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Hydra, the everlasting embryo, confronts aging

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ABSTRACT Existing data imply that the cnidarian *Hydra vulgaris* does not undergo senescence. In contrast, the related species *Hydra oligactis* shows increased mortality and physiological deterioration following sexual reproduction. *Hydra* thus offers the chance to study a striking difference in lifespan in members of the same genus. Adult *Hydra* possess three well-characterized stem cell populations, one of which gives rise to both somatic cells and gametes. The lack of senescence in *Hydra vulgaris* raises the question of how these stem cell populations are maintained over long periods of time. Investigation of the roles in *Hydra* of proteins involved in cellular stress responses in other organisms should provide insight into this issue. Proteins of particular interest include the Hsp70 family proteins and the transcription factor FoxO.

KEY WORDS: *germline, stem cell, heat shock, FoxO, insulin*

"Unlike most metazoa,
Hydra are not subject to death from old age."
W.F. Loomis and H.M. Lenhoff (1956)

Negligible and inducible senescence in *Hydra*

The evolution of multicellularity made possible cell specialization and the evolution of somatic differentiation to form tissue and organs, and ultimately a soma. More than one hundred years ago August Weismann argued that animals lost the "power of unending life" (Weismann 1883) typical of unicellular forms, because of the evolution of division of labor between their cells. Weismann distinguished between the reproductive cells endowed with "molecular" determinants, the germ plasm (Weismann 1885), which rendered them capable of building a new individual; and somatic cells, which differentiate to perform body functions and lost their "heredity" power. The multicellular metazoan body became the vehicle for the life-bearing germ cells and could be disposed of once the germ plasm had been passed down into the next generation.

Only recently we have begun to elucidate the molecular mechanisms that explain Weismann's proposed distinction between somatic and germline cells and to understand their roles in the process of aging. For example, Curran *et al.* (2009) presented evidence that somatic cells of *Caenorhabditis elegans* mutants with increased longevity can express gene programs that are normally limited to the germ line. The authors proposed that the transformation of the somatic cells to a more germline-like state increases genome stability and contributes to the lifespan extension in these mutants. At the same time, germ cells can mediate accelerated aging in somatic tissue. Studies in *C. elegans* and *Drosophila melanogaster* have shown that germ-line ablation extends life span (Hsin and Kenyon 1999; Flatt *et al.,* 2008). Furthermore, overproliferation of germ cells shortens lifespan in *C. elegans* (Curran *et al.,* 2009).

Weismann's characterization of somatic and germ cells applies well to the classical animal models for the study of aging: *C. elegans*, *Drosophila*, and *Mus*. However, the description does not work for all metazoans. One example is the freshwater cnidarian *Hydra*. *Hydra* has a relatively simple bauplan: a cylindrical body with a head at one end and an adhesive basal disk at the other. The head has a dome-like structure with the mouth at its center, the hypostome, surrounded by a ring of tentacles. The *Hydra* body is formed by two epithelial layers (ectoderm and endoderm) separated by extracellular matrix. Ectodermal and endodermal epithelial cells in the body column are actively dividing multipotent stem cells. Body column ectodermal epithelial cells give rise to the differentiated ectodermal cells of the tentacles and basal disk, while body column endodermal epidermal cells give rise to differentiated endodermal cells of the tentacles and basal disk. As epithelial cells divide in the body column, cells are constantly displaced into the tentacles and basal disk, and differentiation constantly occurs (Fig. 1). The average time of residence of an ectodermal cell is four days in the tentacles and 20 days in the body column (Campbell 1967b, a). Dispersed among the epithelial cells is a third population of cells, the interstitial cells. Interstitial stem cells give rise to nerve cells, nematocytes (stinging cells), secretory cells, and gametes (Bode 1996; see also in this issue David 2012; Hobmayer *et al.,* 2012;

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Nishimiya-Fujisawa 2012). Since individual interstitial cells have been shown to give rise to both somatic cells and gametes (Bosch and David 1987), *Hydra* do not possess a germline distinct from the soma, even as adults.

Not only do the interstitial stem cells give rise to both gametes and somatic cells, but, unlike the somatic cells of most animals, *Hydra* epithelial stem cells have the ability both to divide endlessly and to differentiate into several cell types. A well-fed *Hydra* has an epithelial cell turnover time of 3 to 4 days (David and Campbell 1972), so that an individual *Hydra* could have all its epithelial cells replaced within a week (Martínez 2002). This unique ability to discard and replace older cells, and to rejuvenate its soma has led scientists to propose the lack of senescence in *Hydra*—an idea championed by Brien (1953) who reported keeping individual *Hydra* alive for five years without observing any signs of aging or reduction in budding rates.

Existing data suggest that *Hydra vulgaris* does not show senescence (Martínez 1998). Individual *Hydra* maintained in the lab for a period of four years showed age-specific mortality rates of zero or close to zero, suggesting the absence of senescence or at least negligible senescence (Fig. 2). During this period, individual *Hydra* continued to reproduce both asexually and sexually. At the end of four years the experiment was stopped when almost all of the *Hydra* were still alive and reproducing. Four years does not seem a very long time on a human scale but for an animal the size of *Hydra*, which can begin sexual reproduction days after birth, four years is a long time. The question is: should we expect to see significant mortality in four years? In animals there is a positive correlation between age of first reproduction and maximum longevity. Animals the size of *Hydra* that start reproducing a few days after birth show maximum longevities of a few months but not years. One would expect to see significant levels of mortality by four years but we did not. Given that the mortality rate has remained extremely low for four years, the maximum longevity of *Hydra* will certainly be much more than four years, an extremely long time for an animal of that size.

In contrast to *H. vulgaris*, another species of *Hydra*, *H. oligactis,* shows increased mortality and physiological deterioration resembling aging following sexual reproduction (Brien 1953; Noda 1982; Yoshida *et al.,* 2006). *H. oligactis* cultures propagated by asexual reproduction can be maintained in the lab for years. If sexual reproduction is induced, however, individuals show clear signs of physiological deterioration and senescence (Fig. 2). Noda (1982) reports that females of *H. oligactis* transferred from 18ºC to 10ºC became sexual within 22 days and died within 90 days (Fig. 2). Littlefield and collaborators (Littlefield *et al.,* 1985; Littlefield *et al.,* 1991) observed that after three to four weeks of incubation at 10ºC, *H. oligactis* produce differentiated gametes (Fig. 3). In asexually reproducing *H. oligactis* maintained at 18ºC, interstitial stem cells enter the gamete differentiation pathways but fail to produce gametes (Littlefield *et al.,* 1985; Littlefield and Bode 1986), potentially because sperm lineage cells undergo cell death at that temperature (Littlefield *et al.,* 1985; Littlefield *et al.,* 1991). Thus, in *H. oligactis* the presence of gametes is an obvious

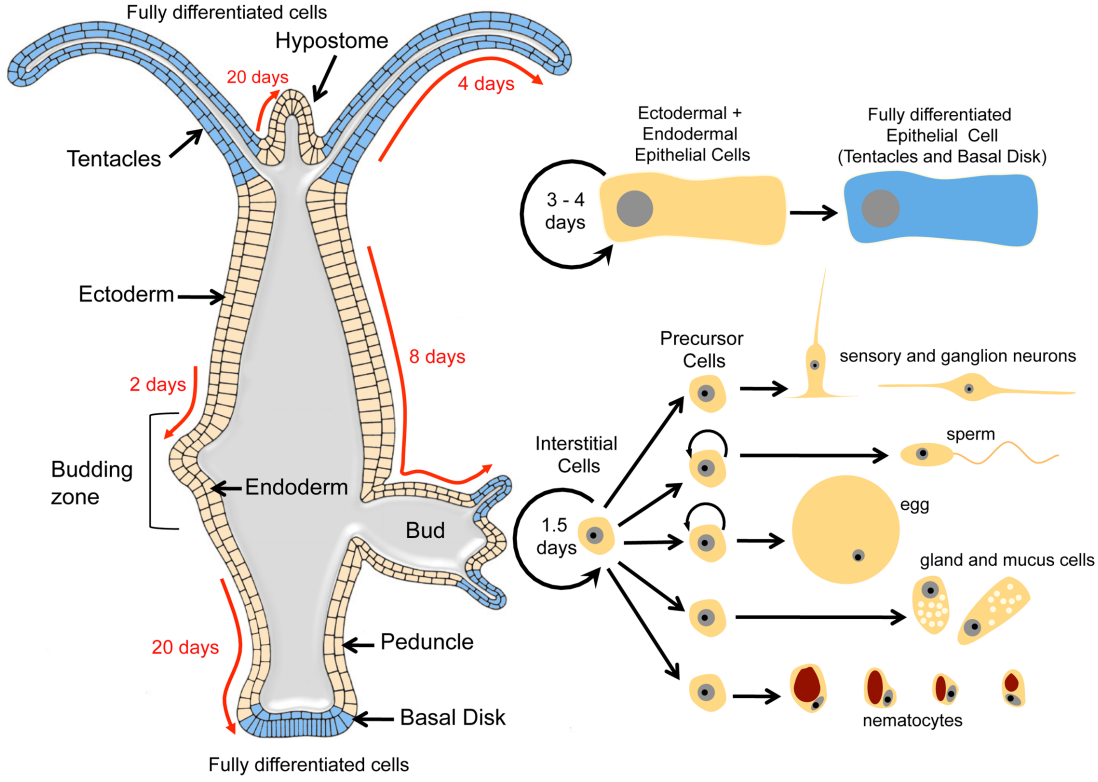


Fig. 1. Cell dynamics in an adult *Hydra*. *Cells divide in the body column, and cell division displaces cells towards the ends of the animal and into buds. Ectodermal and endodermal epithelial cells in the body column give rise to the differentiated ectodermal and endodermal epithelial cells of the tentacles and basal disk. Interstitial stem cells give rise to four classes of differentiated cells. Arrows indicate experimentally determined travelling times of epithelial cells (Campbell 1967b).*

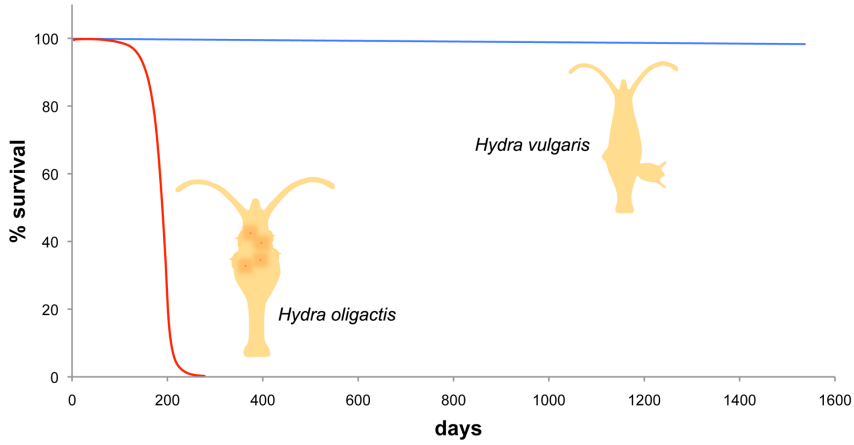


Fig. 2. Survivorship curves for *Hydra vulgaris* and *Hydra oligactis*. *Curves redrawn from Martínez 1998 and Yoshida* et al., *2006. Time 0 for* Hydra vulgaris *represents the time asexually produced polyps separated from the parents. Individual polyps were then followed for a period of four years or until death.* Hydra vulgaris *showed negligible mortality for a period of four years. During that period polyps continue to reproduce asexually and many also produced testes and eggs. Time 0 for* H. oligactis *represents the time of induction to sexual reproduction by transfer into 10ºC. Males developed testes within 3 weeks after temperature induction and females formed eggs within 4 weeks. Both males and females stopped budding upon sexual induction. Mortality was relatively low for the first 60 days but increase significantly thereafter.*

difference between individuals that undergo senescence (when induced to reproduce sexually) and non-sexually-reproducing individuals that do not.

The induction of sexual reproduction and aging has also been observed in two species that are closely related to *H. oligactis*, the North American species *Hydra canadensis (*formerly *Hydra pseudoligactis)* and the European species *Hydra oxycnida* (formerly *Hydra pirardi*), (Burnett and Diehl 1964). A recent molecular phylogeny indicates that these three species form a monophyletic group (known as the Oligactis group) (Schulze 1917; Semal-van Gansen 1954; Campbell 1987) characterized morphologically by their smooth spherical embryotheca and the uniform size of their stenotele nematocysts (Martínez *et al.,* 2010)(Fig. 4). Other species of *Hydra* can be induced to become sexual in laboratory conditions by lowering the culture temperature. For example, *Hydra hymanae* reproduces asexually at 24ºC but becomes sexual 12-35 days after transfer from 24ºC to 15ºC (Davison 1976). *H. hymanae* is hermaphroditic so the sexual state involves the development of both testes (and sperm) and eggs (testes appear earlier than eggs). No clear evidence of inducible aging has been reported for *H. hymanae* although Davison (1976) indicates that the sexual state can be maintained for at least three months and that size and reproductive vigor gradually decrease during this time. The order of divergence of species of *Hydra* (cf. Martínez *et al.,* 2010) suggests that gonochorism (dioecy) may be a derived feature restricted to the Oligactis and the Vulgaris taxonomic groups (Fig. 4). The two most basal groups of *Hydra*, namely Viridissina and Braueri, include hermaphroditic species. Induction of sexual reproduction at low temperatures seems to be restricted to the Brauri and Oligactis groups. Thus, temperature induction of sexual reproduction does not seem to be linked to the actual mode of sexual reproduction (hermaphoditic vs gonochoric). Members of the Oligactis and the Braueri groups are known to thrive in cold water and the induction of sexual reproduction at low temperatures is presumably an adaptation for overwintering (eggs may be able to survive the winter months better than adults). Inducible aging has been only reported in members of the Oligactis group (Fig. 4). An ongoing study in our lab is testing for the presence of both temperature induction and inducible aging in *Hydra circumcincta* and *Hydra utahensis*, two species closely related to *H. hymanae.*

In light of the difference in lifespan between *H. oligactis* and *H. vulgaris*, another difference between the species is particularly intriguing. *H. oligactis* and *H. vulgaris* differ in their tolerance of thermal stress (Bosch *et al.,* 1988). Incubation of *H. oligactis* polyps at 33ºC for 60 minutes results in death and tissue disintegration. *H. vulgaris* polyps exposed to 33ºC for up to 90 minutes fully recover when returned to normal culture temperature (~18ºC). These conditions (33ºC for 90 minutes) cause synthesis of heat shock proteins in *Hydra vulgaris* (Bosch *et al.,* 1988). *H. oligactis,* however, does not produce detectable levels

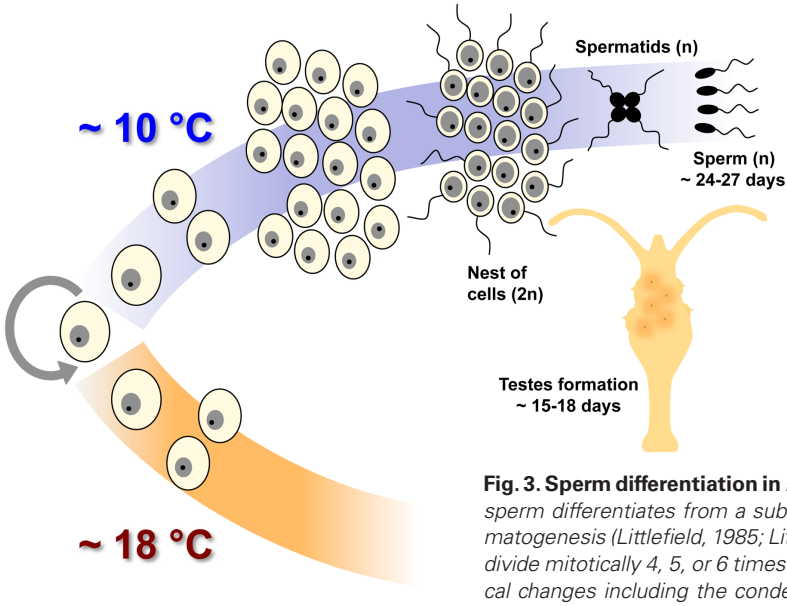


Fig. 3. Sperm differentiation in *Hydra oligactis* under two different temperatures. Hydra oligactis *sperm differentiates from a subpopulation of unipotent interstitial cells that are restricted to spermatogenesis (Littlefield, 1985; Littlefield* et al., *1985; Littlefield and Bode, 1986). These interstitial cells divide mitotically 4, 5, or 6 times to form nests of 16, 32, or 64 cells. Nest cells undergo morphological changes including the condensation of the nucleus, a reduction in size, and the development of flagella. At this point each cell undergoes a complete meiotic division to produce 4 sperm cells (Littlefield* et al., *1991). In both sexual and asexual* H. oligactis *males, cells of the sperm lineage are constantly entering the differentiation pathway to form spermatids and sperm. However, these cells are temperature sensitive and, at temperatures of 18ºC and above, die before fully differentiating. At permissive temperatures (e.g. 10°C) sperm precursors do not die and are able to complete spermatogenesis (Littlefield* et al., *1991).*

of new proteins in response to thermal stresses or other stresses which would be expected to trigger the heat shock response (Bosch *et al.,* 1988; Gellner *et al.,* 1992; Brennecke *et al.,* 1998). *H. oligactis* also produces far lower levels of hsp70 mRNA than *H. vulgaris* under such conditions (Brennecke *et al.,* 1998; Gellner *et al.,* 1992). Heat shock proteins play important roles in maintaining protein quality control in cells. The heat shock response declines with age (Kourtis and Tavernarakis 2011), and levels of misfolded proteins have been found to increase with age in *C. elegans* (Ben-Zvi *et al.,* 2009). Increased levels of heat shock proteins can act to increase lifespan in *C. elegans*, *Drosophila*, and vertebrates (reviewed in Calderwood *et al.,* 2009). The difference between the heat shock response in *H. oligactis* and *H. vulgaris* may well explain the difference in lifespan between the species. It provides a valuable opportunity to study the effects of a major difference in regulation of protein homeostasis.

Interestingly, the difference in heat tolerance between different *Hydra* species may explain their worldwide distribution. Of the four taxonomic groups of *Hydra* only two, the Viridissima and the Vulgaris groups, are present in all continents (with the exception of Antartica). Species of the other two groups (Braueri and Oligactis) have only been found in North America and Eurasia. It has been suggested that the origin and initial radiation of *Hydra* species took place in Laurasia and that only two groups were able to disperse into continents of the Southern Hemisphere due to their thermal/stress tolerance (Martínez *et al.,* 2010). Additional evidence for the presumably limited dispersal ability of *Hydra* of the Braueri and the Oligactis groups is provided by the observed pattern of suspected cases of anthropogenic dispersal. In all cases in which the distribution of a particular *Hydra* strain can be best explained by human introduction (e.g. *Hydra* inhabiting a particular geographic region but with clear affinities to non-endemic *Hydra* or *Hydra* inhabiting oceanic islands), the species involved belong to the Vulgaris or the Viridissima groups (Martínez *et al.,* 2010; Campbell *et al.,* manuscript in preparation).

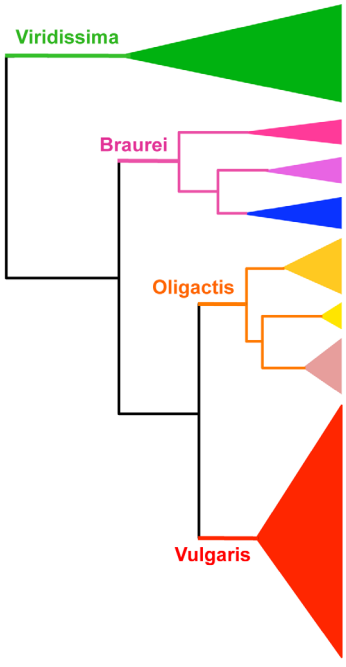
Unlike members of the Oligactis group, *H. vulgaris* show no signs of inducible senescence; sexually reproducing individuals can be maintained in the lab for many years. This system of two species within the same genus exhibiting two different modes of senescence—negligible in *H. vulgaris* and inducible in *H. oligactis*—offers a unique opportunity for aging research. Both species have been studied for many years in laboratories around the world, so we have a very good understanding of many aspects of their cellular, developmental, and reproductive biology.

Hydra vulgaris and *Hydra oligactis* as novel models for aging research

There are several reasons why *Hydra* species are potentially very interesting models for the study of aging. *Hydra* belong to the phylum Cnidaria, one of the earliest arising groups of animals with ectoderm, endoderm, and neurons. Evidence from EST projects suggests that, in spite of their early divergence from the rest of the animals, cnidarians share with mammals many genes that are not present in the genomes of the traditional invertebrate models for the study of aging, *Caenorhabidtis elegans* and *Drosophila melanogaster* (Kortschak *et al.,* 2003). Thus, *Hydra* represents a potential repository of longevity genes of relevance to humans that cannot be studied in worms or flies (Austad 2009).

Due to its regenerative capacity, *Hydra* can be subjected to a plethora of experimental manipulations that are not possible in most animals. *Hydra* can regenerate when cut in pieces. Cells or tissues from different *Hydra* can be combined in several ways. Tissue from one individual can be grafted onto another (MacWilliams 1983; see also in this issue Shimizu 2012). *Hydra* epithelial tissue layers (ectoderm and endoderm) can be separated from each other intact and ectoderm and endoderm from different animals can be combined (Lesh-Laurie 1983). Finally, *Hydra* cells can be dissociated and then centrifuged to form a mass which is able reorganize itself into normal *Hydra* (Flick and Bode 1983). These manipulations allow combination of cells from different strains, from different transgenic lines, or from *Hydra* which have been subject to different experimental treatments.

Key additional experimental



Species	Sexual Reproduction	Temperature Induction	Inducible Aging
H. viridissima	Hermaphroditic	NO	NO
H. hymanae	Hermaphroditic	YES	?
H. circumcincta	Hermaphroditic	?	?
H. utahensis	Hermaphroditic	?	?
H. oligactis	Gonochoristic	YES	YES
H. oxycnida	Gonochoristic	YES	YES
H. canadensis	Gonochoristic	YES	YES
H. vulgaris	Gonochoristic (sex reversal)	NO	NO

Fig. 4. Phylogenetic relationships between different species of *Hydra* and notes on modes of sexual reproduction and inducible aging. *The Temperature Induction column indicates whether or not a* Hydra *species can be induced to become sexually mature by incubation at low temperatures (usually around 10°C). The Inducible Aging column indicates whether or not polyps show signs of physiological deterioration after becoming sexually mature.*

techniques and resources have been developed for *Hydra* more recently. Stably transgenic strains of *H. vulgaris* can be produced by microinjection of plasmid DNA into embryos (Wittlieb *et al.,* 2006), followed by asexual reproduction of transgenic animals. Transgenic lines can be maintained indefinitely. The genome of *Hydra magnipapillata* has been sequenced (Chapman *et al.,* 2010). It should be noted that *H. magnipapillata* is the name used in Japan for the species that in Europe has been called *H. vulgaris*. Morphological studies (Campbell *pers. com.)* and a molecular phylogeny (Martínez *et al.,* 2010) suggest that *H. magnipapillata* and *H. vulgaris* represent a single species that should be called *H. vulgaris* (Pallas).

Differences in genetic background can confound studies of lifespan (Partridge and Gems 2007; Burnett *et al.,* 2011). Because *Hydra* routinely reproduce asexually, they offer the advantage that experiments can be conducted with individuals which are genetically identical. However, when transgenic animals are produced, plasmid DNA presumably integrates into the *Hydra* genome at random locations, potentially causing position effects. For future work, it will be important to develop additional resources for use in producing transgenic lines. Inducible promoters would allow comparison of phenotypes of genetically identical transgenic animals. Strains with landing sites for recombination would allow comparison of transgenic lines with DNA inserted at a known location.

As indicated before, studies have shown that germ-line ablation in *C. elegans* and *Drosophila* extends life span (Hsin and Kenyon 1999; Flatt *et al.,* 2008). Cell-composition manipulations possible in *Hydra* should facilitate investigation of the effects of gametes and other interstitial lineage cells on *Hydra* lifespan. Both *H. vulgaris* and *H. oligactis* can be treated with colchicine (Marcum and Campbell 1978; Marcum and Campbell, 1983) or hydroxyurea (Sacks and Davis 1979; Bode 1983) to generate animals, known as "epithelial *Hydra*", that are devoid of interstitial stem cells and their derivatives. Epithelial *Hydra* look normal to the naked eye and can be maintained in the lab for long periods of time. Special care is required only because the animals lack nematocysts and neurons; they cannot catch prey and must be force-fed (Marcum 1983). Epithelial *Hydra* are unable to produce gametes, even under conditions that would normally induce sexual reproduction. Treatment with hydroxyurea can also be used to generate animals which can which produce gametes but no somatic interstitial lineage cells. Such "pseudoepithelial" *Hydra* provide evidence that interstitial cells include gamete-restricted stem cells as well as multipotent stem cells (Littlefield 1985; Nishimiya-Fujisawa and Sugiyama 1993; see also in this issue Nishimiya-Fujisawa 2012). The alterations of cell composition possible in *Hydra* represent useful tools for dissecting the roles played by specific cell types, including gametes, in *Hydra* aging.

FoxO, insulin and aging in *Hydra vulgaris*

The discovery of a conserved set of cellular pathways involved in the modulation of aging in animals has opened the door for comparative studies of aging mechanisms. FoxO proteins sit at the core of these cellular pathways, regulating the response to a variety of environmental signals. FoxO proteins are transcription factors involved in several cellular processes including apoptosis, the cell cycle, DNA damage repair, oxidative stress, cell differentiation, and glucose metabolism (Huang and Tindall 2007). While mammals posses four distinct FoxO genes *(FoxO1, FoxO3, FoxO4,* and *FoxO6)* only one ortholog has been found in *Drosophila melanogaster (dFoxO)*, and in *C. elegans* (*daf-16).* FoxO proteins have been shown to prolong lifespan in *Drosophila* and *C. elegans* by promoting resistance to oxidative stress and pathogens, and protecting protein structure from damage (Carter and Brunet 2007). Post-translational modifications (e.g. phosphorylation, ubiquitylation and acetylation) tightly regulate the function of FoxO proteins. Two main pathways that affect nuclear localization of FoxO proteins and consequently their function as transcription factors are the insulin/IGF1 and the c-Jun N-terminal kinase (JNK) pathways (Fig. 5A). In response to growth factors like insulin and IGF-1, Akt and the related serum- and glucocorticoid-inducible kinase (SGK) phosphorylate FoxO proteins. This promotes FoxO binding to 14-3-3 proteins and localization of FoxO to the cytoplasm (Huang and Tindall, 2007). In contrast, in response to oxidative stress FoxO proteins translocate into the nucleus in a process that is directly and indirectly regulated by the c-Jun N-terminal kinase (JNK) pathway (Essers *et al.,* 2004; Oh *et al.,* 2005; Wang *et al.,* 2005).

FoxO and several components of the insulin/IGF-1 and the JNK pathways have been identified in *Hydra*. Furthermore, studies have shown that *Hydra* FoxO activity may be regulated in a manner similar to the one observed in *C. elegans, D. melanogaster* and mammals. A single copy of *FoxO* has been identified from the *H. magnipapillata* genome (Bridge *et al.,* 2010). A single *FoxO* ortholog has also been found in another cnidarian species, *Clytia hemisphaerica* (Chevalier *et al*., 2006). An additional cnidarian, *Nematostella vectensis*, has two FoxO genes, one of which may be non-functional (Magie *et al.,* 2005; Chevalier *et al.,* 2006). *Hydra* FoxO predicted structure shows the regular features of FoxO proteins, including the FKH (forkhead) domain and the nuclear localization signal (NLS)—represented by segment of basic amino acids overlapping the end of the FKH domain (Lange *et al.,* 2007). Like other FoxO proteins, *Hydra* FoxO also contains three consensus Akt phosphorylation sites both upstream and downstream of the FKH domain (Alessi *et al.,* 1996; Lin *et al.,* 2001; Burgering and Kops 2002; Jacobs *et al.,* 2003; Junger *et al.,* 2003; Puig *et al.,* 2003). *In situ* hybridization provided evidence that *H. vulgaris FoxO* is expressed in multipotent interstitial stem cells. Interstitial stem cells are the most rapidly dividing of the stem cells present in adult *Hydra* and give rise to gametes as well as some somatic cell types (Fig. 1). FoxO could potentially decrease damage to these cells over time and thus play a key role in the maintenance of the germline. No expression was detected in testes, where proliferation of spermatogonia and their differentiation to produce mature sperm take place (Miller *et al.,* 2000). In oogenesis, nurse cells within an egg field transfer cytoplasm to the developing oocyte, undergo apoptosis, and are phagocytosed by the oocyte (Alexandrova *et al.,* 2005). Low levels of expression were found in developing oocytes. Following oogenesis, the former egg field is depleted of *FoxO*-expressing cells (Bridge *et al.,* 2010).

What do we know about the insulin pathway in Hydra*?*

Three insulin-like peptide genes (Böttger *et al.,* 2006) and a putative insulin/IGF-1 receptor gene (Steele *et al.,* 1996) are present in *H. vulgaris*. High levels of expression of the receptor gene, *HTK7*, are detected in ectodermal epithelial cells at the transition zones at the bases of the tentacles and above the foot basal disk. Cells displaced through those regions differentiate into battery

cells of the tentacles or the squamous cells of the basal disk. A lower level of *HTK7* expression is detected in body column ectodermal cells. Two 14-3-3 genes from *Hydra vulgaris* have also been characterized; mRNA and proteins corresponding to these genes are present in all cell types. Localization of these 14-3-3 proteins within epithelial cells is responsive to starvation, with nuclear localization observed in more cells in starved animals (Pauly *et al.,* 2007).

Hydra FoxO is negatively regulated by insulin/IGF1 signaling

Two independent lines of evidence suggest that the transcriptional activity of *Hydra* FoxO is negatively regulated by insulin/IGF1 signaling. First, when a *Hydra* FoxO-GFP construct is introduced into epithelial cells using a particle gun, 20-60% of the cells expressing FoxO-GFP undergo apoptosis (Lasi *et al.,* 2010). The percentage of apoptotic cells, however, is significantly reduced (5-15%) when the FoxO-GFP construct is co-expressed with the *Hydra* pro-insulin-1 gene (Lasi *et al.,* 2010). These results suggest a direct connection between insulin signaling, FoxO activity, and apoptosis in *Hydra*. Under conditions of variable food availability, apoptosis may be a key mechanism to maintain cellular homeostasis in *Hydra* (Lasi *et al.,* 2010; see also in this issue Reiter *et al.,* 2012). Second, treatment with the PI3K inhibitor LY294002 significantly increased the percentage of interstitial cell derivatives (neurons, nematoblasts) showing nuclear localization of a FoxO-GFP fusion protein (Bridge *et al.,* 2010) (Fig. 5B). This result provides evidence that insulin-IGF1 signaling, mediated by the PI3K/PKB/SGK pathway, negatively regulates FoxO nuclear localization and thus its function as a transcription factor in *Hydra*. FoxO regulation by insulin-like growth factors seems to be, perhaps not surprisingly, a mechanism that appeared early in the evolution of animals.

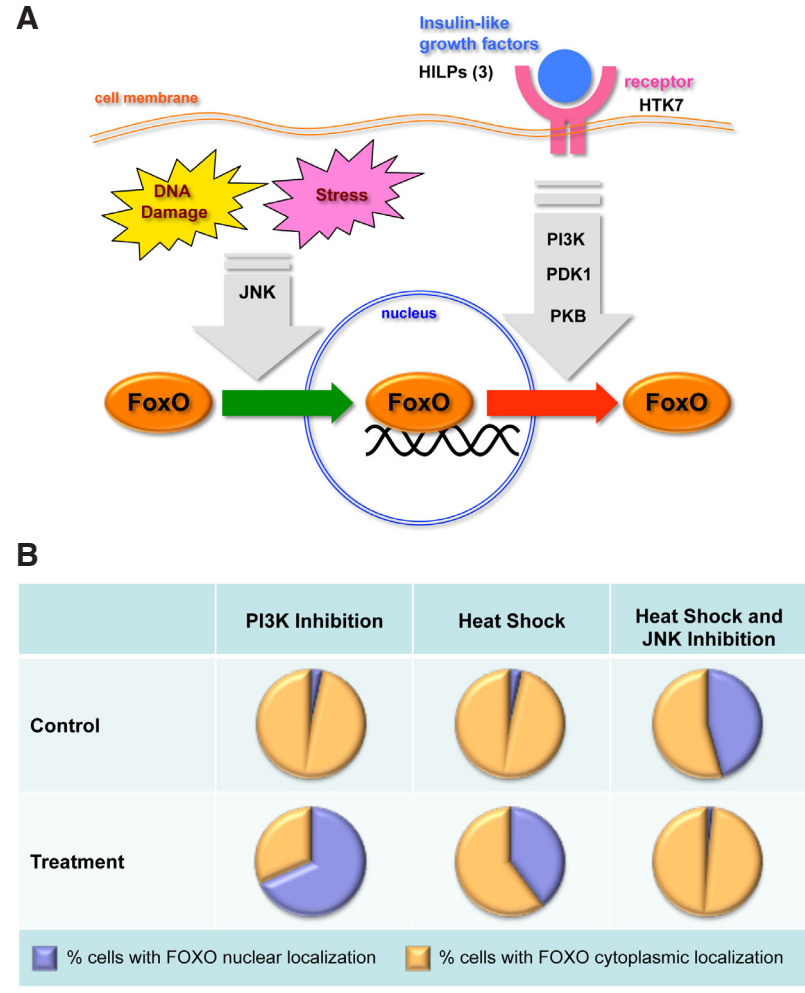


Fig. 5. Regulation of FoxO nuclear localization by the insulin/IGF1 and the JNK pathways. (A) *Insulin signaling results in the nuclear exclusion of FoxO protein. JNK signaling mediates the translocation of FoxO into the cell nucleus as a response to stress stimuli. Putative* Hydra *proteins: HILPs (*Hydra *Insulin-like Peptides, Böttger* et al., *2006), HTK7 (*Hydra *Tyrosine Kinase 7, Steele* et al., *1996), PDK1 (3-phosphoinositide-dependent protein kinase 1, GenBank Accession Number: XM_002157892), PI3K (Phosphoinositide 3-kinase, Manuel* et al., *2006), PKB (Protein Kinase B, Herold* et al., *2002).* **(B)** *Experimental manipulation of FoxO nuclear localization in* Hydra vulgaris *based on data from Bridge* et al., *(2010). Inhibition of insulin signaling by treatment with a PIK3 inhibitor results in an increase in the percentage of cells (stenotele nematocytes, nematoblasts, and ganglionic neurons) showing FoxO-GFP nuclear localization. Heat shock (90 min at 33ºC) results in an increase in the percentage of cells showing FoxO-GFP nuclear localization. Inhibition of the JNK pathway—by treatment with inhibitor SP600125—blocks the response to heat shock treatment. Pie chart values represent averages calculated from three different experiments.*

FoxO, stress-response, and aging in *Hydra vulgaris* and *H. oligactis*

The roles of FoxO proteins in cellular responses to stress, including heat shock in *C. elegans* (Hsu *et al.,* 2003), raise the possibility of a difference in FoxO function between *H. vulgaris* and the less stress tolerant *H. oligactis*. In *H. vulgaris*, FoxO is strongly expressed in cells of the interstitial lineage (Bridge *et al.,* 2010), whose numbers drop rapidly in sexually reproducing *H. oligactis*. Under heat shock conditions, transgenic *H. vulgaris* expressing a FoxO-GPF fusion protein in interstitial cell derivatives show an increase in the percentage of cells showing nuclear localization (Bridge *et al.,* 2010) (Fig. 5B). Furthermore, *H. vulgaris* FoxO nuclear localization in response to heat stress can be significantly reduced by inhibition of the JNK pathway. When subjected to heat shock, polyps treated with the JNK inhibitors SP600125 or AS601245 showed significantly less nuclear localization of FoxO-GFP than heat-shocked control polyps (Bridge *et al.,* 2010). These results provide evidence that *Hydra* FoxO is positively regulated by the JNK pathway under conditions of thermal stress.

As discussed, lifespan extension caused by germline loss involves increased FoxO activity in both *C. elegans* and *Drosophila* (Hsin and Kenyon 1999; Flatt *et al.,* 2008). It will be of interest to determine whether the proliferation of gametes in *H. oligactis* cultured at low temperature promotes senescence at least in part through reduction of FoxO activity.

FoxO proteins can mediate responses to low nutrient levels (Salih and Brunet 2008). Such dietary restriction extends lifespan in a wide range of organisms. In *Hydra*, changes in food availability alter growth rate, with lower food availability leading to lower rates of epithelial cell cycling (Otto and Campbell 1977; Bosch and David 1984), as well as epithelial cell apoptosis (Cikala *et al.,* 1999;

Böttger and Alexandrova 2007) and autophagy (Chera *et al.,* 2009). When nuclear localization of FoxO-GFP expressed in interstitial lineage cells was compared in *Hydra* starved for up to ten days and *Hydra* fed daily, there was no significant difference (Bridge *et al.,* 2010). This may be less surprising than it might seem. Changes in cell cycle length, apoptosis, and autophagy in response to dietary restriction in *Hydra* have only been documented in epithelial cells, not in cells of the interstitial lineage. It is possible that FoxO mediates responses to low nutrient levels in *Hydra* epithelial cells. In *C. elegans*, the FoxA gene *pha-4*, like FoxO plays a key role in regulating longevity in response to dietary restriction (Panowski *et al.,* 2007). FoxA genes are also involved in responses to low food availability in *Drosophila* and mice (Friedman and Kaestner 2006; Bülow *et al.,* 2010). Interestingly, the *Hydra* FoxA gene *budhead* is expressed in the endoderm (Martínez *et al.,* 1997), a location compatible with a role in responses to nutrient levels.

Future prospects

Hydra raises the question of how populations of stem cells may protect themselves from damage over very long periods of time. It is reasonable to expect that genes involved in cellular responses to stress and in reducing damage to cells over time play roles in maintaining these potentially immortal stem cell populations. Thus, elucidating the roles in *Hydra* of genes like FoxO and genes involved in the heat shock response will likely provide insight into the causes of the surprisingly long lifespan of *Hydra vulgaris*. Characterizing the causes of the inducible aging seen in *H. oligactis* should provide valuable information about the regulation of lifespan in *Hydra*. It will also be of interest to characterize differences between the somatic and the interstitial stem cells of *Hydra* to determine what features are unique to the stem cells which produce gametes. *Hydra* promises to provide insight both into mechanisms underlying the long-term survival of stem cell populations, and into the extent to which such mechanisms may be conserved within animals.

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