# Stem cells and aging from a quasi-immortal point of view

*Anna-Marei Boehm[1], Philip Rosenstiel[2] and Thomas C. G. Bosch[1]\**

Understanding aging and how it affects an organism's lifespan is a fundamental problem in biology. A hallmark of aging is stem cell senescence, the decline of functionality, and number of somatic stem cells, resulting in an impaired regenerative capacity and reduced tissue function. In addition, aging is characterized by profound remodeling of the immune system and a quantitative decline of adequate immune responses, a phenomenon referred to as immune-senescence. Yet, what is causing stem cell and immune-senescence? This review discusses experimental studies of potentially immortal *Hydra* which have made contributions to answering this question. *Hydra* transcription factor FoxO has been shown to modulate both stem cell proliferation and innate immunity, lending strong support to a role of FoxO as critical rate-of-aging regulator from *Hydra* to human. Constructing a model of how FoxO responds to diverse environmental factors provides a framework for how stem cell factors might contribute to aging.

**Keywords:**
- aging; FoxO; *Hydra*; immune-senescence; innate immunity; stem cells

*It would not be unexpected if such anti-senescence processes are controlled in a coordinated fashion and are genetically determined by a few master genes.*

(Richard C. Cutler) [1]

## Introduction – the two routes to aging

Aging is a universal phenomenon that affects nearly all animal species and causally influences an organism's

[1] Zoological Institute, Christian-Albrechts-Universität zu Kiel, Kiel, Germany
[2] IKMB, University Hospital Schleswig-Holstein, Kiel, Germany

*Corresponding author:
Thomas C. G. Bosch
E-mail: tbosch@zoologie.uni-kiel.de

lifespan [2]. The biological nature of the aging processes and the genetic biochemical complexity of processes that govern the aging rate are incompletely understood. Is aging controlled by a special set of genes? Aging is the gradual change in an organism that leads to increased risk of weakness, disease, and death [3] (Box 1). Recent data obtained from twin studies show that only 20–30% of the overall variation in lifespan can be attributed to genetic factors [4–8]. Thus, it is very unlikely that there is a single gene or even a few genes that alone induce senescence and lead to mortality. It seems much more likely that an intimate interplay between two major factors controls senescence and lifespan: genetic determinants on one hand and environmental conditions on the other hand, with an astonishingly high environmental contribution [4–8] (Fig. 1).

Although the genetic contribution to aging with 20–30% seems relatively small [5–8] two genes have been shown in genome-wide association studies (GWAS) of different human cohorts to have a strong and direct association with aging and longevity: Apolipoprotein E (Apo-E) and forkhead box O (FoxO) [9, 10] (Fig. 1). APO-E, a major cholesterol carrier that supports lipid transport and injury repair in the brain, is negatively associated with aging and therefore may be considered a mortality factor [9, 10]. In contrast, the transcription factor FoxO3a can be termed a longevity factor since a special variant of the *foxO3a* gene containing three specific single-nucleotide polymorphisms (SNPs) has been demonstrated in independent GWAS from different human populations throughout the world to be a crucial component of the genetic signature of centenarians [11–15]. Because in numerous model organisms (mice, *Drosophila melanogaster* and *Caenorhabditis elegans*) the corresponding FoxO homologue is a major factor capable to delay aging and regulate lifespan [16–19], FoxO function is ancient and well conserved. It, therefore, appears that FoxO makes up a major part of the genetic contribution to aging.

Epidemiological data indicate an increased predisposition of the elderly to infections, caused by profound changes in the immune system and a quantitative decline of adequate immune responses [20]. This gradual deterioration of the immune system, also termed immune-senescence [21] is characterized by a breakdown of the epithelial barriers of the skin, lungs, and gastrointestinal tract [22] as well as by

Problems & Paradigms

## Box 1

## Aging

Gradual change in an organism that leads to increased risk of weakness, disease, and death [118, 119]. Aging can also be defined as a progressive functional decline, or a gradual deterioration of physiological function with age, including a decrease in fecundity, or the intrinsic, inevitable, and irreversible age-related process of loss of viability and increase in vulnerability. Aging is thought to have arisen serendipitously in evolutionary history as a result of a trade-off between the germ line and somatic cells in the distribution of resources. This trade-off has been developed into a formal theory by Thomas Kirkwood and is known as the Disposable Soma Theory of Aging [56].

## Longevity

Total lifespan. Both multiple environmental (~70%) and genetic (~30%) factors play a role in attaining longevity [4, 6].

## Senescence

The process of becoming old [120].

changes in both the adaptive [23–27] and the innate immune system [28–36]. Advanced age is associated with decrease of components of the adaptive immunity including immuno-globulin secretion and effectiveness and numbers of T cells [23–27]. Aging is also accompanied by activation of components of the innate immunity such as pro-inflammatory cytokines and the key immune transcriptional activator Nuclear Factor-kappaB (NF-κB) [28–36], generating a pro-inflammatory profile often referred to as inflammaging [24, 25]. Other innate immune components such as Toll-like
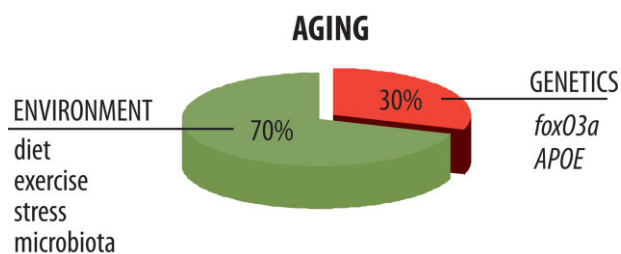


**Figure 1.** Genetic and environmental contribution to aging. Twin studies have indicated that an intimate interplay between both multiple environmental (~70%) and genetic (~30%) factors controls aging and longevity [4–8]. So far, only a few genes have been consistently reported to be associated with human longevity: the ε4 allele of the apolipoprotein E (APOE) gene, a mortality factor [9, 10] and markers in the forkhead box O3A gene (FOXO3A) that show a modest beneficial effect upon survival in nonagenarians and centenarians [11–15].

receptors (TLRs) and nucleotide-binding and oligomerization domain-like receptors (NLRs) are down-regulated during the aging process [32]. Such profound remodeling of the immune system increases the susceptibility to bacterial, viral, and fungal pathogens [2, 32] and causes typical age-related diseases such as atherosclerosis, arthritis, osteoporosis, as well as auto-immune diseases [25, 37]. It also contributes to age-associated frailty, morbidity, and mortality [2]. But, as exemplified by centenarians, some elderly obviously reach old age despite this continuous remodeling process. How?

## We are as old as our stem cells

A hallmark of aging is the decline of cellular and organic functionality over time [38], caused by the accumulation of cellular damages and increasing genetic instability [39]. In young age, when stem cells are highly active, damaged, and worn out cells get regularly replaced. However, with increasing age, functionality and number of somatic stem cells appears to decline [38] (Fig. 2A) as has been shown in several different stem cell systems including the skin [40], hematopoietic [41], neural [42, 43], and muscular stem cells [44]. As seen either in progenitor cell number and/or differentiation potential, the human skin experiences an age-dependent, functional depletion of the stem cells pool [40] (Fig. 2B). Observations in aging humans supported by studies in aging mice show that there is also a significant decrease in the number of functionally competent hematopoietic stem cells (HSCs) [41]. Similarly, old mice experience a twofold reduction in the number of neural stem cells (NSCs) relative to young mice [42, 43]. Moreover, the activity of myogenic stem cells, called satellite cells, is age-dependent and responsible for deterioration of muscle function and muscle regenerative responses in both old human and old mice [44]. Together, this phenomenon termed "stem cell senescence" results in impaired regenerative capacity and reduced tissue function and consequently is leading to organismal aging (Fig. 2). Progress into an understanding of the accelerated aging condition called Hutchinson–Gilford progeria syndrome (HGPS) supports the view that aging is a consequence of stem cell dysfunction. HGPS is caused by a truncated and farnesylated form of Lamin A called progerin [45–47]. HGPS affects mesenchymal lineages, including the skeletal system, dermis, and vascular smooth muscle (VSMC) [48, 49]. The cell type-specific pathologies in HGPS have been attributed to a variety of causes, including progerin-mediated stem cell pool exhaustion [50] and mesenchymal lineage differentiation defects [51]. Recent experiments with induced pluripotent stem cells (iPSCs) from HGPS dermal fibroblasts [49] support the hypothesis that in HGPS patients the mesenchymal stem cell (MSC) pool becomes exhausted [50].

In the aging process, many cellular activities are compromised. One important parameter at the cellular level is the ability of cells to protect themselves against proteome damage due to oxidation (carbonylation) of their amino acids causing loss of protein function and/or decreased precision of interactions among proteins [52]. Intriguingly, the resilience of some rare species (e.g. *Deinococcus radiodurans* bacteria and small aquatic animals such as Rotifers
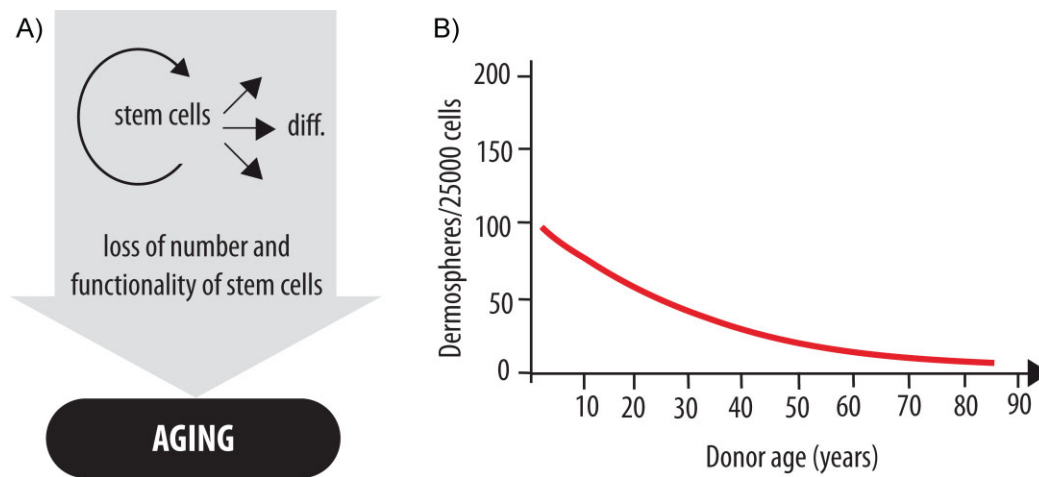
**Figure 2.** Organismal aging is a consequence of stem cell senescence. **A:** In young age, stem cells are numerous and active. Decline of stem cell number and functionality with time leads to aging. **B:** Age-dependent decline of dermosphere-forming precursors derived from skin (modified from [40]).

and Tardigrada) to stresses such as extreme radiation, toxic chemicals, and years of desiccation appears to be due to the evolved prevention of the oxidative damage to the cellular proteome [53, 54].

## Different animal species show different aging patterns and different maximum lifespans

Senescence, defined as progressive declines of physiological functions, leading to an increase in the mortality rate as a function of time, has been found in all metazoans where careful studies have been carried out. Aging and death are integral and intrinsic to the evolution of life [55, 56]. However, both the patterns of aging as well as the maximum lifespans differ widely across the animal kingdom. Comparative studies currently are focused on understanding why some animal species live longer than others [57]. Long-lived animals such as some oysters, clams, and even some species of whales can reach 200 years old. First observations indicate that cultured cells from such long-lived animals are more resistant to stress than those of short-lived species [58]. Most research, however, is still done with model organisms such as yeast [59, 60], fruit flies [61–63], worms [64–66], and mice [67–69], all of which have a short lifespan and mature quickly. With regard to understanding the factors contributing to longevity, probably the most important outcome of numerous elegant genetic studies in these short-lived model organisms was the discovery of the significance of the nutrient-signaling pathways and the impact of environmental growth conditions in longevity [60].

Beside differences in the maximum lifespan there are also great differences in the aging pattern in different animals. For example, in some species including human mortality increases with age while in many reptiles, amphibians, and fish mortality decreases throughout adult life [70]. Unfortunately, research on the evolution of aging so far has brought little light into the factors favoring a pattern of aging with increasing mortality versus alternative patterns with constant or declining mortality [70]. The chemistry of this species-specific biological clock is not yet known although there is good reason to assume that it is DNA chemistry [71]. "Fundamental understanding of why humans deteriorate so sharply compared with other species, why human mortality has fallen so dramatically, and whether aging can be further delayed or even slowed, depends on knowledge of why some species senesce and others do not" (cited from [70]).

## *Hydra* preserves longevity and escapes senescence by having adopted a life cycle in which reproduction can occur asexually by budding

The fresh water polyp *Hydra* (Fig. 3A) seems one of few organisms who escape from aging, are long-lived, and at least in the laboratory may be considered as potentially immortal [57, 70, 72]. The animal was discovered as model organism in the summer of 1740 when a Swiss naturalist and private scholar, Abraham Trembley, used *Hydra*'s extensive regenerative capacity to demonstrate to his disciples [73] that this creature can regenerate itself again and again and therefore – against prevailing belief – not everything in nature is "preformed" but that living creatures and structures can form de novo simply following nature's laws.

The biological reason behind that seemingly unique potential to escape mortality and senescence is simple: the animals have adopted a life cycle in which proliferation and population growth occur exclusively asexually by budding (Fig. 3B). This asexual mode of reproduction demands that each individual polyp maintains continuously proliferating cells [74, 75]. If it would not, the species would lose in number
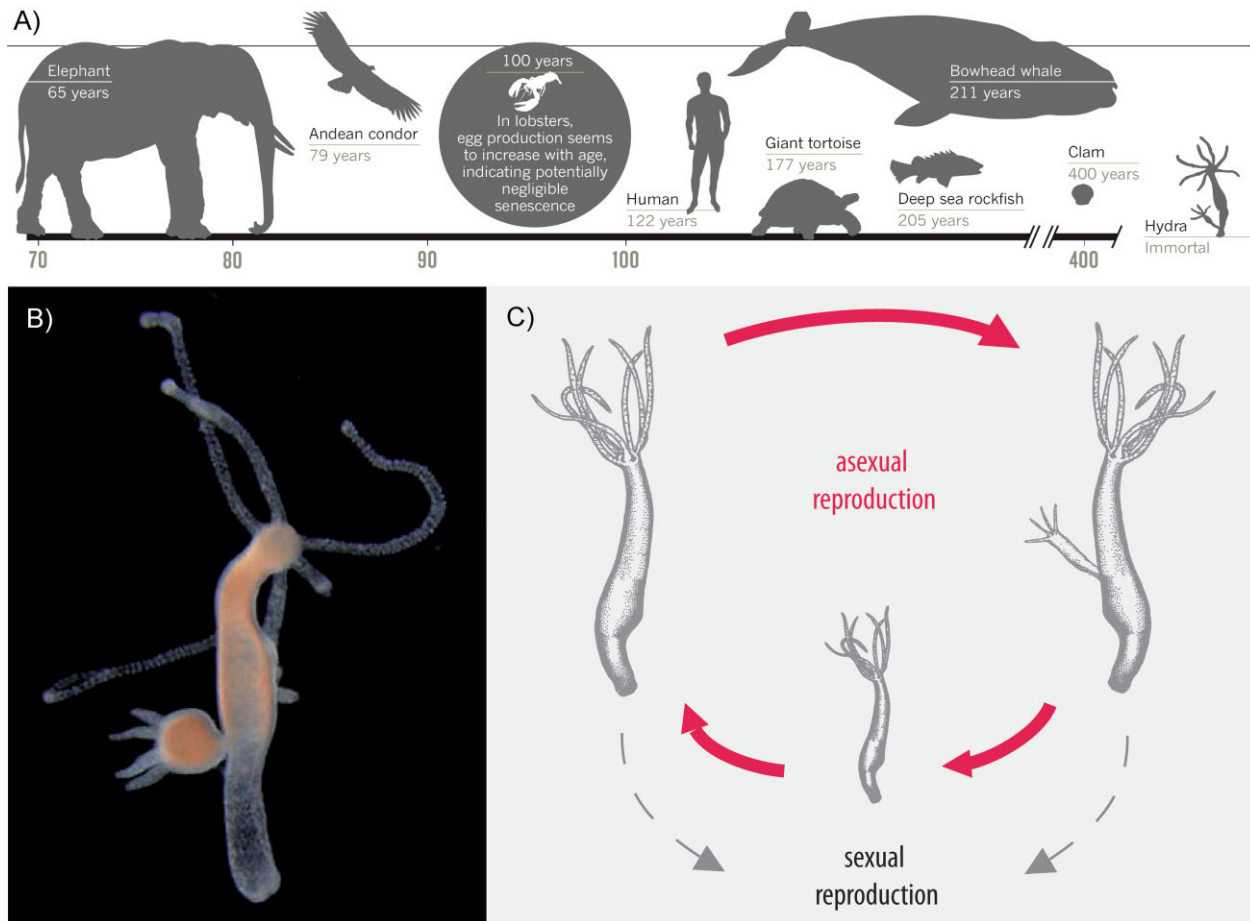
**Figure 3.** *Hydra* does not undergo aging and is potentially immortal. **A:** Different organisms have different maximum lifespans, which are however always limited. In contrast, the fresh water polyp *Hydra* could never be observed to have a limited lifespan and thus can be considered potentially immortal (figure taken from [57]). **B:** The fresh water polyp *Hydra*. **C:** Life cycle of the fresh water polyp *Hydra*. *Hydra* reproduces predominantly asexually by budding.

of offspring and would, sooner or later, be outcompeted by other species. It is generally assumed [57, 76] that *Hydra* polyps achieve this indefinite lifespan only if they reproduce asexually and that they deteriorate if they begin to produce gametes. However, since a number of *Hydra* species such as *Hydra viridis* and *H. vulgaris* produce gametes (either eggs or sperm) and simultaneously reproduce asexually by budding off daughters (Bosch, pers. observation), we question this assumption and take the view that extreme longevity may not be directly linked to an asexual mode of reproduction. Fact is that in *Hydra* there is strong selective constraint to equip the adult polyp's tissue with cells which are capable of continuous proliferation and differentiation. In vivo tracking of individual *GFP*-expressing cells showed unquestionably that epithelial cells [77] as well as interstitial cells [78] in *Hydra* are continuously proliferating and differentiating. Offspring of individual cells were tracked for several years without obtaining evidence for apparent signs of decline in

proliferation rates. These findings added support to an earlier study [72] analyzing the mortality patterns and reproductive rates of four groups of individuals of *Hydra* for a period of four years. The apparent lack of cellular senescence results in an adult polyp which has an indefinite proliferative lifespan. In sum, the key to *Hydra*'s longevity and developmental youth lies in its asexual mode of reproduction and strong evolutionary constraint to continuously maintain the self-renewal capacity of its three somatic stem cell lineages (Fig. 4A). But what drives this continuous stemness in *Hydra*'s cells?

## Factors implicated in *Hydra*'s stem cell homeostasis and longevity

Several recent reviews cover different aspects of a still rather limited knowledge of molecular factors acting in stemness regulation in *Hydra* [75, 79–82]. Wnt signaling is among the best studied signaling systems due to its relevance in embryonic development and cancer. In *Hydra*, canonical Wnt/b-Catenin signaling is a central player in the head organizer and in pattern formation along the oral-aboral body axis [83–86]. Noncanonical Wnt signaling, by comparison, acts in tissue movement and evagination during tentacle and bud formation, providing evidence for an ancestral
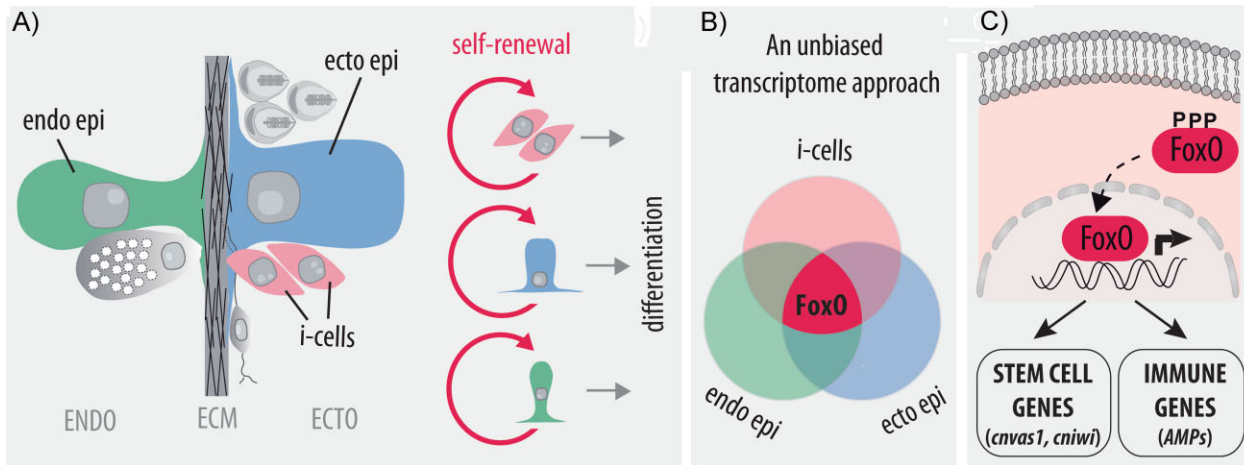
**Figure 4.** *Hydra* stem cells are continuously maintained. **A:** The stem cell system of *Hydra* consists of three independent stem cell lineages: ectodermal and endodermal epithelial stem cells and interstitial stem cells. Ectodermal and endodermal epithelial stem cells are unipotent, interstitial stem cells are multipotent. All stem cells posses a continous self-renewal capacity. **B:** An unbiased transcriptome approach revealed FoxO being expressed by all three stem cell lineages [94]. **C:** The transcription factor FoxO regulates stem cell and immune genes in *Hydra* (modified from [99]).

complexity of the Wnt signaling network [87]. The Wnt/b-Catenin pathway also plays a role in the maintenance and in cell fate decisions of stem cells, and activated Wnt signaling is tightly linked to stemness [88]. Transcription factors of the Myc family may also play a decisive role in the control of proliferation and in the maintenance of stem cells [81, 89, 90]. Moreover, to ensure an error-free genome in continuously proliferating cells, high expression of DNA repair genes such as XPF might be crucial [91]. Screening the collection of genes in a number of early emerging metazoans [92] including the *H. magnipapillata* genome sequence shows that some of the classical stem-cell-specific genes such as *Oct3/4* are missing in *Hydra*; also *Nanog* as well as *REST*, *LIN28*, *Klf4*, and *FGF4* appear to be absent in the basal metazoan taxa [93]. Thus, despite a striking conservation of signaling and transcription-al mechanisms utilized in diverse stem-cell differentiation processes across the animal kingdom, not all of the stem-cell regulators are conserved.

This realization motivated us to search for previously unknown stem-cell regulators. In an unbiased transcriptome profiling approach we, therefore, labeled the three stem cell lineages with the reporter protein GFP, isolated them by fluorescent activated cell sorting (FACS) and submitted them to high throughput sequencing [94]. The approach resulted in uncovering multiple signaling pathways involved in inter-lineage communication of the three stem cell lineages and also identified the *Hydra* epithelium as a signaling center, providing information for interstitial stem cells [94]. Analyz-ing *Hydra*'s stem cell specific transcriptomes also revealed that they express both lineage-specific and common sets of transcription factors [94]. Among the latter, to us the most interesting transcription factor shared by all three stem cell lineages is the forkhead factor FoxO, which is involved in

preventing aging and controlling lifespan in many other organisms.

## The transcription factor *foxO* is a key regulator of stem cell maintenance in *Hydra*

When analyzing the transcriptomes of *Hydra*'s three stem cell lineages we found the *Hydra* homologue of *foxO* being strongly expressed by all three of them (Fig. 4B). This finding in an animal apparently escaping senescence was intriguing since, as outlined above, in mice and flies upregulation of *foxO* has been demonstrated to delay aging and extend lifespan [17] and in human, *foxO3a* has been identified as a crucial component of the genetic signatures of exceptional longevity [12]. In cnidarians, *foxO* previously was identified in *Nematostella* [95] and *Clytia* [96] but never functionally analyzed. *Hydra*, similar to other invertebrates, possesses only a single *foxO* gene [97] which had been suggested to play a role in stress resistance [97] and apoptosis [98]. In gain and loss of function analysis we found [99] that FoxO plays an important role in both controlling stem cells and the innate immune system in *Hydra* [99] (Fig. 4C). Strikingly, *foxO* knock-down polyps showed key features of an aging organism, such as decline in stem cell number and immune functionality. Compared to control polyps they have consid-erably less stem cells due to an increased rate of differentia-tion of epithelial stem cells into foot cells (Fig. 5A), resulting in a reduced population growth rate and delayed bud development (Fig. 5B). Taking into account that these "knock-down" polyps only have a reduced *foxO* level rather than a complete *foxO* knock out mutation, the budding rate might be even more reduced when FoxO activity is completely eliminated in all stem cells. Concurrently to the reduction in stem cell numbers, *foxO* down regulation caused drastic changes in the immune status of the polyps by influencing the expression of antimicrobial peptides (AMPs) [99] (Fig. 4C). AMPs are known as effector molecules of innate immunity in both the animal and plant kingdoms with a major role to fight microbial infections through their strong antimicrobial activity [100]. The finding of AMPs as downstream target
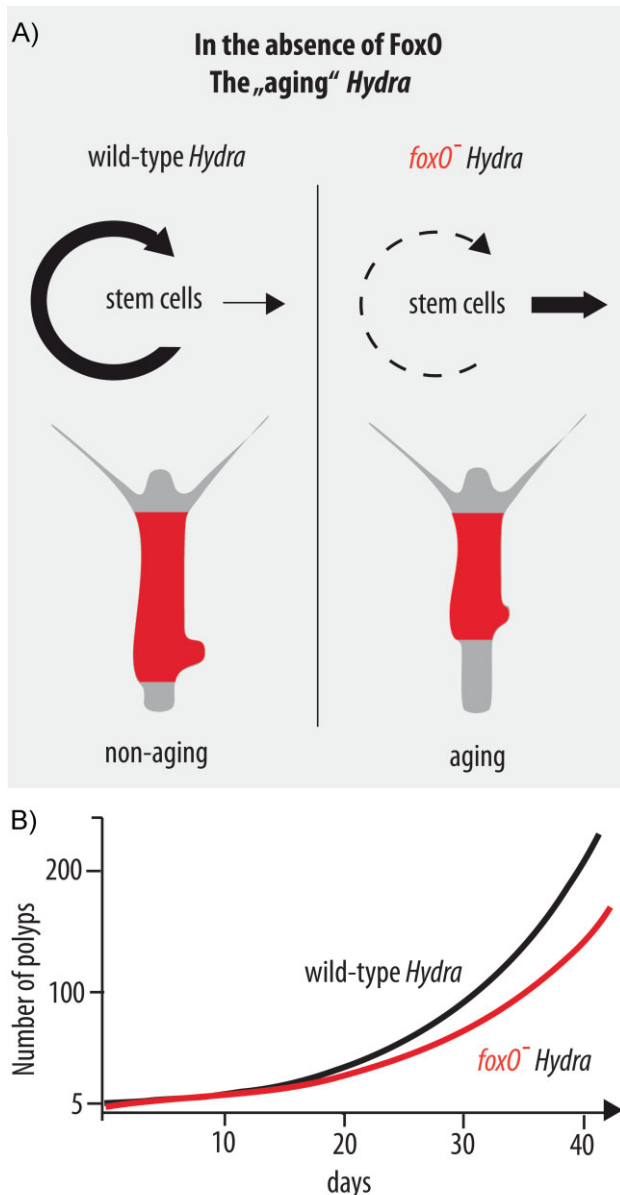
**Figure 5.** *foxO* deficiency results in an "aging" *Hydra*. **A:** *foxO* down-regulation in *Hydra* leads to a reduction of stem cell number [99]. **B:** *foxO* down-regulation results in a slowdown of population growth rate (modified from [99]).

genes of stem cell transcription factor FoxO was therefore unanticipated.

## The question of conservation: FoxO plays a dual role in maintaining both stem cell and immune functionality

The implication of FoxO factors in the regulation of both systems, stem cells and immunity, which are affected by profound age-related changes, seems to be deeply conserved.

In mammals, FoxO1 is essential for the maintenance of ESC self-renewal and pluripotency [101], while FoxO3a is a critical regulator of stem cell homeostasis in neural [102], leukemic [103], and HSCs [104]. Likewise, *foxO3a* knock down in bone marrow cells and NSCs in mice leads to a reduction of colony-forming cells and decrease in stem cell frequencies [102, 104], clearly demonstrating a role of FoxO factors in stem cell maintenance. At the same time, there are strong data implicating FoxO in maintaining immune homeostasis in mammals. In the mouse, knocking down *foxO3a* perturbs cytokine production, induces NF-κB activation, hyperactivates T cells, and causes spontaneous lympho-proliferation, resulting in severe inflammation in several tissues [105, 106]. Other studies showed that FoxO influences the innate immune system by regulating the activity of antimicrobial peptides. In *Drosophila* which are overexpressing *dFoxO*, direct binding of dFoxO to the *drosomycin* regulatory region leads to an induction of AMPs synthesis [16]. Studies in human lung, gut, kidney, and skin cells also revealed a FoxO3a-dependent regulation of AMPs [16]. In *C. elegans*, the FoxO ortholog DAF-16 has been proven to directly activate the saposin-like AMP gene *spp-1* and the lysozyme gene *lys-1* [107, 108] and thereby to confer resistance to several pathogenic bacteria [109]. Another study, focusing on centenarians (age 100+ years), also showed that there is a potential relationship between FoxO activity and the immune status. Being distinguished by their exceptional high age which is correlated with a particular sequence version of the *foxO3a* gene, centenarians at the same time show a remarkable good health status and a reduced pro-inflammatory profile as it is normally typical for elderly people [110]. Despite signs of inflammation, such as high levels of interleukin-6 (IL-6) [111], fibrinogen, and coagulation factors [112, 113], centenarians are remarkably free of most age-related diseases that have an inflammatory component [114]. This might be due to the fact that their pro-inflammatory state is countered by increased expression of anti-inflammatory cytokines such as transforming growth factor-beta1 (TGF-ß1) [110].

## FoxO, a rate-of-aging regulator?

Why do organisms start to show signs of ageing at a particular and species-specific time point? One possibility is that *"over time the level of a rate-of-aging regulator falls, crossing thresholds that trigger various aspects of aging"* [18]. Could FoxO or the FoxO-dependent transcriptome be such a "rate-of-aging regulator"? Experimental evidence in flies, worms, and mice indicates that changes in lifespan indeed can occur through changes in *foxO* expression. Decline of *foxO* expression results in aging and death. Experimental approaches in both *C. elegans* and flies using mutant *DAF-16* and *dfoxO* transgenic lines have shown that the fewer FoxO molecules are present, the earlier the model organisms will die. And vice versa, increase of *foxO* expression delays aging by maintaining stem cell self-renewal and functionality of the immune system. Since we have made similar observations using transgenic *Hydra* [99] we have proposed a model in which in long-lived *Hydra* the high expression of *foxO* in all

three stem cell lineages is crucial for the continuous self-renewal capacity and potentially unlimited lifespan as well as the continuous maintenance of the functionality of the innate immune system. In this model the aging process of most organisms is caused by a progressive reduction of FoxO



**Figure 6.** A scheme illustrating the current scenario on the gene-environmental interactions regulating the aging process of organisms. In response to environmental signals coming from diet, microbiota, exercise, stress, etc., different signaling pathways upstream of FoxO regulate the phosphorylation status of FoxO and thereby its localization and activity. Depending on the FoxO activity the expression of stem cell and immune genes is changed, which has an impact on the maintenance of stem cell and immune system and thereby on the aging process of an organism. Young, non-aged individuals, and potentially immortal organisms such as *Hydra*, have high numbers of active stem cells and an effective immune system. Old, aged individuals, and *foxO*-deficient *Hydra* are characterized by a decline in stem cell number and functionality as well as an increasingly ineffective and unspecific immune system.

activity, which might be due to changes in the upstream regulating signaling cascades. In this view, the amount of FoxO present in a given tissue and organism is age-dependent and when falling below a threshold permits aging to occur. A full understanding of the role of FoxO as a putative "rate-of-aging-regulator" will heavily depend on a more comprehensive knowledge of the mechanisms of aging in other long-lived species. Do all these long-lived organisms share a common "master switch" of longevity? Or will comparative studies show that there are many roads to arrive at a long-lived species?
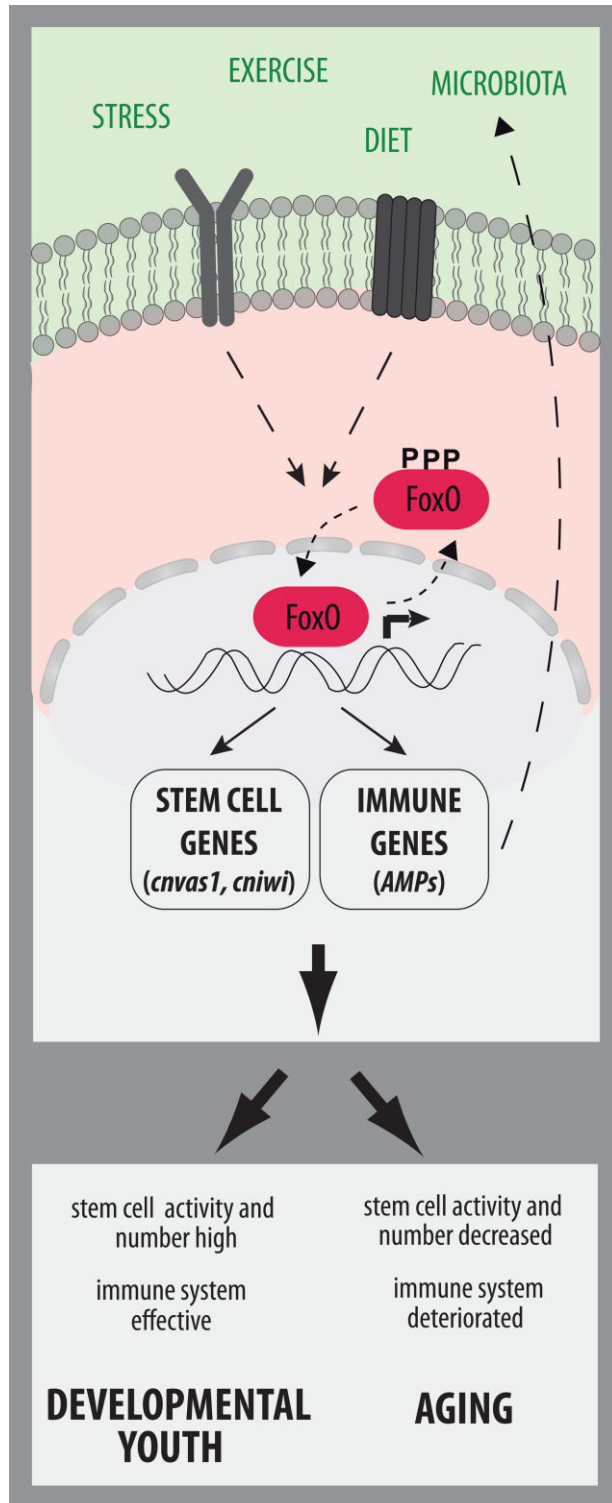
## Impact of environmental conditions on aging

Certainly, the pivotal role of transcription factor FoxO does not rule out the importance of upstream events, and given the impact of environmental factors on the aging process (Fig. 1) it is likely that transcriptional activation of downstream genes must occur with proper coordination of the environmental conditions. A scheme illustrating the current scenario on the gene-environmental interactions regulating the aging process of organisms is presented in Fig. 6. In response to environmental signals coming from diet, microbiota, exercise, stress etc., different signaling pathways upstream of FoxO regulate the phosphorylation status of FoxO and thereby its localization and activity. Depending on the FoxO activity the expression of stem cell and immune genes is changed, which has an impact on the maintenance of stem cell and immune system and thereby on the aging process of an organism. Young, non-aged individuals, and potentially immortal organisms such as *Hydra*, have high numbers of active stem cells and an effective immune system. Old, aged individuals, and *foxO*-deficient *Hydra* are characterized by a decline in stem cell number and functionality as well as an increasingly ineffective and unspecific immune system.

Several studies indicate the impact of environmental conditions on aging. A highly conserved network of nutrient and energy sensing signaling pathways including the insulin/insulin-like growth factor 1 (Ins/IGF-1) [18, 19, 115, 116] and the protein kinase target of rapamycin [68, 116, 117] pathways was

discovered in a number of organisms (*Drosophila*, *C. elegans*, and yeast) to affect the lifespan. Mutations in the gene coding for insulin/IGF-1 receptor were found to double the lifespan in *C. elegans* [64]. This lifespan extension was dependent on the reduction of insulin/IGF-1 receptor activity (DAF-2 in *C. elegans*) and consequently of its downstream effector PI3K (encoded by age-1), and the subsequent activation of FoxO (DAF-16). Similar genetic or pharmacological manipulations in *Drosophila* resulted in identical outcomes [115] indicating the evolutionary conserved roles of these environment-sensing pathways in determining lifespan.

## Conclusions and outlook

Aging is a complex process. The above discussion illustrates that studies in numerous model organisms support a crucial role for two major components in controlling aging and mortality: declining functionality and number of stem cells ("stem cell senescence") as well as profound changes in the immune system ("immuno-senescence"). *Hydra* appears to escape from aging, since its stem cells are continuously active and its immune system is continuously effective [99]. There are many compelling arguments suggesting that the transcription factor FoxO plays a key role in maintaining both stem cell and immune homeostasis [101–106]. And as discussed above, there is also no doubt that environment matters if it comes to aging and longevity.

*Hydra* polyps are potentially immortal and never experience the gradual changes seen in an aging organism leading towards increased weakness, disease, and death. Surprisingly, *Hydra*'s "eternal developmental youth" is unconnected to the prevailing environmental conditions. This is certainly true for polyps kept under controlled feeding conditions in the laboratory where it makes no difference whether the animals are fed ad libidum or strictly limited. *Hydra*'s stem cells in individual polyps never lose their capacity for unlimited self-renewal. Although few ecological field studies have been undertaken so far, there is also no evidence that environmental conditions affect number and/or functionality of *Hydra*'s stem cells in the natural habitat which is characterized by seasonal fluctuations in nutrient abundance. So, why have environmental conditions apparently only limited impact on *Hydra*? Which mechanisms protect them against adverse effects of, e.g. nutrient abundance? Which nutrient and energy sensing pathways are involved? How is stem cell proliferation and tissue growth coordinated with nutritional conditions? Nutrients as well as microbes and stress are among the main extrinsic factors *Hydra* is daily exposed to. How *Hydra* maintains eternal cell lineages and how the cellular activities are coordinated with the prevalent environmental conditions remains enigmatic. How is stem cell proliferation and tissue growth coordinated with nutritional conditions? Combining genetic manipulation of key components of environment-sensing pathways and controlled culture conditions, *Hydra* may emerge as a highly suitable model to study how the interaction between genome and environmental factors affects aging. Most importantly, this information will not only enhance our understanding of genetic and environmental influences on the evolutionary conserved processes controlling aging, but will also be informative to physicians and scientists concerned with human age-associated diseases, and the development of interventions to treat them.

## References

1. **Cutler RG**. 1979. Evolution of human longevity: a critical overview. *Mech Ageing Dev* **9**: 337–54.
2. **Franceschi C, Olivieri F, Marchegiani F, Cardelli M**, et al. 2005. Genes involved in immune response/inflammation, IGF1/insulin pathway and response to oxidative stress play a major role in the genetics of human longevity: the lesson of centenarians. *Mech Ageing Dev* **126**: 351–61.
3. **Britanica Encyclopedia**.
4. **Christensen K, Johnson TE, Vaupel JW**. 2006. The quest for genetic determinants of human longevity: challenges and insights. *Nat Rev Genet* **6**: 436–48.
5. **Herskind A, McGue M, Holm N, Sørensen T**, et al. 1996. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870–1900. *Hum Genet* **97**: 319–23.
6. **vB Hjelmborg J, Iachine I, Skytthe A, Vaupel JW**, et al. 2006. Genetic influence on human lifespan and longevity. *Hum Genet* **119**: 312–21.
7. **Ljungquist B, Berg S, Lanke J, McClearn G**, et al. 1998. The effect of genetic factors for longevity: a comparison of identical and fraternal twins in the Swedish Twin Registry. *J Gerontol A Biol Sci Med Sci* **53**: M441–6.
8. **Skytthe A, Pedersen N, Kaprio J, Stazi M**, et al. 2003. Longevity studies in GenomEUtwin. *Twin Res* **6**: 448–55.
9. **Nebel A, Kleindorp R, Caliebe A, Nothnagel M**, et al. 2011. A genome-wide association study confirms APOE as the major gene influencing survival in long-lived individuals. *Mech Ageing Dev* **132**: 324–30.
10. **Schächter F, Faure-Delanef L, Guenot F, Rouger H**, et al. 1994. Genetic associations with human longevity at the APOE and ACE loci. *Nat Genet* **6**: 29–32.
11. **Anselmi CV, Malovini A, Roncarati R, Novelli V**, et al. 2009. Association of the FOXO3A locus with extreme longevity in a southern Italian centenarian study. *Rejuvenation Res* **12**: 95–104.
12. **Flachsbart F, Caliebe A, Kleindorp R, Blanche H**, et al. 2009. Association of FOXO3A variation with human longevity confirmed in German centenarians. *Proc Natl Acad Sci USA* **106**: 2700–5.
13. **Kojima T, Kamei H, Aizu T, Arai Y**, et al. 2004. Association analysis between longevity in the Japanese population and polymorphic variants of genes involved in insulin and insulin-like growth factor 1 signaling pathways. *Exp Gerontol* **39**: 1595–8.
14. **Li Y, Wang WJ, Cao H, Lu J**, et al. 2009. Genetic association of FOXO1A and FOXO3A with longevity trait in Han Chinese populations. *Hum Mol Genet* **18**: 4897–904.
15. **Pawlikowska L, Hu D, Huntsman S, Sung A**, et al. 2009. Association of common genetic variation in the insulin/IGF1 signaling pathway with human longevity. *Aging Cell* **8**: 460–72.
16. **Becker T, Loch G, Beyer M, Zinke I**, et al. 2010. FOXO-dependent regulation of innate immune homeostasis. *Nature* **463**: 369–73.
17. **Giannakou ME, Goss M, Junger MA, Hafen E**, et al. 2004. Long-lived *Drosophila* with overexpressed dFOXO in adult fat body. *Science* **305**: 361.
18. **Kenyon C**. 2010. A pathway that links reproductive status to lifespan in *Caenorhabditis elegans*. *Ann N Y Acad Sci* **1204**: 156–62.
19. **Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G**. 1997. daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. *Science* **277**: 942–6.

Problems & Paradigms

20. **Dewan SK, Zheng SB, Xia SJ, Bill K**. 2012. Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections. *Chin Med J (Engl)* **125**: 3325–31.

21. **Walford RL**. 1969. *The Immunological Theory of Aging*. Copenhagen: Munksgaard.

22. **Gomez CR, Boehmer ED, Kovacs EJ**. 2005. The aging innate immune system. *Curr Opin Immunol* **17**: 457–62.

23. **Aw D, Silva AB, Palmer DB**. 2007. Immunosenescence: emerging challenges for an ageing population. *Immunology* **120**: 435–46.

24. **Franceschi C, Bonafe M, Valensin S**. 2000. Human immunosenescence: the prevailing of innate immunity, the failing of clonotypic immunity, and the filling of immunological space. *Vaccine* **18**: 1717–20.

25. **Franceschi C, Bonafe M, Valensin S, Olivieri F**, et al. 2000. Inflammaging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci* **908**: 244–54.

26. **Kovaiou RD, Grubeck-Loebenstein B**. 2006. Age-associated changes within CD4+ T cells. *Immunol Lett* **107**: 8–14.

27. **Pawelec G, Hirokawa K, Fulop T**. 2001. Altered T cell signalling in ageing. *Mech Ageing Dev* **122**: 1613–37.

28. **Johnson TE**. 2006. Recent results: biomarkers of aging. *Exp Gerontol* **41**: 1243–6.

29. **Kim HJ, Jung KJ, Yu BP, Cho CG**, et al. 2002. Modulation of redox-sensitive transcription factors by calorie restriction during aging. *Mech Ageing Dev* **123**: 1589–95.

30. **Kim MK, Chung SW, Kim DH, Kim JM**, et al. 2000. Modulation of age-related NF-kappaB activation by dietary zingerone via MAPK pathway. *Exp Gerontol* **45**: 419–26.

31. **Poynter ME, Daynes RA**. 1998. Peroxisome proliferator-activated receptor alpha activation modulates cellular redox status, represses nuclear factor-kappaB signaling, and reduces inflammatory cytokine production in aging. *J Biol Chem* **273**: 32833–41.

32. **Rosenstiel P, Derer S, Till A, Hasler R**, et al. 2008. Systematic expression profiling of innate immune genes defines a complex pattern of immunosenescence in peripheral and intestinal leukocytes. *Genes Immun* **9**: 103–14.

33. **Salminen A, Huuskonen J, Ojala J, Kauppinen A**, et al. 2008. Activation of innate immunity system during aging: NF-kB signaling is the molecular culprit of inflamm-aging. *Ageing Res Rev* **7**: 83–105.

34. **Spencer NF, Poynter ME, Im SY, Daynes RA**. 1997. Constitutive activation of NF-kappa B in an animal model of aging. *Int Immunol* **9**: 1581–8.

35. **Toliver-Kinsky T, Papaconstantinou J, Perez-Polo JR**. 1997. Age-associated alterations in hippocampal and basal forebrain nuclear factor kappa B activity. *J Neurosci Res* **48**: 580–7.

36. **Walter R, Sierra F**. 1998. Changes in hepatic DNA binding proteins as a function of age in rats. *J Gerontol A Biol Sci Med Sci* **53**: B102–10.

37. **Heffner KL**. 2011. Neuroendocrine effects of stress on immunity in the elderly: implications for inflammatory disease. *Immunol Allergy Clin North Am* **31**: 95–108.

38. **Jones DL, Rando TA**. 2011. Emerging models and paradigms for stem cell ageing. *Nat Cell Biol* **13**: 506–12.

39. **Katic M, Kahn CR**. 2005. The role of insulin and IGF-1 signaling in longevity. *Cell Mol Life Sci* **62**: 320–43.

40. **Gago N, Perez-Lopez V, Sanz-Jaka JP, Cormenzana P**, et al. 2009. Age-dependent depletion of human skin-derived progenitor cells. *Stem Cells* **27**: 1164–72.

41. **Rossi DJ, Jamieson CH, Weissman IL**. 2008. Stems cells and the pathways to aging and cancer. *Cell* **132**: 681–96.

42. **Maslov AY, Barone TA, Plunkett RJ, Pruitt SC**. 2004. Neural stem cell detection, characterization, and age-related changes in the subventricular zone of mice. *J Neurosci* **24**: 1726–33.

43. **Molofsky AV, Slutsky SG, Joseph NM, He S**, et al. 2006. Increasing p16INK4a expression decreases forebrain progenitors and neurogenesis during ageing. *Nature* **443**: 448–52.

44. **Cerletti M, Shadrach JL, Jurga S, Sherwood R**, et al. 2008. Regulation and function of skeletal muscle stem cells. *Cold Spring Harb Symp Quant Biol* **73**: 317–22.

45. **Burke B, Stewart CL**. 2006. The laminopathies: the functional architecture of the nucleus and its contribution to disease. *Annu Rev Genomics Hum Genet* **7**: 369–405.

46. **Capell BC, Collins FS**. 2006. Human laminopathies: nuclei gone genetically awry. *Nat Rev Genet* **7**: 940–52.

47. **De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C**, et al. 2003. Lamin a truncation in Hutchinson–Gilford progeria. *Science* **300**: 2055.

48. **Varga R, Eriksson M, Erdos MR, Olive M**, et al. 2006. Progressive vascular smooth muscle cell defects in a mouse model of Hutchinson–Gilford progeria syndrome. *Proc Natl Acad Sci USA* **103**: 3250–5.

49. **Zhang J, Lian Q, Zhu G, Zhou F**, et al. 2011. A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects. *Cell Stem Cell* **8**: 31–45.

50. **Halaschek-Wiener J, Brooks-Wilson A**. 2007. Progeria of stem cells: stem cell exhaustion in Hutchinson–Gilford progeria syndrome. *J Gerontol A Biol Sci Med Sci* **62**: 3–8.

51. **Scaffidi P, Misteli T**. 2008. Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat Cell Biol* **10**: 452–9.

52. **Krisko A, Radman M**. 2010. Protein damage and death by radiation in *Escherichia coli* and *Deinococcus radiodurans*. *Proc Natl Acad Sci USA* **107**: 14373–7.

53. **Krisko A, Leroy M, Radman M, Meselson M**. 2012. Extreme anti-oxidant protection against ionizing radiation in bdelloid rotifers. *Proc Natl Acad Sci USA* **109**: 2354–7.

54. **Krisko A, Radman M**. 2013. Biology of extreme radiation resistance: the way of *Deinococcus radiodurans*. *Cold Spring Harb Perspect Biol* **5**: pii: a012765.

55. **Charlesworth B**. 2000. Fisher, Medawar, Hamilton and the Evolution of Aging. *Genetics* **156**: 927–31.

56. **Kirkwood TB, Holliday R**. 1979. The evolution of ageing and longevity. *Proc R Soc Lond B Biol* **205**: 531–46.

57. **Deweerdt S**. 2012. Comparative biology: looking for a master switch. *Nature* **492**: 10–1.

58. **Harper JM, Salmon AB, Leiser SF, Galecki AT**, et al. 2007. Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. *Aging Cell* **6**: 1–13.

59. **Longo VD**. 2003. The Ras and Sch9 pathways regulate stress resistance and longevity. *Exp Gerontol* **38**: 807–11.

60. **Santos J, Leão C, Sousa MJ**. 2012. Growth culture conditions and nutrient signaling modulating yeast chronological longevity. *Oxid Med Cell Longev* **2012**: 680304.

61. **Pallauf K, Bendall JK, Scheiermann C, Watschinger K**, et al. 2013. Vitamin C and lifespan in model organisms. *Food Chem Toxicol* **58**: 255–63.

62. **Sarup P, Pedersen SM, Nielsen NC, Malmendal A**, et al. 2012. The metabolic profile of long-lived *Drosophila melanogaster*. *PLoS One* **7**: e47461.

63. **Sun J, Tower J**. 1999. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol Cell Biol* **19**: 216–8.

64. **Kenyon C, Chang J, Gensch E, Rudner A**, et al. 1993. A *C. elegans* mutant that lives twice as long as wild type. *Nature* **366**: 461–4.

65. **McCormick MA, Tsai SY, Kennedy BK**. 2011. TOR and ageing: a complex pathway for a complex process. *Philos Trans R Soc Lond B Biol Sci* **366**: 17–27.

66. **Tissenbaum HA**. 2012. Genetics, life span, health span, and the aging process in *Caenorhabditis elegans*. *J Gerontol A Biol Sci Med Sci* **67**: 503–10.

67. **Brown-Borg HM, Borg KE, Meliska CJ, Bartke A**. 1996. Dwarf mice and the ageing process. *Nature* **384**: 33.

68. **Ikeno Y, Hubbard GB, Lee S, Dube SM**, et al. 2013. Calorie restriction and dwarf mice in gerontological research. *Pathobiol Aging Age Relat Dis*, doi: 10.3402/pba.v3i0.20833.

69. **McKee Alderman J, DePetrillo MA, Gluesenkamp AM, Hartley AC**, et al. 2010. Calorie restriction and dwarf mice in gerontological research. *Gerontology* **56**: 404–49.

70. **Baudisch A, Vaupel JW**. 2012. Evolution. Getting to the root of aging. *Science* **6107**: 618–9.

71. **Elez M, Murray AW, Bi LJ, Zhang XE**, et al. 2010. Seeing mutations in living cells. *Curr Biol* **20**: 1432–7.

72. **Martinez DE**. 1998. Mortality patterns suggest lack of senescence in hydra. *Exp Gerontol* **33**: 217–25.

73. **Trembley A**. 1744. Memoires pour servir a l'histoire d'un genre de polypes d'eau douce, a bras en forme de cornes.

74. **Bosch TCG**. 2009. Hydra and the evolution of stem cells. *BioEssays* **31**: 478–86.

75. **Bosch TCG, Anton-Erxleben F, Hemmrich G, Khalturin K**. 2010. The Hydra polyp: nothing but an active stem cell community. Review. *Dev Growth Differ* **52**: 15–25.

76. **Yoshida K, Fujisawa T, Hwang JS, Ikeo K**, et al. 2006. Degeneration after sexual differentiation in hydra and its relevance to the evolution of aging. *Gene* **385**: 64–70.

77. **Wittlieb J, Khalturin K, Lohmann JU, Anton-Erxleben F**, et al. 2006. Transgenic Hydra allow in vivo tracking of individual stem cells during morphogenesis. *Proc Natl Acad Sci USA* **103**: 6208–11.

78. **Khalturin K, Anton-Erxleben F, Milde S, Plotz C**, et al. 2007. Transgenic stem cells in Hydra reveal an early evolutionary origin for key elements controlling self-renewal and differentiation. *Dev Biol* **309**: 32–44.

79. **Bosch TCG** (Ed.). 2008. *Stem Cells in Immortal Hydra. In Stem Cells: from Hydra to Man*. Netherlands: Springer, pp 37–58.

80. **David CN**. 2012. Interstitial stem cells in Hydra: multipotency and decision-making. *Int J Dev Biol* **56**: 489–97.

81. **Hobmayer B, Jenewein M, Eder D, Eder MK**, et al. 2012. Stemness in Hydra – a current perspective. *Int J Dev Biol* **56**: 509–17.

82. **Watanabe H, Hoang VT, Mattner R, Holstein TW**. 2009. Immortality and the base of multicellular life: lessons from cnidarian stem cells. *Semin Cell Dev Biol* **20**: 1114–25.

83. **Broun M, Gee L, Reinhardt B, Bode HR**. 2005. Formation of the head organizer in Hydra involves the canonical Wnt pathway. *Development* **132**: 2907–16.

84. **Gee L, Hartig J, Law L, Wittlieb J**, et al. 2010. Beta-catenin plays a central role in setting up the head organizer in Hydra. *Dev Biol* **340**: 116–24.

85. **Hobmayer B, Rentzsch F, Kuhn K, Happel CM**, et al. 2000. WNT signalling molecules act in axis formation in the diploblastic metazoan Hydra. *Nature* **407**: 186–9.

86. **Nakamura Y, Tsiaris CD, Özbek S, Holstein TW**. 2011. Autoregulatory and repressive inputs localize Hydra Wnt3 to the head organizer. *Proc Natl Acad Sci USA* **108**: 9137–42.

87. **Philipp I, Aufschnaiter R, Ozbek S, Pontasch S**, et al. 2009. Wnt/beta-catenin and noncanonical Wnt signaling interact in tissue evagination in the simple eumetazoan Hydra. *Proc Natl Acad Sci USA* **106**: 4290–5.

88. **Wend P, Holland JD, Ziebold U, Birchmeier W**. 2010. Wnt signaling in stem and cancer stem cells. *Semin Cell Dev Biol* **21**: 855–86.

89. **Ambrosone A, Marchesano V, Tino A, Hobmayer B**, et al. 2012. Hymyc1 downregulation promotes stem cell proliferation. *PLoS One* **7**: E30660.

90. **Hartl M, Mitterstiller AM, Valovka T, Breuker K**, et al. 2010. Stem cell-specific activation of an ancestral Myc proto-oncogene with conserved basic functions in the early metazoan Hydra. *Proc Natl Acad Sci USA* **107**: 4051–6.

91. **Barve A, Ghaskadbi S, Ghaskadbi S**. 2013. Conservation of the nucleotide excision repair pathway: characterization of Hydra Xeroderma Pigmentosum Group F Homolog. *PLoS One* **8**: E61062.

92. **Hemmrich G, Bosch TCG**. 2008. Compagen, a comparative genomics platform for early branching metazoan animals, reveals early origins of genes regulating stem-cell differentiation. *BioEssays* **30**: 1010–8.

93. **Chapman JA, Kirkness EF, Simakov O, Hampson SE**, et al. 2010. The dynamic genome of Hydra. *Nature* **464**: 592–6.

94. **Hemmrich G, Khalturin K, Boehm AM, Puchert M**, et al. 2012. Molecular signatures of the three stem cell lineages in Hydra and the emergence of stem cell function at the base of multicellularity. *Mol Biol Evol* **29**: 3267–80.

95. **Magie CR, Pang K, Martindale MQ**. 2005. Genomic inventory and expression of Sox and Fox genes in the cnidarian *Nematostella vectensis*. *Dev Genes Evol* **215**: 618–30.

96. **Chevalier S, Martin A, Leclere L, Amiel A**, et al. 2006. Polarised expression of FoxB and FoxQ2 genes during development of the hydrozoan *Clytia hemisphaerica*. *Dev Genes Evol* **216**: 709–20.

97. **Bridge D, Theofiles AG, Holler RL, Marcinkevicius E**, et al. 2010. Foxo and stress responses in the cnidarian *Hydra vulgaris*. *PLoS One* **5**: E11686.

98. **Lasi M, David CN, Bottger A**. 2010. Apoptosis in pre-Bilaterians: Hydra as a model. *Apoptosis* **15**: 269–78.

99. **Boehm AM, Khalturin K, Anton-Erxleben F, Hemmrich G**, et al. 2012. FoxO is a critical regulator of stem cell maintenance in immortal Hydra. *Proc Natl Acad Sci USA* **109**: 19697–702.

100. **Bevins CL, Salzman NH**. 2011. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol* **9**: 356–68.

101. **Zhang X, Yalcin S, Lee DF, Yeh TY**, et al. 2011. FOXO1 is an essential regulator of pluripotency in human embryonic stem cells. *Nat Cell Biol* **13**: 1092–9.

102. **Renault VM, Rafalski VA, Morgan AA, Salih DA**, et al. 2009. FoxO3 regulates neural stem cell homeostasis. *Cell Stem Cell* **5**: 527–39.

103. **Naka K, Hoshii T, Muraguchi T, Tadokoro Y**, et al. 2011. TGF- beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. *Nature* **463**: 676–80.

104. **Miyamoto K, Araki KY, Naka K, Arai F**, et al. 2007. Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell* **1**: 101–12.

105. **Lin L, Hron JD, Peng SL**. 2004. Regulation of NF-kappaB, Th activation, and autoinflammation by the forkhead transcription factor Foxo3a. *Immunity* **21**: 203–13.

106. **Snoeks L, Weber CR, Wasland K, Turner JR**, et al. 2009. Tumor suppressor FOXO3 participates in the regulation of intestinal inflammation. *Lab Invest* **89**: 1053–62.

107. **Evans EA, Chen WC, Tan MW**. 2008. The DAF-2 insulin-like signaling pathway independently regulates aging and immunity in *C. elegans*. *Aging Cell* **7**: 879–93.

108. **Murphy CT, McCarroll SA, Bargmann CI, Fraser A**, et al. 2003. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* **424**: 277–83.

109. **Wang J, Nakad R, Schulenburg H**. 2012. Activation of the *Caenorhabditis elegans* FOXO family transcription factor DAF-16 by pathogenic *Bacillus thuringiensis*. *Dev Comp Immunol* **37**: 193–201.

110. **Franceschi C, Capri M, Monti D, Giunta S**, et al. 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev* **128**: 92–105.

111. **Passeri G, Pini G, Troiano L, Vescovini R**, et al. 2003. Low vitamin D status, high bone turnover, and bone fractures in centenarians. *J Clin Endocrinol Metab* **88**: 5109–15.

112. **Coppola R, Mari D, Lattuada A, Franceschi C**. 2003. Von Willebrand factor in Italian centenarians. *Haematologica* **88**: 39–43.

113. **Mari D, Mannucci PM, Coppola R, Bottasso B**, et al. 1995. Hypercoagulability in centenarians: the paradox of successful aging. *Blood* **85**: 3144–9.

114. **Franceschi C, Bonafe M**. 2003. Centenarians as a model for healthy aging. *Biochem Soc Trans* **31**: 457–61.

115. **Tatar M, Kopelman A, Epstein D, Tu MP**, et al. 2001. A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**: 107–10.

116. **Partridge L, Alic N, Bjedov I, Piper MD**. 2011. Ageing in Drosophila: the role of the insulin/Igf and TOR signalling network. *Exp Gerontol* **46**: 376–81.

117. **Wei Y, Zhang YJ, Cai Y**. 2013. Growth or longevity: the TOR's decision on lifespan regulation. *Biogerontology* **14**: 353–63.

118. **Partridge L, Mangel M**. 1999. Messages from mortality: the evolution of death rates in the old. *Trends Ecol Evol* **14**: 438–42.

119. **Lopez-Otin C, Blasco MA, Partridge L, Serrano M**, et al. 2013. The hallmarks of aging. *Cell* **153**: 1194–217.

120. *Dorland's Medical Dictionary for Health Consumers*. 2007. Philadelphia: Saunders, Elsevier, Inc.

**Problems & Paradigms**