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Review

The free-living flatworm *Macrostomum lignano*: A new model organism for ageing research

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ABSTRACT

To study the several elements and causes of ageing, diverse model organisms and methodologies are required. The most frequently used models are *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster* and rodents. All have their advantages and disadvantages and allow studying particular aspects of the ageing process. During the last few years, several ageing studies focussed on stem cells and their role in tissue homeostasis. Here we present a new model organism which can study this relation where other model systems fail. The flatworm *Macrostomum lignano* possesses a dynamic population of likely totipotent somatic stem cells known as neoblasts. Several characteristics qualify *M. lignano* as a suitable model system for ageing studies in general and more specifically for gaining more insight in the causal relation between stem cells, ageing and rejuvenation. In this review, we will briefly describe the species and its life history, and discuss the role of its stem cells in ageing and rejuvenation. We also give an overview of the available experimental tools that allow a multidisciplinary approach for studying ageing in *M. lignano*.

1. Introduction

Ageing is a complex process that affects a wide variety of functions (Arking, 1998). This complexity is shown by the countless ageing theories that have been published, most of which are monistic in nature and focuse on one particular element (Semsei, 2000). Studying the different elements and causes of ageing requires diverse methodologies and model organisms, such as *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster* and rodents. All of these models have their characteristic strengths and weaknesses (Fig. 1).

During the last few years, several ageing studies have focussed on stem cells and their role in tissue homeostasis (Rando, 2006; Rossi et al., 2008; Sharpless and DePinho, 2007). These studies have shown that ageing is invariably accompanied by a diminished capacity to adequately maintain tissue homeostasis or to repair tissues after injury. Furthermore, the ageing of tissue-specific stem cells and progenitor cell compartments is believed to play a pivotal role in the decline of tissue and organ integrity and function in the elderly (Rossi et al., 2008). However, studying the reciprocal influence between stem cells and the ageing process, especially in vivo, is difficult in the current model organisms. This is partly due to the following problems: the adult soma of *C. elegans* and *D. melanogaster* are either completely or largely post-mitotic, and the complexity and relative inaccessibility of the vertebrate stem cell population restricts in vivo analysis of stem cell functionality (Sánchez Alvarado et al., 2002; Sharpless and DePinho, 2007).

These limitations are not present in flatworms, which possess a dynamic population of likely totipotent somatic stem cells known as neoblasts (Dubois, 1948; Stephan-Dubois and Gilgenkrantz, 1961; Lange and Gilbert, 1968; Lange, 1968a; Baguñà, 1981). In the past, several researchers already used flatworms to gain more insight into the causal relation between neoblasts, ageing and rejuvenation (see references in Haranghy and Balázs, 1964; Lange, 1968a). While most of these studies used triclad species, we introduce *Macrostomum lignano* (Macrostomorpha) as a new model. We focus on this species because it has several advantages in comparison to triclads, making it possible to expand and improve the flatworm ageing research. These advantages are discussed below in the appropriate sections.

In the past, *M. lignano* has already been put forward as a model organism for stem cell biology, development, regeneration, and the study of sexual selection (Ladurner et al., 2005a, 2008). In this review, we will briefly describe the species and its life history,

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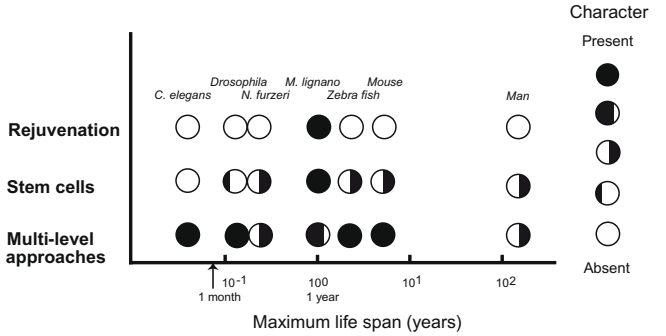

Fig. 1. Suitability of model organisms for studying the relation between stem cells and age. Essential characteristics for studying this conceptual theme are plotted against the maximum lifespan of the models (presented on a logarithmic scale). For each characteristic of interest, its presence, absence and intermediate state are schematically illustrated. A good model should have a versatile experimental toolbox allowing multi-level approaches. While *M. lignano* has entered the era of functional genomics, the molecular approaches found in *C. elegans*, for example, are still in development. Stem cells are well characterised and experimentally accessible in *M. lignano*, while they are entirely (*C. elegans*) or almost entirely (*Drosophila*) absent, or not readily accessible (mouse, zebrafish) in the other models. Rejuvenation is a fascinating and emerging field of interest. It can only be studied in a limited number of species, such as cnidarians, annelids and flatworms, which have the ability to regenerate and renew their body. As discussed in the text, it can be easily studied in *M. lignano*. *M. lignano* has a shorter maximum lifespan than the vertebrate models, except for *Nothobranchius furzeri*. This annual fish is a short-lived vertebrate which may be of biomedical relevance but does not have such an accessible stem cell system as *M. lignano*.

and discuss the role of its stem cells in ageing and rejuvenation. We will also give an overview of the available experimental tools that allow a multidisciplinary approach for studying ageing in *M. lignano*.

2. *Macrostomum lignano:* description

Macrostomum lignano (Macrostomorpha, Rhabditophora) is a marine, free-living flatworm that can be found in the high-tide interstitial sand fauna on beaches of, for example, the Northern Adriatic Sea (Ladurner et al., 2000, 2005a). In the laboratory, it can be easily cultured and allows stocking in high densities due to its small size. *M. lignano* is cultured individually, in pairs or in groups in Petri dishes or multiwell plates in f/2, a nutrient-enriched artificial seawater medium at a salinity of 32% (Guillard and Ryther, 1962). The dishes are incubated at 20 °C, with a 60% relative humidity and a 14:10 h or 13:11 h light:dark cycle. Animals are fed with the diatom *Nitzschia curvilineata* that can be obtained from culture collections (Ladurner et al., 2008). The diatoms are grown under the same conditions as the worms and, if necessary for an experiment with controlled feeding, they can be easily quantified (Vizoso and Schärer, 2007).

Macrostomum lignano is a small animal of about 1–1.5 mm length consisting of roughly 25,000 cells (Ladurner et al., 2000). Although individuals may appear brownish because of the gut content, they are highly transparent in squeeze preparations, allowing easy observation of all major tissues and organ systems (Fig. 2A) (Ladurner et al., 2008). The several organ systems are described in detail in Ladurner et al., (2005a), and here we focus only on the stem cell system.

The neoblasts are located in two bands along the lateral sides of the animal, merging in the tail plate (Fig. 2B). Somatic neoblasts are also present along the post-pharyngeal commissure and a few, most likely gastrodermal neoblasts, can be found scattered along the midline of the body. Neoblasts are entirely absent in the rostrum which is the region anterior to the eyes. The neoblasts are located within the parenchyma and are the only dividing cells in the adult (see Ref. in Ladurner et al., 2008). They can differentiate

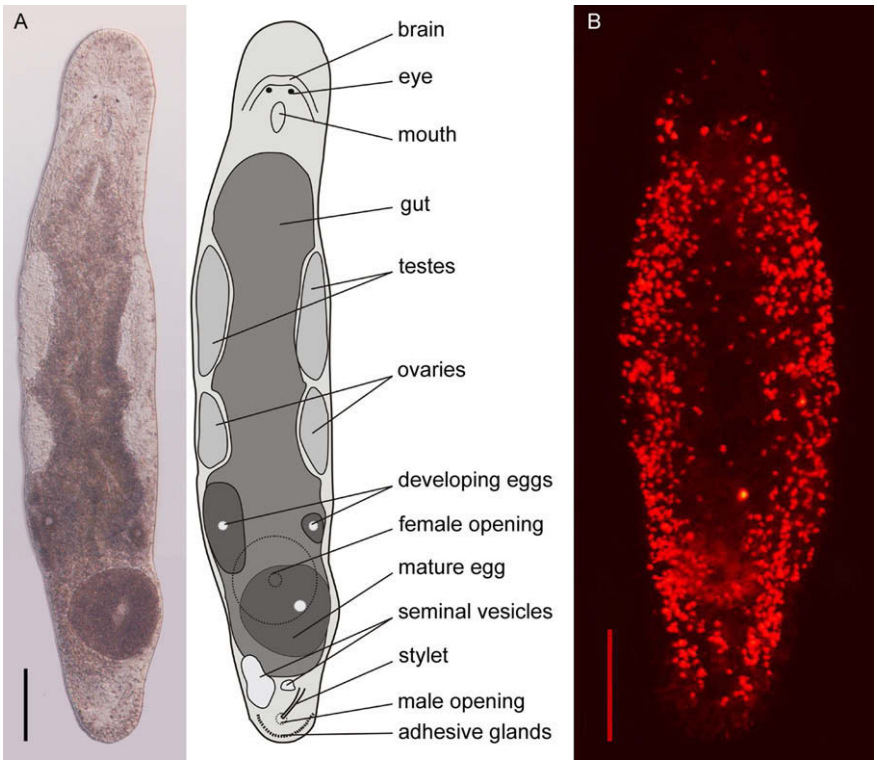

Fig. 2. (A) Interference contrast photomicrograph and schematic drawing of *M. lignano*. The length of the animal is about 1 mm. Scalebar: 100 μm. (B) BrdU picture of *M. lignano*. The red dots are labelled S-phase neoblasts. Scalebar: 100 μm.

Table 1
Selected *Macrostomum lignano* ESTs with homology to genes known to play a role in ageing.

Category	Clone	BLAST Homologs	E-value
Telomerase	ANGU5132	Telomerase-associated protein TP-1 [Homo]	4e-10
	ANGU2599	Flotillin 2	2e-62
Insulin	ANGU6390	Insulin induced gene 1 [Danio rerio]	4e-39
	ANGU7494	Insulin receptor substrate protein	4e-15
	ANGU793	Insulin receptor-related precursor	4e-11
	ANGU7967	Insulin-degrading enzyme [Homo]	8e-54
	KN-30_A06_T7	Insulin precursor	9e-05
	Ml_aW_004_E10	Insulin-like growth factor 2 mRNA-binding protein 1	3.0e-16
Stress	ANGU1456	Stress protein HSP70	4e-19
Heat shock	ANGT5211	Hsc70 protein [Danio rerio] >gi	1e-131
	ANGU1138	Similar to Wolf-Hirschhorn synd...	2e-16
	ANGU2041	S21175 dnaK-type chaperone hsc71	9e-32
	ANGU2436	GR75_CRIGR stress-70 protein	1e-116
	ANGU2666	Stress protein HSC70 [Xiphophorus]	1e-64
	ANGU6242	PDX1_SUBDO probable pyridoxin	4e-66
	AZWT1718	Err-related and stress induced protein	1e-121
Superoxid	ANGT5713	Copper/zinc superoxide dismutase [Anemonia]	7e-52
Dismutase	ANGT6742	SODC_IPOBA superoxide dismutase [Cu-Zn]	5e-26
	ANGU1264	Superoxide dismutase 2, mitochondrial	2e-76
	ANGU5058	Cytosolic Cu/Zn-superoxide dismutase [Taenia]	1e-51
Deacetylase	ANGU1759	Histone deacetylase 11 [Mus musculus]	6e-59
	ANGU4714	Transcriptional regulator *sir*2 family prot	7e-34
	ANGU824	LOC432017 protein [Xenopus laevis]	4e-29
Longevity	ANGU2720	HrPET-1 [Halocynthia roretzi]	9e-05
	ANGU6685	LAG1 longevity assurance homolog 2 isoform	1e-18
*Sir*2	ANGU4714	Transcriptional regulator *sir*2	7e-34
	Ml_aW_002_rv_K01	*Sir*2-like protein	8.0e-28
	ANGU8000.g2	Sirtuin	9.0e-23
Others	ANGT6056	C. elegans methuselah protein MTH-1	0.46*
	ANGU7232	p53-related protein kinase [Homo sapiens]	4e-22
	ANGU7644.g2	Target of rapamycin (TOR) kinase	9.0e-58
	ANGU7677.g2	DNA repair protein	2.0e-22
	Ml_aW_007_I23	DNA double-strand break repair	8.0e-04
	KN-32_H11_T7	DNA-repair protein	7.62e-44

into all cell types, including germ cells, and maintain their own population (Ladurner et al., 2000). Therefore, neoblasts play a key role during reproduction, development, tissue turnover and regeneration. In an adult animal there are about 1600 neoblasts, which is 6.5% of the total cell number (Bode et al., 2006). The average number of somatic S-phase neoblasts is 435 ±79 (Ladurner et al., 2000), which is 27% of the total somatic neoblast population (Bode et al., 2006), and only a small fraction of about 20–40 neoblasts are in mitosis (Ladurner et al., 2008). The several techniques to study the neoblasts are discussed in the section 5.

During the last years, *M. lignano* has clearly entered the era of functional genomics. More than 15,000 expressed sequence tags (ESTs) have been generated (Morris et al., 2006; Ladurner unpublished; Pfister pers. com.), in which a number of candidate genes known to be involved in ageing processes in other organisms were found (Table 1). This opens possibilities to study these ageing genes in *M. lignano*. There are ongoing efforts to increase the number of ESTs, to perform large-scale whole mount in situ hybridization studies (Pfister et al., 2007) and systematic mutagenesis screens.

3. Life history

M. lignano is a hermaphrodite with a five-day embryonic development and a generation time of about 18 days (see references in Ladurner et al., 2008). Mature worms generally lay one egg per day, allowing fine-graded culturing which is essential for survival studies and experiments.

The fecundity and fertility pattern during adult lifespan are important parameters for ageing studies (Terzibasi et al., 2007). Preliminary data in *M. lignano* suggest that fertility declines with advancing age, although a post-reproductive period has not yet been observed: 40-week-old individuals are still able to produce eggs, but the number of offspring is lower in comparison to young adults.

M. lignano has a median lifespan of 205 ± 13 days (29.6 weeks) and a 90th percentile (90% mortality) of 362 ± 41 days (51.7 weeks), determined from three replicate cultures each of 100 individuals. M. lignano is the first flatworm species in which the complete survival curve has been established and a basic set of demographic data is analysed. In the existing flatworm ageing literature, only the maximum lifespan of the studied species, often triclads with a maximum lifespan of several years, is reported (Haranghy and Balázs, 1964; Lange 1968a). The availability of a basic set of demographic data is, however, a prerequisite for ageing research and this is now obtained for M. lignano. Furthermore, the culture conditions of M. lignano are very standardised, making it possible to distinguish between physiological and pathological ageing, which was in the past often difficult in the used triclad species (Haranghy and Balázs, 1964).

4. Chronological and replicative ageing

It is known that post-mitotic cells experience chronological ageing, whereas proliferative cells experience both chronological and replicative ageing (Rando, 2006). Like all flatworms, *M. lignano* consists of both differentiated post-mitotic cells and totipotent proliferating neoblasts. Replicative ageing and the limit of the replicative potential of neoblasts, however, have not yet been studied in *M. lignano* and other flatworms.

During tissue homeostasis, the neoblasts produce daughter cells that differentiate and replace aged and injured post-mitotic cells. Because different tissues have a different cellular turnover, the extent of cell renewal of the different tissues will vary. However, the

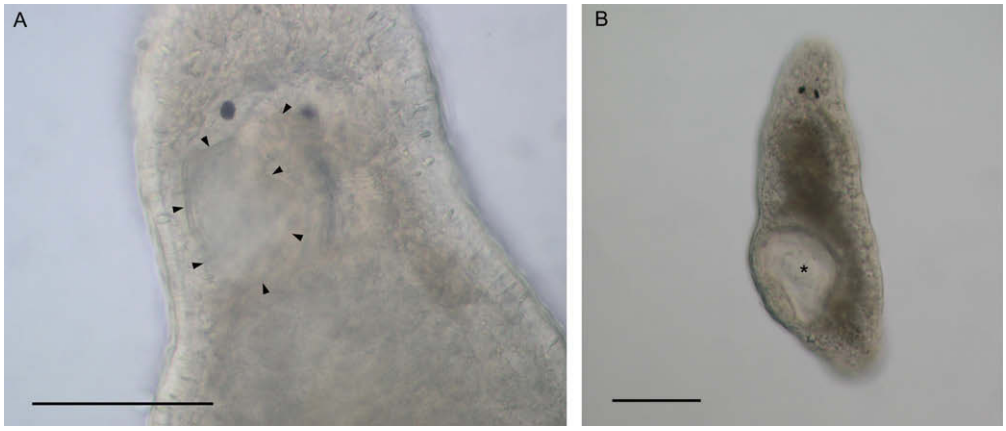

Fig. 3. Body deformities in 238 days old individuals of *M. lignano*. Scalebars: 100 μm. (A) The presence of a groove (black arrowheads) in the head region is a common deformity in old individuals. Grooves are external structures at the epidermis. It should be noted that in the literature both the terms 'urns' (Ladurner et al., 2005) and 'grooves' (Egger et al., 2006) are used for these structures. (B) Another frequently observed deformity is the presence of a cyst (∗). This is an internal structure which can be located anywhere in the body. Besides the presence of a cyst, this individual is also shrinking. This can be observed because the animal is small and the gonads are degenerating.

turnover rates of the different tissues affect the proliferation rate of the neoblasts only as a total demand for new cells from the neoblast pool because the neoblasts pool is likely totipotent.

Already in 1930, Abeloos hypothesized that the main cause of ageing in flatworms is the insufficiency of tissue renewal to assure the permanent equilibrium between young and old cells in the somatic post-mitotic tissues. Our morphological ageing data support this hypothesis. With advancing age, the transparent body changes to an opaque grey, but inner organs can still be observed under a microscope. Body deformities appear such as small bulges in the epidermis, the presence of grooves in the head region, and liquid-filled cysts that tend to be present in all body regions (Fig. 3). Furthermore, it is several times observed that individuals shrink during their last months of life, i.e. they decrease in size and lose their gonads (Fig. 3B). These observations suggest that there is indeed an overall decline in tissue maintenance potential with advancing age.

Tissue homeostasis can be studied by using continuous 5-bromo-2′-deoxyuridine (BrdU) exposure. By using this technique, Nimeth et al., (2002) demonstrated that about one-third of all epidermal cells are replaced every 2 weeks in adults of *M. lignano*. Further studies in which epidermal replacement is used as a parameter for measuring tissue homeostasis over the complete lifespan are underway.

5. Experimental toolbox

Stem cell function is regulated by both intrinsic (cell autonomous) and extrinsic factors (for example surrounding tissue, stem cell niche and environment) during ageing. Moreover, these factors also interact with each other (Rando, 2006). Therefore, a large-scale multidisciplinary approach at all the levels of organisation, from molecules and genes to tissues, organs and ultimately the whole organism is necessary. The essential experimental toolbox for this kind of research is available in *M. lignano* and will be discussed in this section.

M. lignano has a well characterised and easily stainable stem cell system (Bode et al., 2006; Egger et al., 2006; Ladurner et al., 2000; Nimeth et al., 2004). The morphology of the neoblasts has been described in detail by means of light and electron microscopy (see references in Ladurner et al., 2008). S-phase neoblasts can be single or double stained with the thymidine analogs BrdU, IdU and CldU. Dividing cells can be labelled with the anti-phospho-histone H3 mitosis marker and double labelling with both this marker and a thymidine analog can be performed (Ladurner et al., 2008). These markers make studying the distribution, migration and differentiation of the neoblasts possible by performing pulse, pulse-chase and continuous labelling experiments. Moreover, the number of neoblasts can be easily quantified by using the 3D counting method presented by Schärer et al., (2004). The fact that this can be done at any given time point is a major advantage to assess the relation between age and neoblasts. Such studies have already been undertaken and preliminary results suggest that the number of neoblasts does not decline with age in *M. lignano*. However, it remains to be tested whether the dynamics of neoblast proliferation change with age. For this, animals can be incubated in colchicine to determine mitotic rate and hydroxyurea can be used to arrest neoblasts in early S-phase (Nimeth et al., 2004).

Molecular germ line/stem cell markers (vasa, piwi) are also available (Ladurner et al., 2008; Pfister et al., 2007, 2008) and can be used for studying the mRNA expression by in situ hybridization and protein localization by specific antibody labelling (Pfister et al., 2008).

In most mammalian tissues, the relative inaccessibility of the stem cell population in vivo has made it difficult to determine whether a decline in stem cell function is correlated to the decline of regenerative capacities with ageing (Sharpless and DePinho, 2007). In flatworms, however, the neoblast population is accessible, and purification of the neoblast cell fraction is well established (Hayashi et al., 2006). In fact, the rescue of lethally X-irradiated hosts after transplanting neoblasts from healthy donors is one of the most convincing experiments that suggests totipotency of triclad stem cells (Dubois, 1948; Stephan-Dubois and Gilgenkrantz, 1961; Lange, 1968b, c, 1969a, b; Lange and Gilbert, 1968; Baguñà et al., 1989). In *M. lignano* it would be possible to perform parabiotic pairings of injected "old" or "young" neoblasts into young or old X-ray irradiated hosts, respectively. This would allow testing whether the putative diminished replicative potential of the neoblasts is primarily due to intrinsic ageing or to the effect of the ageing somatic environment. Similar experiments have recently been performed in mice and have shown the importance of a youthful systemic milieu to activate aged muscle stem cells (Rando, 2006). In flatworms, the importance of the somatic environment was shown by Lange (1968a) who studied ageing in the triclad *Dugesia lugubris*. The experimental toolbox available for *M. lignano* allows

studying the causal relationship between the age of the somatic environment and the declining stem cell functionality in greater detail.

Other advantages of *M. lignano* are its small size, its simple well-described anatomy, and its transparency. This allows a relatively fast assessment of (1) the number of cells, (2) the general physical condition of the animal, and (3) the in vivo study of cells and organ systems during ageing. Moreover, the recent production and characterization of cell- and tissue-specific monoclonal antibodies (mAB) for this species (Ladurner et al., 2005b) will certainly favour future studies on ageing, especially in studies concerning cell differentiation potential and cell and tissue quantification during lifespan. For example, the number and the condition of gut cells, nerve fibres and clusters, epidermal cells, gland cells, muscle fibres, and spermatids can now be easily quantified and visualized over time. Because protocols for electron microscopy are well established for *M. lignano*, it is further possible to analyze tissues and cells ultrastructurally (Bode et al., 2006). Three life stages (i.e. 1, 6, and 9 months old) of *M. lignano* are currently being analyzed by means of electron microscopy and future analyses will aim at following the ultrastructural morphology of cells, tissues and body deformities during its entire lifespan. Recently, high pressure freezing was used as a primary fixation technique for ultrastructural preparations in *M. lignano* (Egger et al., in review). This new tool will improve future ultrastructural immunocytochemistry studies aimed at characterizing ageing specific markers such as the p53 protein.

6. Lifespan extension and rejuvenation

The main goal of ageing research is to understand and eventually manipulate processes which lead to senescence and death. Essentially, increased lifespan can be achieved either by slowing down or even by reversing the ageing process. The latter phenomenon is called rejuvenation, known as the "fountain of youth" concept in myth and legend. In several flatworm species, a lifespan extension induced by starvation or repeated regeneration is observed. Several authors even suggest that starvation and repeated regeneration induce rejuvenation and bring individuals to a "younger" condition than at the start of the experiment (see Ref. in Haranghy and Balázs, 1964). The possibilities of *M. lignano* for studying lifespan extension and especially for studying rejuvenation will be discussed in this section.

By using *M. lignano*, the relationship between reproduction and longevity can be readily addressed. This hermaphrodite flatworm allocates a substantial amount of energy into reproduction (Schärer and Ladurner, 2003; Vizoso and Schärer, 2007). Analogous to recent *C. elegans* work, efforts are being undertaken to use RNA interference for genes involved in gonad formation, which may make it possible to generate animals with reduced or absent spermatogenesis or oogenesis, or to generate animals completely devoid of gonads (Sekii et al., unpublished). These manipulations are expected to open avenues to contribute to our understanding of how reproduction might regulate lifespan in this species. Known lifespan-extending pathways (e.g. insulin/insulin-like growth factor 1 signalling pathway) could also be studied by using the same molecular technique.

One of the most interesting advantages of *M. lignano* as a stem cell model is the possibility of experimentally inducing additional stem cell proliferation through amputation and regeneration. In addition, somatic damage can be removed and post-mitotic tissues and the gonads can be renewed by inducing regeneration. *M. lignano* is capable of regenerating lost tissue anterior to the brain and posterior to the pharynx (Egger et al., 2006). Repeated amputation and subsequent regeneration of the same individual is not only possible, but apparently extends the lifespan as well (Egger et al., 2006). Over a period of more than two years (26.5 months), up to 59 consecutive amputations of the posterior part of single individuals were successfully performed and animals were consistently able to regenerate lost tissues between amputations (Egger, unpublished observations; Egger et al., 2006; Ladurner et al., 2008). Only the head, including the rostrum, brain and eyes, and the pharynx region were not amputated in the repeatedly regenerating animals. Interestingly, after about seven months, some animals showed signs of deterioration in the head region: grooves appeared in the rostrum and the pigmented eyes were lost one after the other. After about 10 months, all test animals had lost both pigmented eyes and these were never found to be repaired or replaced during the remaining months of the experiment (Egger, unpublished observations). The additional stem cell proliferation possibly leads to proliferation-induced damage, and the loss of eyes and deterioration in rostrum could be an indication of distorted maintenance of the head, possibly causing the eventual death of the animal. However, it is important to note that at the end of the experiment the individuals were still able to regenerate. The

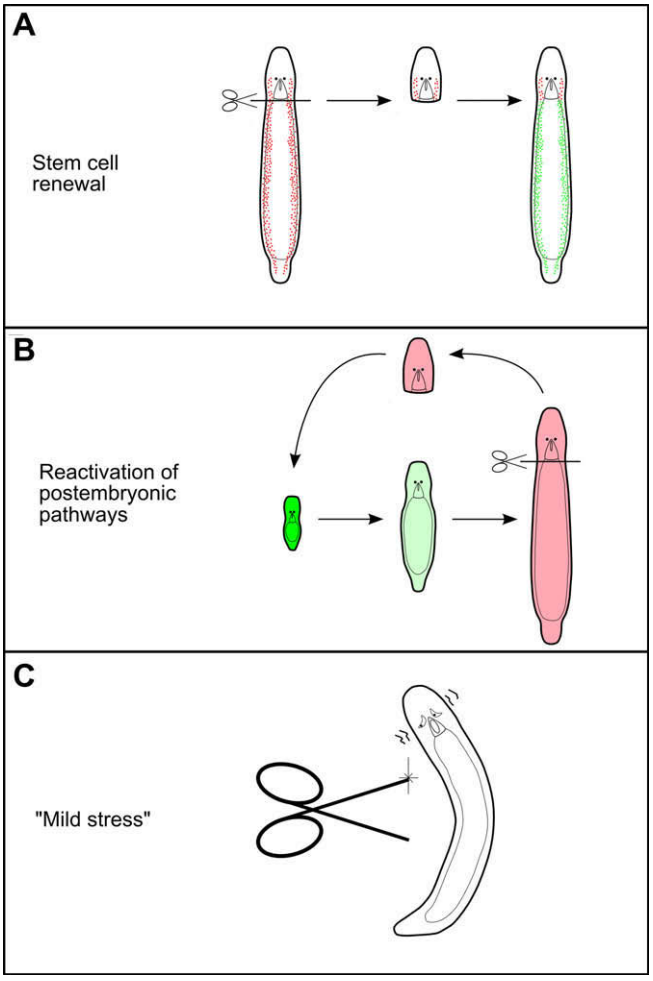

Fig. 4. Possible explanations for increased longevity (rejuvenation) by means of repeated regeneration in *M. lignano*. All three presented hypotheses can play a role at the same time. (A) Adult animal with aged stem cells (red spots). Amputating a posterior body part causes the animal to regenerate lost tissues, including stem cells (green spots). (B) Genetic pathways (green) expressed during postembryonic development are possibly reactivated during regeneration and subsequent growth; some of these pathways are probably not expressed in adults (red). (C) Amputation itself can be considered a form of "mild stress" to animals with pronounced regeneration capacity and thus increase the lifespan.

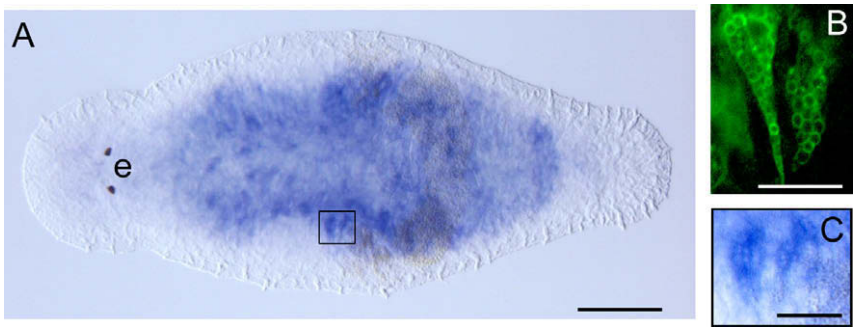

Fig. 5. In situ hybridization of a *sir2*-like gene of *M. lignano*. (A) The signal (blue) is located in the gut. (C) Higher levels of *sir2*-like mRNA are located in a special cell type called goblet cells. (B) This cell type can also be labelled with a specific monoclonal antibody (see Ladurner et al., 2005). The scale bars are 100 μm in (A) and 20 μm in (B) and (C).

renewal of all amputated tissues, including neoblasts, suggests an actual rejuvenation of the regenerated tissues. The next step is to assess whether the number of amputations, the amputation level and the age of amputated individuals influence the lifespan. Different hypotheses can be formulated as for the reasons for the extended lifespan due to repeated regeneration in *M. lignano*:

1) During regeneration, neoblasts lost by amputation are being renewed (Fig. 4A). This is in contrast to normal tissue maintenance, where differentiated cells, but not necessarily neoblasts, are renewed. The possibility of stem cell renewal taking place in adult organisms can also be observed in obligatorily asexually reproducing animals. For example, the annelid *Dorvillea bermudensis* (Åkesson and Rice, 1992) has been known to reproduce in laboratory cultures exclusively by transverse fission for more than 30 years.
2) During regeneration, all amputated tissues are completely renewed. Morphologically, the regeneration of small fragments of *M. lignano* presumably passes through several stages of postembryonic development again: after wound healing and blastema formation, the remaining fragment undergoes morphallactic changes, shrinking the remaining tissues (rostrum, pharynx) to proportions similar to a freshly hatched juvenile (Egger et al., 2006, 2007). Subsequently, the juvenile and the regenerating animal face identical challenges: both must increase their size considerably by forming new tissue, and need to develop gonads and copulatory organs (Fig. 4B). It appears likely, therefore, that during regeneration some of the same genetic pathways are used as in postembryonic and possibly even as in embryonic development (see Egger et al., 2007). Monitoring gene expression during postembryonic development and regeneration will clarify this question.
3) Animals are experiencing so-called "mild stress" during amputation and regeneration (Fig. 4C). Exposure to mild stress including, for example, irradiation, starvation, heat stress, hypergravity and free radicals, has been shown to increase the lifespan of cells and organisms (Rattan, 2008). Amputation can be considered a form of mild stress, at least in animals capable of regeneration.

All of these hypotheses are non-exclusive and could very well act together to increase the individual lifespan.

In some flatworms, starvation was also found to increase longevity. It is suggested that the smaller the fragment left to regenerate, or the longer the period of starvation is, the more pronounced the rejuvenation effect is (see references in Haranghy and Balázs, 1964). Like most flatworms, *M. lignano* can cope with extended periods of starvation (months) by shrinkage (termed 'degrowth' in Baguñà, 1976; Nimeth et al., 2004; Pfister et al., 2008). During starvation, the animals shrink, absorb reproductive organs and other tissues, and assume a shape that strongly resembles a hatchling. The expression of the *vasa*-like gene *macvasa* in gonads and stem cells is greatly reduced but animals recover to normal morphology and gene expression upon refeeding (Pfister et al., 2008). However, for *M. lignano*, it still needs to be tested whether starvation has a similar influence on longevity as regeneration.

In addition to starvation, the effects of dietary restriction can also be studied in *M. lignano* because it is possible to manipulate calorie uptake by limiting the food source without inducing starvation (=malnutrition) (Vizoso and Schärer, 2007). Dietary restriction (defined as caloric restriction without malnutrition) slows ageing in yeast, flies, nematodes, fish and rodents. *Sir*2 is conserved from yeast to flies, worms and mammals (Fabrizio et al., 2005) and may be involved in dietary restriction mediated determination of lifespan. We have studied the expression of a *sir*2-like gene in *M. lignano*. Surprisingly, *sir*2 mRNA was found in a certain cell type – called goblet cells – in the gastrodermis (Fig. 5). These cells can also be identified with the monoclonal antibody MDr-2 (Fig. 5) that was raised for *M. lignano* (Ladurner et al., 2005b).

7. Conclusion

M. lignano is a flatworm that can be easily cultured. It has a relatively short lifespan and an accessible stem cell population. Because an experimental toolbox is available for all the levels of organisation, this species is an ideal model organism for obtaining more insight into the reciprocal influence between stem cells and the ageing process. Furthermore, this species is a suitable model system for studying lifespan extension and rejuvenation after starvation and repeated regeneration.

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