peter et al., 2004; Shibata et al., 2010; Tanaka and Reddien, 2011; Rink, 2013). These stem cells are probably present in all kinds of flatworms, including acoels (Fig. 6; De Mulder et al., 2009a, b), tapeworms (Toledo et al., 1997; Brehm, 2010) and trematodes (Collins et al., 2013). These are small, round cells with little cytoplasm, spread through the mesenchyme around the organs and making up about 1/4 of planarian cells (Baguña and Romero, 1981; Reddien and Sánchez Alvarado, 2004). Neoblasts form all of the somatic cells and continuously replace them so that the entire planarian body turns over in a few weeks (Rink, 2013; similar to hydras as discussed in the previous

section). This is particularly evident in a study by Wagner et al. (2011), who lethally irradiated planaria of a sexual strain of *Schmidtea mediterranea* (because the sexual strain is more tolerant of radiation than asexual ones) and then implanted a single neoblast derived from an asexual strain. Some of these planaria survived and regenerated lost parts and fed, and ultimately were expanded into entire asexual strains. This indicates that the single neoblast transformed the recipient (sexual strain) into an asexually reproducing animal with the donor genotype. More importantly, it shows that an individual neoblast is truly pluripotent, capable of producing all kinds of somatic cells. Imagine if a very old and sick person could inject a single stem cell, frozen away since youth, under his/her skin and rejuvenate their body – youthful and disease-free!

The molecular genetics of neoblasts and planarian regeneration has been studied widely and we can only discuss it briefly in this review. In addition to the somatic pluripotent neoblasts that we have been discussing, flatworms also have some germline neoblasts; these may cycle at a slower rate than somatic neoblasts and contain the gene *nanos*, associated with gonad development and germ cell development and regeneration (Wang et al., 2007; Rink, 2013). Interestingly, both sexual and asexual strains have germ cells expressing *nanos*, so that inhibition of development of mature reproductive organs in asexual strains must occur downstream of *nanos* function. Generally for animals, viability of populations of germline stem cells is maintained through many cell divisions by overcoming the end-replication problems of chromosomes via telomere elongation, which occurs during embryogenesis. So how do asexual planaria strains maintain the viability of their somatic neoblasts? These cells can produce higher levels of telomerase via alternative splicing of active telomerase so that telomere length is maintained during the cell replication events that occur during fission or regeneration (Tan et al., 2012). Thus, this might explain how asexual planaria achieve potential immortality. These neoblasts also express several *Piwi* homologues, more typically associated with germline stem cells (see discussion in earlier sections of this review); these homologues help to maintain the neoblast genome integrity (Rink, 2013). Neoblasts also have chromatoid bodies and their numerous associated proteins, classically linked to germ cell function; this is probably indicative of their close link in evolution. Related to this, we described above how stem cells of sponges and Cnidaria and other groups are involved in both somatic and germ cell lineages (Rink, 2013). Most likely, in flatworms of both kinds, acoels and Platyhelminthes, germ cells and pluripotent somatic neoblasts are both derived from totipotent neoblasts that self-renew (Bely and Sikes, 2010; Solana, 2013).

8. How does aging in higher animals differ from that of simple animals?

Aging strategies among animals fall roughly into two or three groups, related to cell and tissue maintenance and regeneration, and to aging of the individual cells; proposed mechanisms vary according to the author (Shostak, 2008; Iaseva, 2011; Tanaka and Reddien, 2011; Solana, 2013; Vilchez et al., 2014). Solana (2013) examined the relative amount of totipotency found in some cell populations among all animal groups, using this to define a population of primordial stem cells (PriSCs; Fig. 7) that can form all germ and somatic cell types. In early metazoan evolution, these unlimited PriSCs continue into the adult; they include especially the archeocytes of sponges, and the I-cells of many or most cnidaria and neoblasts of flatworms; i.e., probably all of the groups that we have discussed

in detail in this review. These PriSCs allow many members of this first group to reproduce by asexual reproduction (fission, budding, etc.), often imparting potential immortality as has been discussed in previous sections; but the PriSCs also can give rise to germ cells for sexual reproduction (however, germ cells also can form earlier in development and be retained into the adult). The second group has restricted PriSCs and includes some annelids (segmented worms) and some molluscs (snails and clams, etc.). In these animals, an early embryonic cell, commonly the 4d blastomere, divides to form primordial germ cells that form adult germ cells, but this blastomere also produces somatic stem cells that continue into the adult (notably as the MPGZ [mesodermal posterior growth zone] of some polychaete annelids), allowing limited pluripotency in the adult animal. The third group has rudimentary PriSCs. Solana (2013) includes in this group the oyster, *Crassostrea gigas* (a mollusk), as well as *C. elegans* (a nematode), *Drosophila* (an arthropod), and *Xenopus* (a frog) and other vertebrates. In these organisms, some early cell stages in embryogenesis give rise to primordial germ cells and thus adult germ cells (Extavour and Akam, 2003; Solana, 2013), as in the second group, but only some initial somatic cells; thus there are no adult pluripotent somatic stem cells (Solana, 2013). In fact, numerous studies in fish and amphibians and other vertebrates support the idea that vertebrates normally lack adult pluripotent somatic stem cells (Tanaka and Reddien, 2011). Interestingly, mammalian embryonic stem cells have the same basic molecular determinants of pluripotency that are found in planarian neoblasts, suggesting that the mechanism is evolutionarily conserved (Önal et al., 2012).

Thus, there was a trend of reduced incidence of asexual reproduction as more complex metazoans evolved. Within the third group described above, we can recognize two different strategies in the evolution of more complex animal forms from the simple metazoans that show continuous rejuvenation (and potential immortality) in the adult (Pearson and Sánchez Alvarado, 2008; Tanaka and Reddien, 2011; Rink, 2013; Solana, 2013). First, for vertebrates and some other higher metazoans, there is maintenance of some limited rejuvenation abilities in the adult resulting in a moderately long lifespan. In contrast, for *C. elegans* and some other animals, rejuvenation is restricted to sexual reproduction and the embryo, resulting in a short lifespan and an increase in generations per unit time.

Another way of dividing metazoan reproductive/aging strategies is given by Shostak (2008). Shostak argues that basal metazoans have determinative development with narrow potencies, but also, as we have noted, with continuous proliferation of cells followed by asexual reproduction to get rid of the excess cells. Simple metazoans get rid of excess cells, due to proliferation, by asexual reproduction to reduce body size. The best example of this is the hydra as described in a previous section; hydras utilize three kinds of stem cells to proliferate and replace all of the cells of their body in a short time, with excess cells either used to make daughter buds or simply sloughed off at both ends of the body. In this case at least, two of the three kinds of stem cells, i.e., the ones producing the two epithelial cell layers, appear to have a limited potency. Other relatively simple metazoans such as the nematode *C. elegans*, also have determinative growth but without cell proliferation in the adults (except for the germline and gametogenesis), and with a consequently short lifespan (Extavour and Akam, 2003; Solana, 2013). This probably is not possible in more complex animals that are thus in danger of developing cancer (see also Pearson and Sánchez

Alvarado, 2008; López-Otin et al., 2013). One solution is to constrain proliferation using a more regulative type of development: 1) adult stem cells have asymmetric distribution of DNA strands to constrain steady state populations (Fig. 8; Conboy et al., 2007); 2) a subset of cells can have a count-down timer, possibly involving telomere length (capping ends of chromosomes), so that after receiving only freshly-replicated DNA strands for the adult stem cells, precursor cells revert to symmetric division and for a limited number of divisions. In addition, López-Otin et al. (2013) note that some mechanisms promote cellular senescence, which "...protects the organism from cancer but which, in excess, can promote aging." (Fig. 1). However, strategies to regulate cell and tissue development as a strategy to prevent tumors are not limited to higher metazoans; even planaria can get tumors (as noted in a previous section) and thus have developed molecular mechanisms to avoid them (Oviedo et al., 2008; Pearson and Sánchez Alvarado, 2008).

Within the complex bodies of vertebrates and other higher Metazoa, we can see that different stem cell populations showing unipotency or oligopotency utilize one of two strategies to maintain their cell populations (Vilchez et al., 2014). Either the stem cells have a high quality of proteostasis (maintenance of proteome homeostasis as noted above) and produce differentiated cells with a high turnover/short lifespan (e.g., blood and epithelial cells), or the stem cells have a lower quality of proteostasis but produce differentiated cells with a low or no turnover/long lifespan that show a corresponding higher quality of maintenance of proteostasis (e.g., neurons, cardiomyocytes; see also Rando, 2006).

Stem cell maintenance in hydras depends on the transcription factor, FoxO, and may account for the potential immortality of hydras (Boehm et al., 2012, 2013). The FoxO gene is expressed at high levels in all three kinds of stem cells of the hydra. FoxO may promote longevity in hydras both by maintaining the stem cells and by supporting innate immune pathways that defend the animal against microbe invasion. FoxO also has been found in other cnidarians, as well as in sponges and placozoans (Boehm et al., 2012, 2013), so it probably is a key longevity factor in basal metazoans. FoxO also has been associated with aging regulation in bilaterians, including *C. elegans*, *Drosophila* and humans. Interestingly, in humans, more polymorphisms in the FOXO3A gene may be associated with people in their 100s compared to those in their 90s or younger (Flachsbart et al., 2009). So why does FoxO not promote immortality in humans and other higher bilaterians? As animal life evolved into more complex forms, FoxO seems to have taken on several other functions and has become coupled to pathways involved mainly with the maintenance of post-mitotic cells as the animal ages (Schaible and Sussman, 2013). Thus, Schaible and Sussman (2013) suggest that these other pathways distract FoxO from its original function of stem cell renewal; some of these pathways may even lead to inhibition of FoxO. Ultimately, in higher bilaterians, FoxO may help maintain the aging body at the expense of lost immortality.

Thus, the groups of basal metazoans and simple flatworms that we have emphasized in this review, and as we have noted previously, have a simple structure that allows them to retain adult pluripotent or totipotent stem cells and to reproduce using both asexual and sexual mechanisms. Adult pluripotency and asexual reproduction seem to work together as a single strategy that is successful mainly for the simplest metazoans, and both are lost in most of the higher metazoans. However, there are exceptions: Isaeva (2011) reviews studies of

pluripotent gametogenic stem cells of colonial parasitic rhizocephalan crustaceans (phylum Arthropoda, i.e., the phylum that includes *Drosophila*) and in colonial ascidians (types of tunicates or sea squirts, i.e., invertebrates in the phylum, Chordata, that includes vertebrates).

9. Conclusions

The evolutionary goal of any species presumably is the continuance of that species into the future. This can be accomplished by extending the life of the individual organism or by maximizing reproduction of juveniles, i.e., the next generation. Protozoans opt for a short individual cell life and a continuation of the identical cell clone or a modification of that cell clone through sexual reproduction. This mechanism became more complicated with the evolution of multicellular animals. Now there were many different interdependent cell clones all living together as one organism. The earliest strategy was to maintain the total organism by developing pluripotent stem cells that could replace all cell types as needed, for regeneration and rejuvenation of the individual organism. But sexual reproduction has been present from the beginning of multicellularity also, and so we see a great variety of combinations of sexually reproducing individuals that sometimes have short lives, and asexually reproducing ones that are potentially immortal. Then, as we move up the evolutionary tree to more complex animals including humans, we see more and more losses in longevity and a complete loss in potential immortality. People have long hoped for lifespan extension, and even the potential immortality of simple animals. There are many aging and rejuvenation mechanisms as we have discussed briefly, and all of these may have to be considered in this quest for “immortality.”

Ultimately, we may be able to derive benefits to humans from research on phenomena such as dedifferentiation and regeneration in the simplest animals (Table 3); these studies may provide valuable information useful to overcome human diseases and other problems of aging. Stem cell research already has shown many advances, especially in mouse models of human diseases, and including the formation of various specialized cell types and even the generation of entire organs in vitro (reviewed in Sánchez Alvarado and Yamanaka, 2014; Tanabe et al., 2014) and possible genetic rejuvenation of aging muscles in mice in vivo (Sousa-Victor et al., 2014). Also, recent studies have put more emphasis on the influence of the stem cell niche, i.e., the physical microenvironment where stem cells reside in the organism, studied so far mainly in *Drosophila* and vertebrates (reviewed by Scadden, 2014), but noted also for sponges (Müller, 2006) and flatworms (Mouton et al., 2009). This will be an important consideration in future studies that utilize simple animals as models of stem cell function in human aging and disease. Thus, the knowledge gained from studying the simplest animals may someday provide keys for the extension of healthy human life to ages far beyond what is possible today.

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Highlights

- Basal metazoans like sponges, jellyfish, corals and hydras often show great longevity and potential immortality.
- These animals can have many pluripotent stem cells that maintain somatic cell lineages central to regeneration and rejuvenation.
- These stem cell clones represent a holdover from immortal cell clones of single-celled organisms.
- Early bilaterians such as flatworms often retain this system of stem cell-based immortality.
- Higher bilaterians including humans have traded immortality for greater complexity and safeguards against unregulated cell growth.

Figure 1.

Mechanisms of aging. The diagram illustrates the nine hallmarks of aging as described by López-Otin et al (2013); several of these mechanisms are described in section 2 of this review. Aging animals often display noticeable changes in external appearance, body shape and posture. The center shows a stereotypical silhouette of an aging person with balding head and stooped posture due to various degenerative diseases. Some aging flatworms can show comparable changes such as a surface head groove (asterisk) and a body deformed by cysts (c); note how the digestive tract or gut (g) is pushed to the side. The 2 black dots represent the eyes. The drawing is an original based on micrographs of *Macrostomum lignano* (Mouton et al., 2009; see section 7).

Figure 2.

Misfolding of proteins can lead to degenerative changes associated with aging. This diagram illustrates energy changes associated with protein folding, and shows how misfolding can favor protein aggregation involved in some diseases of aging. “Protein folding allows an amino acid chain to become a functional molecule. Multiple factors in this dynamic process can result in misfolding. A mutation can stabilize an intermediate in the folding process, causing this misfolded state to be favored over the properly folded protein. Furthermore, for many proteins, the functionally folded form is not the lowest energy state of the protein. Many proteins can form stable amyloid fibers or amorphous aggregates that can accumulate and disrupt cellular processes. Given the importance of proper folding to protein function, it is not surprising that each of these misfolding events can be associated with disease.” The figure and text are reprinted from part of a figure in the open-access review of Valastyan and Lindquist (2014).

Figure 3.

Asymmetric asexual reproduction and senescence. All drawings are original and based on drawings and micrographs in the cited references. A–E. These are examples of asymmetric cell/body division where the “mother” shows signs of senescence (see text for details). A. The sessile, stalked bacterium, *Caulobacter crescentus*, produces motile swarmer cell progeny (Ackerman et al., 2003). B. Budding yeast (*Saccharomyces cerevisiae*) mother cells (m) get bigger as they age and produce more ellipsoidal daughters (d; Lee et al, 2012). C. In contrast, fission yeast, such as *Schizosaccharomyces pombe*, divide symmetrically under

normal conditions. But under stress, there is asymmetric accumulation of protein aggregates (p) leading to aging of the cell that retains most of these aggregates. D. Stalked mother cells (m) of some sessile suctorians ciliates, such as *Acineta tuberosa* (Bardele, 1970) and *Tokophrya infusionum* (Rudzinska, 1961; Millecchia and Rudzinska, 1970; Karakashian et al., 1984), produce an offspring in a brood pouch (bp) that is born through a birth pore (po). The brood pouch is associated with the contractile vacuole/excretory system (cv; the smaller contractile vacuole of the offspring is shown also, but not labeled). Also note how a portion of the mother's macronucleus (nu) pinches off into the offspring. E. The flatworm, *Stenostomum incaudatum* (Sonneborn, 1930; Nuttycombe and Waters, 1938; Newmark and Sánchez Alvarado, 2002), produces a caudal chain of developing offspring. Arrows show locations rostral to the heads of the forming juveniles (note the pinching in of the digestive tract), where they will separate, eventually, from the mother. mo, mouth. F,G. In contrast to the previous examples, asymmetric asexual reproduction in some planaria by fission (F; arrow; Saló, 2006) and hydras via budding (G; b; Bosch, 2009) may not lead to increased senescence of the mother. In these cases, rapid cell proliferation continuously replaces all cells of the body in a short time period, thus continuously renewing the "mother."

Figure 4.

Evolution of choanoflagellate colony to basal metazoans with choanocytes and archeocytes (as found in modern sponges). Single-celled choanoflagellates (A) evolved into colonial forms made of clusters or spheres of cells (B), as still living today. Note the characteristic single flagellum (f) surrounded by a collar (c) made of microvilli. The ancestral metazoan may have evolved from a similar colony. Initially, cell proliferation may have become restricted to a subset of the cells. As the metazoan evolved, cells became specialized for a variety of functions (i.e., division of labor for bodily functions; C). Continued replacement of these various cell types became the task of the ancestral somatic stem cells; in the case of sponges, these were probably choanocytes - cells that retained the basic morphology of the ancestral choanoflagellates (asterisks). As these metazoans evolved into more complex forms with both surface and interior cell types of various kinds (e.g., skeleton-forming cells such as sponge sclerocytes; s), an additional population of stem cells evolved that could migrate inside the body (asterisks; these would be the archeocytes in sponges). This combination of exterior and interior pluripotent stem cells could form an immortal cell clone, e.g., thus allowing some sponges and other basal metazoans to live for thousands of years (see text for details). Based on Funayama, 2010.

Figure 5.

An “immortal” jellyfish? In the hydrozoan, *Turritopsis nutricula* (Piraino, 1996), asexual reproduction by budding of hydroid polyps (p) from a stolon (s) follows rejuvenation from the sexual medusoid (jellyfish) stage (m) in a unique mechanism of reverse metamorphosis. In theory, these animals could continue this forever, hydroid colonies producing sexual medusae that eventually return to the hydroid stage and then repeat the cycle, leading to the popular name in news accounts of “The Immortal Jellyfish.”

Figure 6.

“The stem cell system of *Isodiametra pulchra* (A,B). Morphology (C–E) and distribution (F–I) of neoblasts. (A) Schematic drawing. (B) Differential interference contrast image. (C) Typical neoblast with nucleus (red) and thin rim of cytoplasm (yellow). (D–D’’) Macerated BrdU labeled cells show typical neoblast-like morphology (E) BrdU labeled neoblast, as shown by immunogold staining after a 30 min BrdU pulse; arrowheads point to gold particles. (F) Histological cross section; brown spots are BrdU labeled S-phase cells. (G,H) Confocal projection overview (G) and detail of lateral body margin (H) after 30 min BrdU pulse; the red spot in (H) is a mitotic figure. Note that S-phase cells [green fluorescence] were lacking in the epidermis (between dotted lines). (I) Electron microscopic image of a posterior-lateral body margin.” “bwm, body wall musculature; cc, condensed chromatin; cs, central syncytium; e, egg; de, developing eggs; ep, epidermis; g, Golgi; m, mitochondria; mo, mouth opening; st, statocyst. Scale bars (A,B,G) 100 μ m; (C,E) 1 μ m; (D,H) 10 μ m; (F) 25 μ m; (I) 5 μ m.” Figure and legend are reprinted (with some terms excluded from the quoted term list) from Fig. 1A–I of the open-access report of De Mulder et al. (2009a).

Figure 7.

“The germline cycle in freshwater planarians. Freshwater planarians possess a population of stem cells, the so-called neoblasts, which represents the primordial stem cells in these organisms (PriSC). Neoblasts are able to give rise to the germ cells and to somatic cells. Germ cells give rise to oocytes and sperm, which jointly give rise to the zygote. The zygote gives rise to both somatic cells and the PriSCs. The planarian PriSCs have unlimited self-renewal and both germ potential (GP) and somatic potential (SP). Green dots represent the presence of nuage granules and germ plasm components. The vertical blue line represents

the position of the germ-to-soma boundary, as classically understood. The horizontal green line represents the proposed position of the germ-to-soma boundary as postulated in the Primordial Stem Cells hypothesis (of Solana, 2013); this coincides with the Weismann barrier (solid black line) in freshwater planarians.” This figure is an original simplified drawing, based on Fig. 2 of Solana (2013; open access; see that paper and this review text for details; abbreviations and some label designations were changed in the quoted legend). Note that the Weismann barrier essentially prevents somatic cell mutations from affecting the germline and future generations. The PriSCs can allow some planaria to renew all of the cells of their bodies continuously, thus potentially avoiding senescence.

A

B

Figure 8.

“Divergent cell fates associated with asymmetric segregation of template strands. (A) Pairs of cells were co-immunostained for BrdU and the myoblast marker Desmin; representative images are shown. (B) The data from experiments as in (A) were quantified. Pairs with Desmin expression and asymmetric BrdU labeling were distinguished based on the pattern of Desmin expression (asymmetric and coincident with BrdU, asymmetric and mutually exclusive with BrdU, or symmetric). The legend for individual cells is shown to the right, and the legends for the cell pairs are shown below. Data represent mean $\pm$ SEM (n=4).”

Figure and legend reprinted from Fig. 3 of the open-access review of Conboy et al. (2007; doi:10.1371/journal.pbio.0050102.g003.). In this study of dividing mouse muscle stem cells (satellite cells) we see that the daughter cells inheriting the older templates (no BrdU) usually retain the more immature phenotype, thus apparently remaining in the stem cell population, while the daughter cells inheriting the younger template (BrdU positive) show that they are forming the differentiated cell type, in this case, the Desmin-containing myoblast. This kind of asymmetric division is different from the examples shown in figure 3 of the present review. The strategy of asymmetric segregation of template strands may ensure that the stem cells remain as an immortal cell clone, while the fate of the differentiated cell is only eventual senescence.

Table 1

List of major animals discussed in this review.

Protozoa	Ciliates (e.g., Paramecium, Tetrahymena, suctorians) Choanoflagellates
Basal Metazoans	Porifera (sponges) Placozoa Ctenophora (comb jellies) Cnidaria (hydras, jellyfish, sea anemones, colonial hydroids, corals) Myxozoa
Bilateria	Acoelomorpha (acoel flatworms) Platyhelminthes (planaria and other free-living flatworms, flukes, tapeworms) Other groups included for comparison: Nematoda (Caenorhabditis elegans) Annelida (polychaete worms) Mollusca (snails, clams, oysters) Arthropoda (Crustacea, Drosophila) Chordata (sea squirts, vertebrates such as fish, frogs, mice, humans)

Table 2

Stem cell terminology.

Stem cells	Undifferentiated cells that can differentiate into specialized cell types and can divide through mitosis to produce more stem cells, either for a limited number of divisions or potentially as an immortal cell clone.
Unipotent/oligopotent stem cells	Stem cells that can differentiate into only one/few cell types. For example, vertebrate muscle satellite cells are stem cells that produce myoblasts that form into muscle cells (see Fig. 8).
Multipotent stem cells	Stem cells that can differentiate into many cell types. An example would be the I-cells of hydras (see text).
Pluripotent somatic stem cells	Stem cells that can differentiate into all known somatic cell types of an animal.
Primordial germ cells	The initial cells of a developing germline, which is capable only of forming germ cells (gametogenesis).
Totipotent stem cells	Pluripotent stem cells that also are capable of gametogenesis. Examples would be mammalian zygotes and early embryos. Choanocytes and archeocytes of sponges and neoblasts of planaria may be totipotent or pluripotent, depending on various authors' definitions and descriptions (see text).

Table 3

Mechanisms affecting aging and longevity in the simplest animals.

Ciliate Protozoa	asexual clonal immortality asymmetric budding cycling between dedifferentiation and differentiation encystment regeneration
Porifera	asexual reproduction including gemmule formation, budding and stolon formation degeneration and regeneration dissociation and reorganization
Cnidaria	asexual budding clonal colonial growth continuous cellular renewal dedifferentiation of cells dissociation and reorganization regeneration by morphallaxis rejuvenation
Flatworms (Acoelomorpha and Platyhelminthes)	alternating regression and growth asexual reproduction via uneven fission controlled shrinkage during starvation regeneration from body fragments

Some of these phenomena are associated with negligible senescence, and have been largely lost in the evolution of the higher Metazoa; they may be worth exploring in the search for solutions to extend the human life span and avoid the diseases of aging. See text for discussion.