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Abstract

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A need for additional model systems for aging studies

Aging is the process where the accumulation of changes in physiological processes that occurs over the life span of the organism leads to a decrease in fitness, increased sensitivity to environmental challenges and 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. Since the discovery of age-1, the first gene that increases the lifespan of nematode when mutated (Friedman and Johnson, 1988), much progress was achieved in our understanding of factors and processes that contribute to aging (reviewed in Gems and Partridge, 2013; Lopez-Otin et al., 2013).

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 human (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 and Galliot, 2013a). Altogether these observations suggests that cnidarian model organisms might lead to the identification of new candidate regulators of human aging.

The Hydra model system

Hydra is a small freshwater cnidarian polyp (Fig. 1A), which exhibits a low senescence and maintains throughout its life astonishing regenerative and budding capabilities, as first described by Trembley in 1744 see in (Galliot, 2012). In fact this animal not only regenerates any lost part of its body after bisection, but also it regenerates from re-aggregates after tissue dissociation. A recent orthologome analysis showed that Hydra shares at least 6,071 genes with humans. In contrast, Drosophila and C. elegans only share 5,696 and 4,571 respectively (Wenger and Galliot, 2013a). Therefore Hydra fulfills the conditions for providing a new potent model system for aging studies.
The anatomy of the *Hydra* polyp is rather simple, basically 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 (Fig. 1A). *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 of 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 (Fig. 2A).

Well fed *Hydra* reproduce asexually by budding. Excess of 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

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**Figure 1:** Discovery of inducible aging in *Hydra oligactis* by Brien 1953, reproduced with modifications. (A) Phylogenetic relationship between three *Hydra* species drawings by Brien, 1953, nematode, *Drosophila* and human. (B) *Hydra oligactis* born on February 26th 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 May 27th when bud 84th detached. (C) *Hydra oligactis* born on January 17th 1949 and transferred to 10°C on February 22nd 1949 red arrowhead exhibited a slowing down of budding until it completely ceases on March 10th after the detachment of bud 19th red arrow. In parallel the polyp started developing ovaries and produced 16 eggs over the next two weeks until egg production declined. Then *Hydra* became “exhausted” from oogenesis, producing a last egg on May 20th 1949. In B, C the ordinate axis corresponds to the number of buds or eggs produced by the same animal on a given day.
Aging in Hydra  
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Efficient physiological and developmental functions, including regeneration (Marcum and Campbell, 1978).

Over the past years “omics” strategies applied to Hydra yielded the H. magnipapillata genome (Chapman et al., 2010), extensive transcriptomes (Boehm et al., 2012; Wenger and Galliot, 2013b), and a detailed repertoire of small RNAs (Krishna et al., 2013). In addition, tools to study quantitatively and qualitatively cell proliferation (Plickert and Kroicher, 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 model system. Genes of interest can be transiently or stably 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). Indeed the establishment in 2006 of stable AEP H. 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-XXth 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, H. viridissima and H. oligactis polyps which had been observed individually for several years (Brien, 1953) (Fig.1B, 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 was observed in H. oligactis polyps. In this species induction of gametogenesis by transferring the animals from 18°C to 10°C ceased budding, caused exhaustion and subsequent degeneration (Fig. 1B, lower).

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

These findings indicate that asexual polyps from most Hydra species have extraordinarily long lifespans, whereas in a unique species, H. oligactis, 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). This author 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 (Fig.2B). 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 fibres in the myoepithelial cells, likely 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).

To further test 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. We identified three different types of response to cold exposure 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 healthy. Finally some cold insensitive strains CI did not develop sexual traits at all upon 10°C transfer (Tomczyk et al. in preparation).

To assess the impact of aging on the apical nervous system, we immunodetected nerve cells with the anti-RFamide neuropeptide antibody (Grimmelihiuizien, 1985). In H. oligactis polyps triggered for aging by cold treatment, we observed a drastic decrease in the number of RFamide neurons and

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<tr>
<th>Aging in humans</th>
<th>Aging in Hydra oligactis (CS strain)</th>
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<tbody>
<tr>
<td>Neurodegeneration</td>
<td>Disorganization of the apical nervous system and loss of neurons, loss of the feeding behavior (Yoshida et al. 2006; this work)</td>
</tr>
<tr>
<td>Loss of adult somatic stem cells</td>
<td>Dramatic reduction in the number of interstitial somatic stem cells (Littlefield et al. 1985; Yoshida et al. 2006)</td>
</tr>
<tr>
<td>Sarcopenia</td>
<td>Disorganization of the actin myofibers, loss of contractility, loss of food transfer to the gastric cavity (Yoshida et al. 2006)</td>
</tr>
<tr>
<td>100% mortality within 100 years after sexual maturity</td>
<td>100% mortality within 120 days after sexual differentiation (Brien, 1953; Noda, 1982; Yoshida et al. 2006)</td>
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Table 1: Table listing the criteria that support aging in H.o. CS polyps undergoing sexual differentiation. For aging in humans, see in Lopez-Otin et al. 2013; Rai et al. 2014; Sousounis et al. 2014.
a disorganization of the apical nervous system (Fig. 2C). Actin fibres visualized by phalloidin labeling were characterized by disorganization of the network similar to that observed by Yoshida et al. (data not shown). These observations confirm that aging phenotypes can be easily induced in the H. oligactis species, although with varying degrees depending on the strain.

Molecular aspects of aging versus non-aging in Hydra

H. oligactis as a model present all 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.

As an alternative approach to aging studies, several studies aimed at dissecting the mechanisms that underlie the lack of senescence in Hydra, focusing on FoxO, an evolutionarily-conserved transcription factor, which, in bilaterian organisms, regulates the response to stress, the proliferation of stem cells, and modulates lifespan (reviewed in Salih and Brunet, 2008). In nematodes and fruit flies the knockdown of FoxO significantly shortens the lifespan. In Hydra, FoxO is expressed in stem cells, and appears to respond to stress (Bridge et al., 2010). Reduction of 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

Hydra oligactis presents a potent model system for aging research. It offers a unique experimental setting where aging can be induced and observed over a four months period Table 1. 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 pattern of aging. Recently a comprehensive review stressed nine general hallmarks of the aging process (Lopez-Otin 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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