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Cellular and molecular mechanisms of negligible senescence: insight from the sea urchin

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Sea urchins exhibit a very different life history from humans and short-lived model animals and therefore provide the opportunity to gain new insight into the complex process of aging. Sea urchins grow indeterminately, regenerate damaged appendages, and reproduce throughout their lifespan. Some species show no increase in mortality rate at advanced ages. Nevertheless, different species of sea urchins have very different reported lifespans ranging from 4 to more than 100 years, thus providing a unique model to investigate the molecular, cellular, and physiological mechanisms underlying both lifespan determination and negligible senescence. Studies to date have demonstrated maintenance of telomeres, maintenance of antioxidant and proteasome enzyme activities, and little accumulation of oxidative cellular damage with age in tissues of sea urchin species with different lifespans. Gene expression studies indicate that key cellular pathways involved in energy metabolism, protein homeostasis, and tissue regeneration are maintained with age. Taken together, these studies suggest that long-term maintenance of mechanisms that sustain tissue homeostasis and regenerative capacity is essential for indeterminate growth and negligible senescence, and a better understanding of these processes may suggest effective strategies to mitigate the degenerative decline in human tissues with age.

Keywords: echinoderm; aging; telomeres; oxidative stress; tissue homeostasis; regeneration

Aging in humans and other animals is a well-defined process characterized by a progressive functional decline and increasing mortality over time. However, there are a number of different animals that show negligible senescence, with no increase in mortality rate or decrease in fertility, physiological function, or disease resistance with age (Finch 1990; Finch and Austad 2001; Bodnar 2009). Studying these animals may suggest effective defenses against the degenerative process of aging, and sea urchins provide an ideal model to investigate mechanisms of longevity and negligible senescence.

Sea urchins have served as model organisms for scientific research for more than a century and have contributed significantly to our understanding of many biological processes including gene regulation, molecular embryology, fertilization biology, cell biology, evolutionary biology, population genetics, and toxicology. Part of their value as a model organism is their close phylogenetic relationship to humans. Sea urchins (Phylum: Echinodermata) are nonchordate deuterostomes and are more closely related to humans than other common invertebrate model organisms such as worms (e.g. *Caenorhabditis elegans*) and flies (e.g. *Drosophila melanogaster*). Sequencing the genome of *Strongylocentrotus purpuratus* confirmed the close genetic relationship between sea urchins and humans, revealing that sea urchins have an estimated 23,300 genes including representatives of most vertebrate gene families (Sodergren et al. 2006). Sea urchins are commercially fished, and there are considerable data available regarding their growth, survival, longevity, susceptibility to disease, and reproductive patterns as this information is essential for effective fisheries management (Lawrence 2007). From these data, it has been noted that different species of sea urchins exhibit very different natural lifespans, and some have extreme longevity and negligible senescence. For example, the red sea urchin *Strongylocentrotus franciscanus* is one of the earth's longest living animals, living in excess of 100 years with no age-related increase in mortality rate or decline in reproductive capacity (Ebert and Southon 2003; Ebert 2008). In contrast, *Lytechinus variegatus* has an estimated lifespan of only 4 years (Moore et al. 1963; Beddingfield and McClintock 2000), while the most widely studied species of sea urchin, *S. purpuratus*, has an estimated maximum lifespan of more than 50 years (Ebert 2010). Comparisons between long-, intermediate-, and short-lived species may provide insight into mechanisms involved in lifespan determination and negligible senescence. Thus, sea urchins represent an interesting alternative model for aging research (Bodnar 2009).

Aging is a complex and multifactorial process, and many theories have been proposed to explain this phenomenon at the molecular, cellular, systemic, and evolutionary levels (Weinert and Timiras 2003). These theories should not be considered mutually exclusive,

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but an interconnected network of processes that contribute to the overall progression of aging. Nevertheless, they provide a useful framework from which we can begin to understand the fundamental differences between key processes in animals that age and those that show negligible senescence. This mini-review will present studies characterizing the biology of sea urchins in the context of these well-established theories of aging.

Telomere loss theory

The telomere loss theory states that telomere attrition contributes to both cell and organismal aging (Weinert and Timiras 2003). At a cellular level, shortened telomeres can lead to replicative senescence or genomic instability whereas maintenance of telomeres, through the activity of telomerase, confers immortality on cells. At an organismal level, aging is accompanied by telomere attrition in humans, dysfunctional telomeres accelerate aging in mice and humans, and reactivation of telomerase can delay aging in telomerase-deficient mice (López-Otín et al. 2013). Telomere biology has been investigated in the tissues of three species of sea urchin: short-lived *L. variegatus*, long-lived *S. franciscanus*, and *Echinometra lucunter lucunter* which has an intermediate lifespan, estimated to be about 40 years (Ebert et al. 2008). There was no evidence of telomere shortening when terminal restriction fragment (TRF) lengths were compared between tissues of young and old sea urchins or between somatic and germ tissues within adult animals (Francis et al. 2006; Ebert et al. 2008). Telomerase activity was detected in the investigated tissues suggesting a mechanism for telomere maintenance (Francis et al. 2006; Ebert et al. 2008). Maintenance of telomeres in tissues of species with different lifespans suggests a lack of telomere-directed senescence in sea urchins. Although the levels of telomerase activity were not quantified in the tissues of species used in these studies, it is interesting to note that TRF length was inversely correlated with life expectancy such that short-lived *L. variegatus* had the longest mean TRF length. This suggests that telomere length is not a determinant of maximum lifespan of sea urchin species and is consistent with the observation that average telomere length across species does not generally correlate with interspecific variation in maximum lifespan (Monagham 2012).

Oxidative stress or free radical theory

The oxidative stress or free radical theory is one of the most studied hypotheses for the molecular basis of aging. This theory proposes that the accumulation of cellular damage caused by reactive oxygen species (ROS) plays a key role in the aging process, as well as in determining organismal longevity (Weinert and Timiras 2003). Oxidative stress results from an imbalance between the production of ROS and the cell's ability to mitigate damage through antioxidant pathways or mechanisms that repair or eliminate damaged molecules. Although many studies have shown that oxidative damage increases with age in the cells and tissues of a variety of organisms (Martin and Grotewiel 2006), it is not yet clear whether this is a cause or effect of aging. A general age-related increase in markers of oxidative damage was not observed in sea urchin tissues from three species with different lifespans: *L. variegatus*, *S. purpuratus,* and *S. franciscanus* (Du et al. 2013). Levels of protein carbonyls and 4-hydroxynonenal measured in tissues (muscle, nerve, esophagus, gonad, coelomocytes, and ampullae) and 8-hydroxy-2′-deoxyguanosine measured in cell-free coelomic fluid showed no general increase with age. The fluorescent age-pigment lipofuscin measured in muscle, nerve, and esophagus did increase with age; however, it was not evenly distributed and not punctate as one would expect if it was confined to lysosomes, but often appeared as patches of autofluorescence in areas devoid of nuclear staining (Du et al. 2013). This extracellular staining suggests that export of damaged material from cells may be a protective mechanism; however, how this affects tissue function remains to be determined. Comparisons between species with different lifespans indicated that markers of oxidative damage (protein carbonyls and 4-hydroxynonenal) were generally higher in the tissues of short-lived *L. variegatus* compared to long-lived *S. purpuratus* and *S. franciscanus* at all ages, and lipofuscin was higher in *L. variegatus* than the other two species at comparable ages (Du et al. 2013). It is tempting to speculate that levels of oxidative damage contribute to the maximum lifespan potential of sea urchins; however, the sea urchins used in this study were collected from different geographic locations and variable environmental factors (e.g. temperature, light, and pollution) can stochastically modify the production of ROS and the accumulation of damage (Tully et al. 2000; Lesser 2006). Therefore, differences across species may not be solely related to lifespan, and further studies are required to explore the relationship between oxidative damage and longevity in sea urchins.

To explore potential mechanisms for the lack of accumulation of oxidative damage, the activity of a variety of cellular antioxidant systems and proteasome enzyme activities was investigated (Du et al. 2013). Superoxide dismutase activity and total antioxidant capacity (measured by the ability of cell and tissue extracts to reduce Cu^{2+} to Cu^{+}) were generally maintained in sea urchin tissues with age, and there was little difference between species with different lifespans (Du et al. 2013). The degradation of oxidized proteins by the proteasome pathway constitutes another important part of the cell's defense against oxidative stress (Löw 2011).

There is evidence that proteasome activity decreases with age in human tissues and short-lived model organisms (i.e. flies and worms) contributing to the process of aging (Löw 2011). In contrast, there was no age-related decrease in the activity of proteasome enzymes (trypsin-, chyomotrypsin-, and caspase-like activities) in most tissues of *L. variegatus*, *S. purpuratus,* and *S. franciscanus* (Du et al. 2013). This suggests a general maintenance in proteasome function with age which may contribute to the lack of increase in protein carbonyls and perhaps sustained protein homeostasis. These results suggest that maintenance of antioxidant capacity and proteasome enzyme activities may be important mechanisms to mitigate the accumulation of oxidative damage in the tissues of animals with negligible senescence. However, antioxidant and proteasomal activities were not generally higher in the tissues of long-lived species, and it may be important to investigate interspecific differences in ROS production to explain the higher levels of oxidative damage in *L. variegatus*.

Gene regulation theory

The gene regulation theory of aging proposes that senescence results from changes in gene expression (Weinert and Timiras 2003). Although many genes show changes in expression with age and certain genes have been shown to affect longevity, the idea that aging is a programmed mechanism directly governed by genes is widely debated. Nevertheless, there have been many studies characterizing changes in gene expression that accompany aging in flies, worms, mice, and humans (Weindruch et al. 2002; McCarroll et al. 2004; Zahn et al. 2006). These studies have revealed distinct transcriptional profiles in different tissues and various organisms; however, comparison of gene expression patterns across tissues of a species or across divergent species has identified some common biological processes that are altered with age. Comparison between *C. elegans* and *D. melanogaster* shows changes in transcription of genes involved in mitochondrial metabolism, DNA repair, catabolism, peptidolysis, and cellular transport (McCarroll et al. 2004). In humans, the common aging signature in different tissues involves six genetic pathways including genes encoding subunits of the mitochondrial electron transport chain, components of the extracellular matrix, components of the cytosolic ribosome, factors involved in complement activation, cell growth, and chloride transport (Zahn et al. 2006). Comparisons of gene expression data from humans, mice, and flies found that components of the electron transport chain decrease in expression with age suggesting that this may be a general marker for aging across species (Zahn et al. 2006). Using a microarray and qRT-PCR, age-related changes in gene expression were examined in three tissues (muscle, esophagus, and nerve) of the sea urchin *S. purpuratus* (Loram and Bodnar 2012). Results indicated age-related changes in gene expression involving many key cellular functions such as the ubiquitin–proteasome pathway, DNA metabolism, signaling pathways, and apoptosis. Although there were tissue-specific differences in gene expression profiles, there were some characteristics that were shared between tissues, and some aspects that differ from short-lived model organisms, providing insight into potential mechanisms that promote lack of senescence in sea urchins. For example, *S. purpuratus* did not show an age-related decline in expression of genes involved in energy production as has been described as a hallmark of aging across a number of species (McCarroll et al. 2004; Zahn et al. 2006). In fact, there was an age-related increase in expression of several mitochondrial genes including components of the electron transport chain in radial nerve tissue of *S. purpuratus* suggesting maintenance of energy production with age (Loram and Bodnar 2012). In addition, in contrast to the age-related decline in the function of the ubiquitin–proteasome pathway in some tissues of mammals, flies, and worms (Löw 2011), an up-regulation in expression of several components of the proteasome pathway was observed in radial nerve and muscle of *S. purpuratus* with age which may contribute to maintenance of protein homeostasis (Loram and Bodnar 2012).

Significant changes in expression of key regulatory genes have been reported in humans and model animals during aging, but the complex interplay of the various signaling pathways in normal aging is not fully understood (Carlson et al. 2008). It is interesting that there was increased expression of genes encoding components of the Notch signaling pathway with age in muscle, nerve, and esophagus of *S. purpuratus* (Loram and Bodnar 2012). The Notch signaling pathway is known to play a role in development and organogenesis but is also important in adult tissue regeneration and repair (Conboy et al. 2003; Carlson et al. 2009). A decrease in Notch signaling accompanies aging in some mammalian tissues which may be responsible for the age-related loss of tissue regenerative potential (Carey et al. 2007). The increased expression of components of the Notch signaling pathway in tissues of *S. purpuratus* with age suggests a mechanism to retain tissue regenerative potential. Some studies have revealed a functional antagonism between the Notch and Wnt signaling pathways, and others have demonstrated that continuous or increased Wnt exposure promoted accelerated aging in mouse models and cultured cells (Brack et al. 2007; Liu et al. 2007). It is therefore interesting that *Wnt*1 gene expression was significantly down-regulated in *S. purpuratus* nerve and muscle tissue with age. Further, proteomics analysis indicated that the ectodomain of low-density lipoprotein receptor-related protein 4, an antagonist of

Wnt-signaling, was increased with age in coelomic fluid of sea urchins suggesting a more general suppression of the Wnt signaling pathway (Bodnar 2013). The interplay between the Notch and Wnt pathways may be an important mechanism to maintain regenerative potential with age in sea urchin tissues. Continual replacement of damaged cells and the sustained ability to regenerate tissues would ensure life-long growth and homeostasis and may be an important property of animals with indeterminate growth and negligible senescence (Vogt 2011).

Echinoderms are known to have tremendous regenerative capacity and can regenerate both external appendages and internal organs (Carnevali 2006). Regeneration in echinoids (i.e. sea urchins) is less well studied than other Echinoderm classes; however, they are known to regenerate spines and pedicellariae (Dubois and Ameye 2001). Current work is focused on understanding regenerative potential in sea urchin tissues and how this process is affected by age. Quantitative analysis of cell proliferation (BrdU incorporation) and apoptosis (assessed by TUNEL and the Apo ssDNA™ assays) in sea urchin tissues (muscle, nerve, esophagus, and coelomocytes) indicate a low level of tissue renewal that is maintained with age in sea urchin species with different lifespans (*S. franciscanus*, *S. purpuratus,* and *L. variegatus*) (Bodnar laboratory, unpublished data). Regenerative capacity, assessed by measuring the regrowth of amputated spines and tube feet (motor and sensory appendages) in *L. variegatus*, is maintained with age (Bodnar laboratory, unpublished data). This result is contrary to what is observed in mammals where regenerative processes decline with age (Ho et al. 2005). As the decline in regenerative potential in mammals is linked to decline in stem cell function, it stands to reason that animals with a high regenerative potential either have an abundance of stem cells or can dedifferentiate specialized tissue cells into stem or progenitor cells (Ho et al. 2005). There is currently no evidence for the existence of true stem cells and distinct stem cell niches in non-reproductive organs of echinoderms (Vogt 2011). However, there is evidence for the potential to produce multipotent progenitor cells by dedifferentiation in various tissues (Vogt 2011). Further study is needed to understand the mechanisms of regeneration in these animals and how these processes are maintained with age.

Conclusion

Studying animals with indeterminate growth and negligible senescence offers the opportunity to understand the protective and regenerative processes employed to prevent the degenerative decline with age. Because they exhibit life-long growth and the ability to regenerate damaged tissues, sea urchins are ideally suited to understanding mechanisms that maintain tissue homeostasis and regenerative capacity with age. The occurrence of species with different lifespans, including some with extreme longevity and negligible senescence, allows investigation of the role of these processes in lifespan determination and mitigation of aging. Long-term maintenance of tissue homeostasis relies on the accurate regulation of somatic and stem cell activity to balance growth and repair of damage while at the same time avoiding overproliferation. As neoplasms are rarely seen in sea urchins (Jangoux 1987; Bodnar 2009; Robert 2010), they provide an additional unique opportunity to understand the regulatory factors involved in long-term tissue homeostasis and regeneration without conferring predisposition to cancer development.

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