

Hydra, a powerful model for aging studies

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Cnidarian *Hydra* polyps escape senescence, most likely due to the robust activity of their three stem cell populations. These stem cells continuously self-renew in the body column and differentiate at the extremities following a tightly coordinated spatial pattern. Paul Brien showed in 1953 that in one particular species, *Hydra oligactis*, cold-dependent sexual differentiation leads to rapid aging and death. Here, we review the features of this inducible aging phenotype. These cellular alterations, detected several weeks after aging was induced, are characterized by a decreasing density of somatic interstitial cell derivatives, a disorganization of the apical nervous system, and a disorganization of myofibers of the epithelial cells. Consequently, tissue replacement required to maintain homeostasis, feeding behavior, and contractility of the animal are dramatically affected. Interestingly, this aging phenotype is not observed in all *H. oligactis* strains, thus providing a powerful experimental model for investigations of the genetic control of aging. Given the presence in the cnidarian genome of a large number of human orthologs that have been lost in ecdysozoans, such approaches might help uncover novel regulators of aging in vertebrates.

Keywords: *Hydra oligactis*; Cold-sensitive strain; sexual differentiation; loss of somatic stem cells; neurodegeneration; inducible aging

Introduction: a need for additional model systems for aging studies

Aging is the process whereby changes in physiological processes that accumulate over the lifespan of the organism lead to a decrease in fitness, an increased sensitivity to environmental challenges, and an increase in pathological processes. As a result, an exponential increase in death rate fitting a Gompertzian representation is observed (Finch 1990). Since antiquity people noted that closely related animals may have very different maximal lifespans. According to Aristotle “The reasons for some animals being long-lived and others short-lived, and, in a word, causes of the length and brevity of life call for investigation.” However, it was only when two short-lived invertebrate organisms, the fruit fly *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*, became available as genetically tractable model systems that aging studies began, uncovering the complexity of the aging process (reviewed in Gems & Partridge 2013; López-Otín et al. 2013). Since the discovery of *age-1*, the first gene that increases the lifespan of nematode when mutated (Friedman & Johnson 1988), much progress has been achieved in our understanding of factors and processes that contribute to aging.

Although the impact of the nematode and the fruit fly models in aging research is indisputable, both organisms have critical shortcomings (Austad 2009). Firstly,

except for the *Drosophila* gut, the somatic adult tissues of these two organisms have no regenerative capabilities with scarce to no cell proliferation. Therefore, they mimic poorly the mammalian processes that depend on stem cell renewal and tissue repair to maintain tissue homeostasis. Secondly, in response to stress, *C. elegans* larvae and *D. melanogaster* adults can enter a non-aging stage, suggesting that modulation of lifespan observed in corresponding adult organisms may be mediated by stress response mechanisms that have no equivalent in humans (Larsen et al. 1995; Tatar et al. 2001). Thirdly, *D. melanogaster* and *C. elegans* belong to Ecdysozoa, a superphylum where a large fraction of human orthologs are missing, although present in Cnidaria (Kortschak et al. 2003; Wenger & Galliot 2013a). Altogether these observations suggest that cnidarian model organisms might be more likely to lead to the identification of new candidate regulators of human aging.

The *Hydra* model system

Hydra is a small freshwater cnidarian polyp (Figure 1(A)) that exhibits a low senescence and an astonishing regenerative and budding capabilities throughout its life, as first described by Trembley in 1744, and reviewed by Galliot (2012). In fact, *Hydra* not only regenerates any lost part of its body after bisection, but also it

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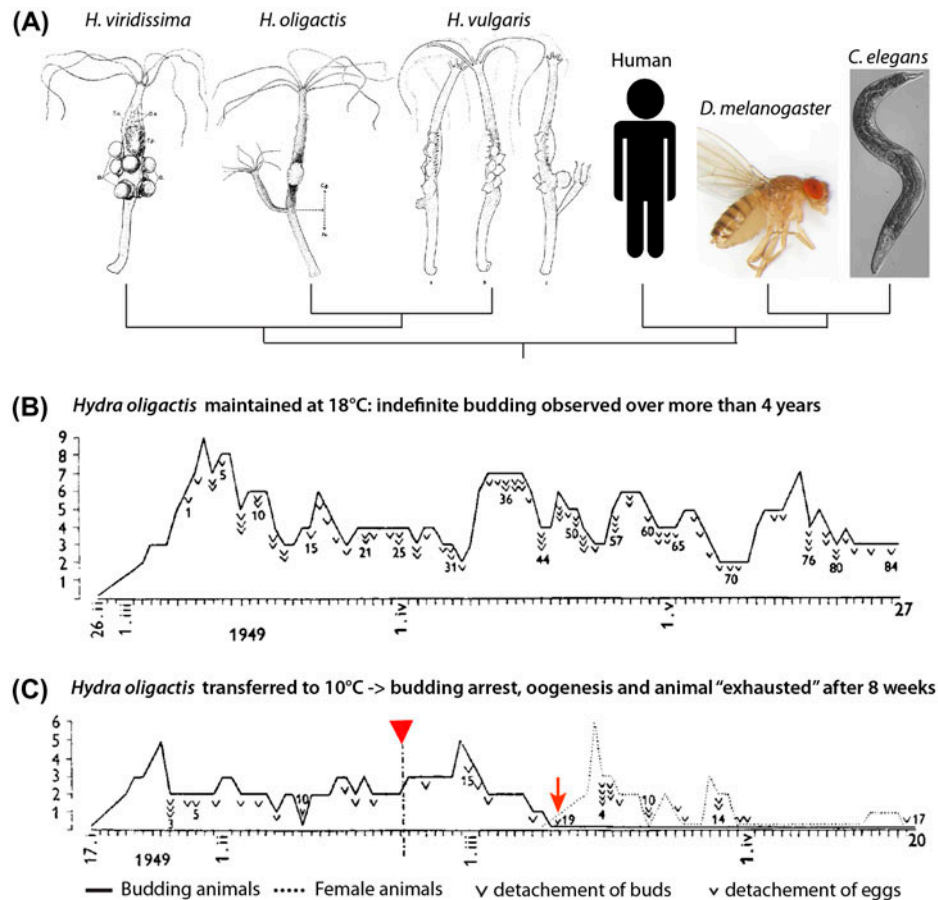


Figure 1. Discovery of inducible aging in *H. oligactis* by Brien 1953, reproduced with modifications. (A) Phylogenetic relationship between three *Hydra* species (drawings by Brien 1953), nematode, *Drosophila*, and human. (B) *H. oligactis* born on 26 February 1949 and maintained at 18 °C continuously produced buds with no sign of aging over four years, shown here over a three months period up to 27 May when bud 84 detached. (C) *H. oligactis* born on 17 January 1949 and transferred to 10 °C on 22 February 1949 (red arrowhead) exhibited a slowing down of budding until it completely ceases on 10 March after the detachment of bud 19 (red arrow). In parallel, the polyp started developing ovaries and produced 16 eggs over the next two weeks until egg production declined. Then, *H. oligactis* became “exhausted” from oogenesis, producing a last egg on 20 May 1949. In (B) and (C), the ordinate axis corresponds to the number of buds or eggs produced by the same animal on a given day.

regenerates from re-aggregates after tissue dissociation. A recent orthologome analysis showed that *Hydra* shares at least 6071 genes with humans, in contrast to *Drosophila* and *C. elegans*, which share only 5696 and 4571 genes, respectively, with humans (Wenger & Galliot 2013a). Therefore, *Hydra* fulfills the conditions for providing a new potent model system for aging studies.

The anatomy of the *Hydra* polyp is simple, comprised basically of a digestive tube terminated at the oral pole by the mouth/anus opening surrounded by a ring of tentacles, and at the aboral pole a basal disc (Figure 1(A)). *Hydra* possesses two innervated body layers, ectoderm and endoderm, separated by an extracellular matrix named mesoglea. A single animal is composed of 50,000 to 100,000 cells, with three distinct stem cell populations, interstitial, ectodermal epithelial, and endodermal epithelial, that altogether give rise to a

dozen cell types (Hobmayer et al. 2012). Regardless of age, these stem cells constantly self-renew in the body column, giving rise to terminally differentiated cells located predominantly at the extremities of the animal, where they are sloughed off (Steele 2002). Interestingly, interstitial stem cells are multipotent, providing progenitors for somatic (gland cells, neurons, stinging cells named nematocytes) as well as germ cells (Figure 2(A)).

Well-fed *Hydra* reproduce asexually by budding. Excess dividing cells escape the parental body column forming a bud, which, in few days, develops into a new fully formed *Hydra*, which eventually detaches from the parental polyp. *Hydra* polyps can also be chemically or genetically depleted of their interstitial stem cells, becoming a so-called epithelial *Hydra*. If such epithelial animals are force fed, they can be maintained for several months, with efficient physiological and developmental

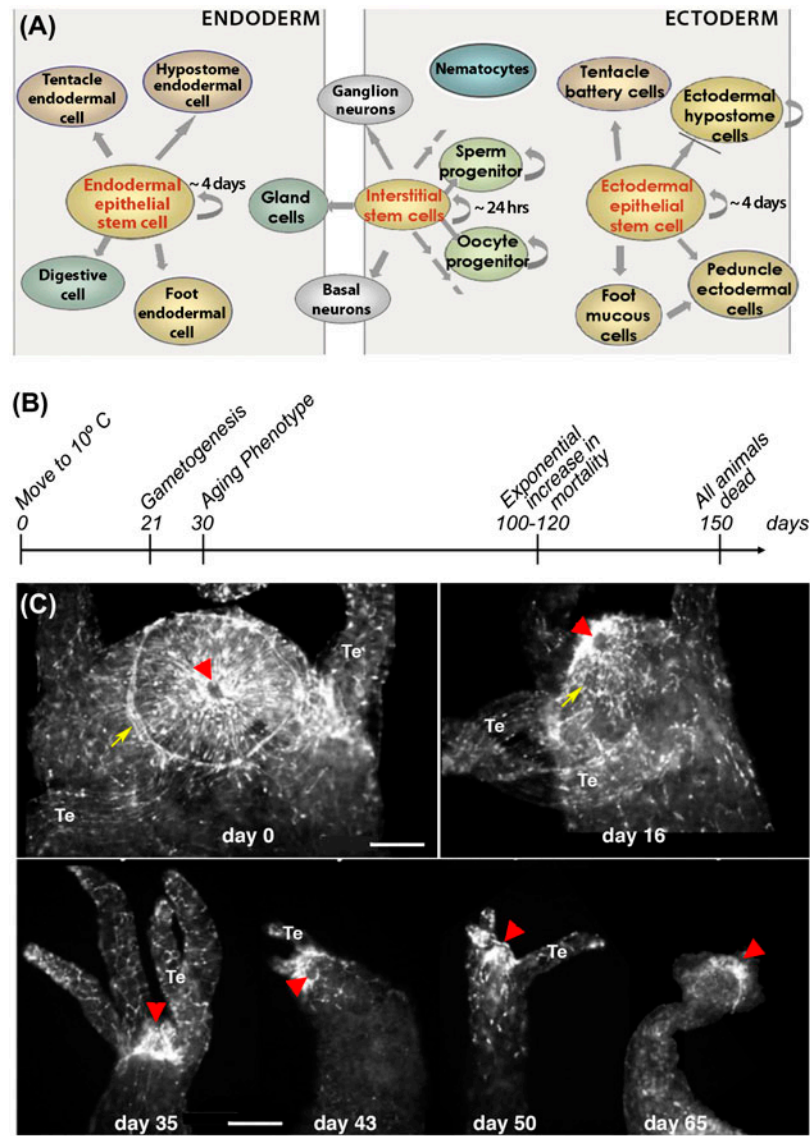


Figure 2. (A) Scheme depicting the different cell types in *Hydra*. Endodermal and ectodermal epithelial stem cells permanently self-renew along the body column and once displaced at the extremities differentiate into foot- and head-specific epithelial cells reprint from (Galliot 2013). Interstitial stem cells cycle faster and give rise to all other cell types including germ cells. (B) Timeline of aging in *H. oligactis* maintained at 10°C as described by (Yoshida et al. 2006). (C) Progressive disorganization of the apical nervous system in *H. oligactis* as evidenced by RFamide immunodetection of the mature neurons. Arrows: nerve net; arrowheads: mouth opening, Te: tentacle. Scale bar: 100 μm.

functions, including regeneration (Marcum & Campbell 1978).

Over the past years “omics” strategies applied to *Hydra* yielded the *Hydra magnipapillata* genome (Chapman et al. 2010), extensive transcriptomes (Boehm et al. 2012; Wenger & Galliot 2013b), and a detailed repertoire of small RNAs (Krishna et al. 2013). In addition, tools to study quantitatively and qualitatively cell proliferation (Plickert & Kroiher 1988), apoptosis (Lasi et al. 2010; Reiter et al. 2012), autophagy (Chera et al. 2009), or stem cell biology (Hobmayer et al. 2012) are

available. Genetic functional approaches also emerged, reinforcing the strength of *Hydra* as a model system. Genes of interest can be transiently introduced either by gene gun or by electroporation (Bottger et al. 2002; Miljkovic et al. 2002), or silenced through RNA interference upon feeding the animals with bacteria expressing dsRNA (Chera et al. 2006). Moreover, the establishment in 2006 of stable *AEP Hydra vulgaris* transgenic lines by microinjection of fertilized eggs (Wittlieb et al. 2006) opened a new era for dissecting cellular processes and molecular regulations in *Hydra*.

Low senescence in asexual *Hydra* and inducible aging in *Hydra oligactis*

In the mid-twentieth century, Paul Brien, while investigating the impact of sexual and asexual reproductions on *Hydra* lifespan, reported the lack of senescence in asexually reproducing *H. vulgaris*, *Hydra viridissima*, and *H. oligactis* polyps that had been observed individually for several years (Brien 1953) (Figure 1(B), upper). Moreover, he noticed that *H. vulgaris* and *H. viridissima* polyps could go through several rounds of sexual differentiation without any loss of fitness. This is in contrast to what he observed in *H. oligactis* polyps. In this species, induction of gametogenesis by transferring the animals from 18 to 10 °C caused budding to cease, accompanied by exhaustion and subsequent degeneration (Figure 1(B), lower).

Decades later, Brien's observations were confirmed. The polyps of the Japanese species *Pelmatohydra robusta* (*P. robusta*), closely related to *H. oligactis*, when maintained asexual over three years, did not show any sign of aging, but died within 90 days after induction of gametogenesis (Noda 1982). Also cohorts of asexual *H. vulgaris* polyps followed for four years in North America survived without any obvious signs of aging (Martínez 1998).

These findings indicate that asexual polyps from most *Hydra* species have extraordinarily long lifespans, whereas in a unique species, *H. oligactis/P. robusta*, induction of gametogenesis correlates with aging. The fact that asexual *Hydra* display negligible senescence or even escape senescence is usually interpreted as a consequence of the constant self-renewal of stem cells (Jones et al. 2014). By contrast in *H. oligactis*, gametogenesis is associated with loss of interstitial stem cells and their somatic derivatives (Littlefield et al. 1985; Yoshida et al. 2006), mimicking the loss of somatic stem cells reported in aged humans (Sousounis et al. 2014).

The aging phenotype of *H. oligactis*

This aging-like phenomenon of *H. oligactis* was investigated in more detail by Yoshida et al. (2006). These authors reported that polyps from three *H. oligactis* strains transferred to 10 °C differentiate gonads by day 21 and undergo severe morphological degeneration by day 30. Mortality began after 60 days, increasing exponentially after 100 days to affect all animals by day 150 (Figure 2(B)). The observed mortality pattern fits the Gompertzian mortality function, the most commonly used model to describe mortality rates and lifespan in aging populations (Finch 1990).

Yoshida and colleagues reported, in addition, a decline in physiological functions such as prey capture, spontaneous contractions, and transfer of food to the gastric cavity. Detailed cell counting confirmed the

disappearance of most interstitial stem cells and their derivatives after day 30, the polyps being composed almost exclusively of epithelial cells and persisting germ cell derivatives. Finally, this study also reported on the disorganization of the actin fibers in the myoepithelial cells, which likely is the cause for the observed reduction in movement in these *Hydra* and reminiscent of sarcopenia observed in the aging skeletal muscles of bilaterians (Yoshida et al. 2006; Rai et al. 2014).

As a further test of the potential of *Hydra* as a model for aging research, we induced sexual differentiation in several *H. oligactis* strains with phylogenetic affiliation verified by barcoding. Three different types of response to cold exposure were identified among these strains. The cold-sensitive strain (CS) used by Yoshida et al. exhibited the highest response, with nearly 100% polyps developing sexual traits and dying within 100–120 days. In contrast, the cold-resistant strain (CR) exhibited limited sexual differentiation, evidenced in only 30% of the polyps, which, after releasing gametes, reverted to asexual state, and continued living in a healthy condition. Finally, some cold insensitive strains (CI) did not develop sexual traits at all upon 10 °C transfer (Tomczyk & Galliot unpublished).

To assess the impact of aging on the apical nervous system, we immunodetected nerve cells with an anti-RFamide neuropeptide antibody (Grimmelikhuijzen 1985). In *H. oligactis* polyps triggered for aging by cold treatment, a drastic decrease in the number of RFamide neurons and a disorganization of the apical nervous system occurred (Figure 2(C)). In myoepithelial cells, the actin fibres, visualized by phalloidin labeling, are highly disorganized, similar to that observed by Yoshida et al. (data not shown). These observations confirm that aging phenotypes can be easily induced in *H. oligactis*, although with varying degrees depending on the strain.

Molecular aspects of aging vs. non-aging in *Hydra*

H. oligactis is a model that has numerous features that complement the drawbacks of existing invertebrate model systems used for aging research, namely hundreds of human orthologs that were lost in nematode and fruit fly ancestors. To identify the putative aging genes present in *Hydra* but missing in *C. elegans* and *D. melanogaster*, we analysed the hydra-human orthologs associated with aging. Among 259 human aging genes retrieved from The Human Ageing Genomic Resources (<http://genomics.senescence.info>), we found that 207 (80%) were conserved in *Hydra* (E-value of the best BLAST hit of *Hydra* below 1E-10). Interestingly, some of these genes are missing or poorly conserved in *D. melanogaster* and *C. elegans*, such as the p53 regulator MDM2 or the TGFβ inhibitor noggin. The aging-induced regulation of these genes is currently under investigation.

Table 1. Criteria that support aging research in *H. oligactis* CS polyps undergoing sexual differentiation. For aging in humans, see (Lopez-Otin et al. 2013; Rai et al. 2014; and Sousounis et al. 2014).

Aging in humans	Aging in <i>Hydra oligactis</i> (CS strain)
Neurodegeneration	Disorganization of the apical nervous system and loss of neurons, loss of the feeding behavior (Yoshida et al. 2006; this work)
Loss of adult somatic stem cells	Dramatic reduction in the number of interstitial somatic stem cells (Littlefield et al. 1985; Yoshida et al. 2006)
Sarcopenia	Disorganization of the actin myofibers, loss of contractility, loss of food transfer to the gastric cavity (Yoshida et al. 2006)
100% mortality within 100 years after sexual maturity	100% mortality within 120 days after sexual differentiation (Brien 1953; Noda 1982; Yoshida et al. 2006)

As an alternative approach to aging studies, several studies aimed at dissecting the mechanisms that underlie the lack of senescence in *Hydra* focused on FoxO, an evolutionarily conserved transcription factor. In bilaterian organisms, FoxO regulates the response to stress, the proliferation of stem cells, and modulates lifespan (reviewed in Salih & Brunet 2008). In nematodes and fruit flies, the knockdown of FoxO significantly shortens lifespan. In *Hydra*, FoxO is expressed in stem cells, and appears to respond to stress (Bridge et al. 2010). Reduction in FoxO levels in the *H. vulgaris* AEP strain negatively affected the proliferation of stem cells, the speed of the budding process, the growth of *Hydra* population, and the production of immune peptides (Boehm et al. 2012). However, no mortality was observed in FoxO deficient polyps, suggesting that other factors contribute to negligible senescence in *H. vulgaris*.

Conclusions

H. oligactis presents a potent model system for aging research. As summarized in Table 1, comparing hallmarks of aging in *H. oligactis* and humans, *H. oligactis* offers a unique experimental setting, where aging can be induced and observed over a time span of four months. Moreover, we have recently characterized different *H. oligactis* strains that make possible the comparison of cellular and molecular processes in animals from the same species, submitted to the same stress conditions, but exhibiting different patterns of aging. Recently, a comprehensive review stressed nine general hallmarks of the aging process (López-Otín et al. 2013). Each of these hallmarks is potentially testable in *Hydra*. Such studies should help dissect the genetic circuitry underlying aging in *Hydra*, and thus potentially identify some new regulators of aging to be subsequently tested in mammalian cells and organisms.

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