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UNUSUAL MODEL SYSTEMS FOR CELL DEATH RESEARCH

Autophagy and apoptosis in planarians

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Abstract Adult planarians are capable of undergoing regeneration and body remodelling in order to adapt to physical damage or extreme environmental conditions. Moreover, most planarians can tolerate long periods of starvation and during this time, they shrink from an adult size to, and sometimes beyond, the initial size at hatching. Indeed, these properties have made them a classic model to study stem cells and regeneration. Under such stressful conditions, food reserves from the gastrodermis and parenchyma are first used up and later the testes, copulatory organs and ovaries are digested. More surprisingly, when food is again made available to shrunken individuals, they grow back to adult size and all their reproductive structures reappear. These cycles of growth and shrinkage may occur over long periods without any apparent impairment to the individual, or to its future maturation and breeding capacities. This plasticity resides in a mesoderm tissue known as the parenchyma, which is formed by several differentiated non-proliferating cell types and only one mitotically active cell type, the neoblasts, which represent approximately 20–30% of the cells in the parenchyma. Neoblasts are generally thought to be somatic stem-cells that participate in the normal continuous turnover of all cell types in planarians. Hence, planarians are organisms that continuously adapt their bodies (morphallaxis) to different environmental stresses (i.e.: injury or starvation). This adaptation involves a variety of processes including proliferation, differentiation, apoptosis and autophagy, all of which are perfectly orchestrated and tightly regulated to remodel or restore the body pattern. While neoblast biology and body re-patterning are currently the subject of intense research, apoptosis and autophagy remain much less studied. In this review we will summarize our current understanding and hypotheses regarding where and when apoptosis and autophagy occur and fulfil an essential role in planarians.

Keywords Planarian · Autophagy · Apoptosis · Cell death · Regeneration · Remodelling

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Introduction

Autophagy or macroautophagy is a process that occurs at a basal level in most cells as a mechanism to eliminate protein aggregates and damaged organelles. These cargos are sequestered within cytoplasmic double-membrane vesicles called autophagosomes that are finally delivered to lysosomes for bulk degradation. Autophagy is also activated during starvation and it contributes to the recycling of nutrients to maintain protein synthesis, to produce substrates for oxidation and for ATP synthesis and to contribute to the inhibition of apoptosis. Thus, autophagy is a cell survival mechanism. However, under certain circumstances, autophagy can be a cell death process, which morphologically is clearly different from classical apoptosis, because it starts by degrading the cell's own organelles. Cytoplasmic disorganization with electron-dense autophagic vesicles and blebbing of the plasma membrane are the main features of autophagy, while degradation of

the DNA is not a necessary condition. Apoptosis is the best described mechanism of programmed cell death in Metazoans, and it often leads to rapid removal of cells via phagocytosis. It involves the activation of proteases by signalling cascades, leading to stereotypical morphological characteristics which include: nuclear chromatin condensation, nuclear fragmentation, cell shrinkage, blebbing of the plasma membrane, and maintenance of organelle integrity. This was just a quick overview of these two very complex processes that we will explore in detail and in the context of planarian homeostasis and regeneration.

The term planarians refers to the free-living freshwater flatworms in the phylum Platyhelminthes, Order Tricladida and Class Turbellaria, which belong to the Lophotrocozoan clade of the Protostomes [1]. Planarians are dorso-ventrally flattened bilaterians that lack a coelom, as well as a circulatory, respiratory and skeletal system (Fig.1). The three-branched digestive system that is characteristic of the triclads is connected to the pharynx (the mouth opening) in the middle of the ventral side of the planarian, although the gut is blind and thus, these animals lack an anus. The connective tissue between the body wall musculature and the gut is called the parenchyma or mesenchyme, and it contains diverse cell types such as: epidermal cells, gland cells, fixed parenchymal cells that connect different cell types and tissues, pigment cells, and neoblasts. Neoblasts are somatic stem cells that represent the only proliferating cell type in planarians and they can give rise to all other planarian cell types [2–7]. In the anterior part of the body, a pair of eyes (or more, depending on the species), can be distinguished on the dorsal side that consist of photoreceptor cells and pigment cells. The axons of these photoreceptor cells project to the brain, producing a partial chiasm that is bilobed and located on the ventral side of the animal. The brain is further connected to the two ventral nerve cords that run longitudinally along the lateral sides of the planarian body and that fuse in the tail [8, 9]. The animal is not segmented, although the digestive system and the nervous system show some iteration. The body lacks a circulatory system and the diffusion of factors such as oxygen and nutrients takes place over short distances in the flat body tissue. The movement of planarians is produced by a combination of muscle movements, slime secretion and the use of the ciliated ventral epidermal tissue and adhesive glands. Indeed, they represent one of the largest organisms to employ ciliary locomotion since freshwater planarians can reach lengths of up to 2.5 cm or even 40–50 cm in the case of some species in Lake Baikal [2].

Planarians are usually hermaphrodites and some often possess the ability to reproduce both asexually and sexually. In the period of asexual reproduction, planarians do not have mature reproductive organs and these must be formed *de novo* when the animal enters periods of sexual reproduction [10, 11]. However, recent molecular data suggests that the asexual organism does in fact possess primordial germ cells [11–14]. The reproductive organs degenerate when planarians shift from sexual to asexual reproduction as well as during regeneration, starvation and

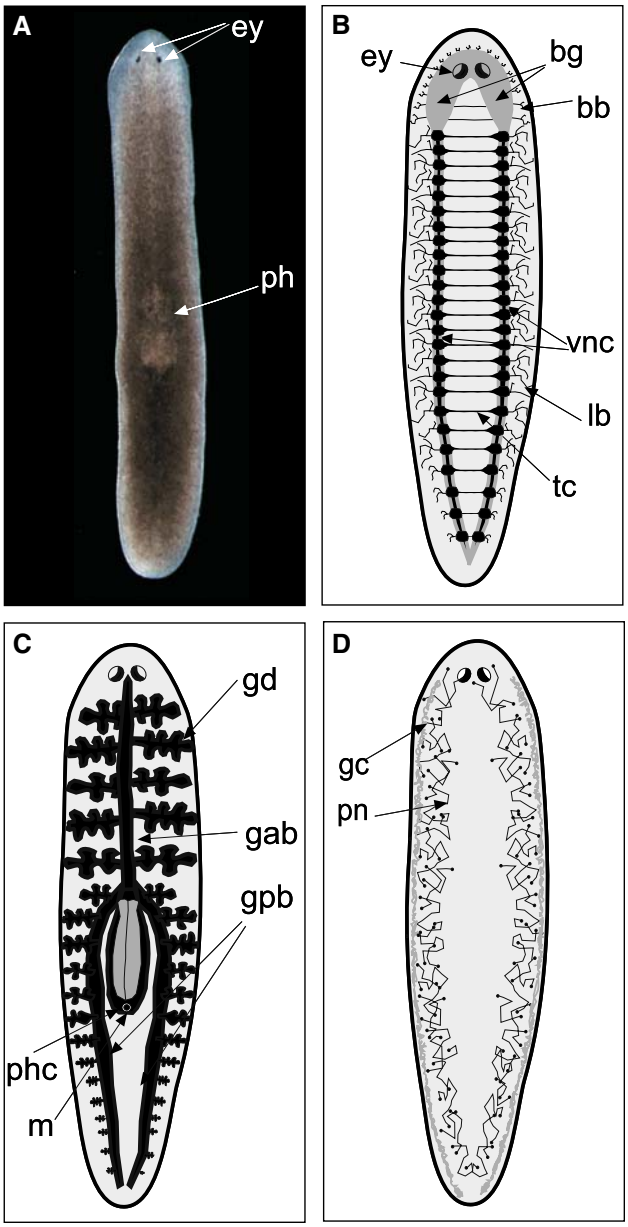

Fig. 1 *Schmidtea mediterranea* asexual strain morphology. **a** Dorsal view of the external morphology. The eyes (ey) and the pharynx (ph) are characteristic external features. **b** Central nervous system. *bb* brain branches, *bg* brain ganglia, *ey* eyes, *lb* lateral branches, *tc* transversal commissures, *vnc* ventral nerve cord. **c** Digestive system. *gab* gut anterior branch, *gd* gut diverticula, *gpb* gut posterior branches, *m* mouth, *phc* pharynx cavity. **d** Excretory system. *gc* subepidermal ventral and marginal adhesive gland cells, *pn* protonephridial network. **b**, **c** and **d** are systems that have to be remodelled during regeneration, degrowth during starvation and in the normal turnover of the adult organism

after non-lethal doses of X-rays [2, 15–17], although the reproductive organs will grow back when such stressful conditions remit. The use of two reproductive strategies is an adaptation to external conditions, especially temperature and food availability [18]. Asexual reproduction occurs by transverse fission whereas sexual reproduction normally occurs by cross-fertilization between the two hermaphroditic planarians. The fertilized ectolecithal polyembryonic egg capsule (the yolk cells are located outside the egg) is then laid and attached to a given surface. Subsequently, the juveniles hatch after a period of 10 days to several weeks, depending on the species and the temperature [18].

Planarians have been a favoured model to study regeneration for more than two centuries. Almost any piece of a planarian can regenerate a full organism and this amazing plasticity is also reflected in their ability to control their morphology and cell turnover. To maintain tissue homeostasis there is a continuous renewal of all cell types, and degrowth (shrinkage) of planarians occurs by reducing the total body cell number as a natural consequence of restricted food availability [19]. When food becomes available again, the planarian grows back to its original size by again increasing cell number [19]. Accordingly, growth and degrowth seem to always exist in a dynamic equilibrium. However, the plasticity referred to in these animals relies on a single cell type: the neoblast. Neoblasts are somatic stem cells that represent approximately 20–30% of the cells in the parenchyma. Until very recently, stem cells were only recognized by: (1) their morphology, as they are small cells of about 5–10 μm with a high nuclear-cytoplasmic ratio; (2) their location, as they are distributed throughout the parenchyma but not in the most anterior part of the head and pharynx; and (3) the fact that they are the only dividing cell in planarians [20–23]. In the last 10 years, a large effort has focused on investigating the nature of the neoblast and as a result, there are currently many specific neoblast markers available and BrdU incorporation can be examined in planarians. Moreover, FACS technology has recently been applied to the study of planarian neoblasts and the first lineage tracing experiments have been done [24–34]. These new techniques show that neoblasts are a very heterogeneous population. Together with the fast advance in the adaptation of molecular tools to planarians [35–40] and the sequencing of the genome of the species *Schmidtea mediterranea* [41, 42], all these studies have helped to establish this planarian as a model organism that is ideal to study stem cell biology and regeneration.

Of all the plastic events that occur in planarians, regeneration is by far the best studied process. If a planarian is cut into smaller pieces, each piece will close the wound and neoblast proliferation will commence to produce the regenerative blastema. In about 2–3 days, the blastema will start differentiating the missing structures, beginning in the most distal region while the old tissue will be remodelled to obtain new proportions. For instance, *Schmidtea mediterranea* can restore all the missing structures in about 7 days and it will need only 7 more days to completely rescale to its new size (Fig. 2). Thus, regeneration involves the production of new tissue (epimorphosis) and the remodelling of the old tissue (morphallaxis) to generate a complete, proportioned and functional planarian [43].

Wound healing is a rapid process that takes place in the first 30 min following injury; without wound healing, regeneration will not succeed. Initially, an epidermal layer of existing tissue completely covers the wound thanks to muscle contraction in the wound area and the ensuing relaxation [44, 45]. The fast and intense proliferation and the local migration of neoblasts to the wound then generate new tissue, or a blastema, in which the neoblasts stop dividing and initiate their differentiation. This proliferation occurs in two mitotic peaks, one 4–8 h after injury and the other, at around 2–3 days [46, 47]. De-differentiation processes have not been found to date, except in one case where it was shown that the germ cells could contribute to blastema formation and differentiate into new structures [48, 49]. However, this process has been considered as transdetermination of partially determined stem cells, the germ line [50]. Finally, both classic observations and the results of more recent experiments suggest that the blastema cannot be formed without neoblasts. Cell renewal does not occur after X-ray irradiation, impeding the animal regenerating and finally leading to death. This lethal phenotype can be rescued by injecting a neoblast-enriched cell suspension, whereas the injection of a cell suspension enriched in differentiated cells does not rescue this effect and no mitosis is observed [3]. More recent experiments involving BrdU-incorporation have confirmed these observations [31].

Patterning during planarian regeneration occurs soon after wound healing. This has been demonstrated by grafting experiments that take advantage of the ability of the head and the pharynx, once determined, to inhibit the formation of another head and pharynx, respectively [4, 50]. In these experiments determination occurs very rapidly, within the first 24 h in the case of the head and up to 36 h for the pharynx, at a temperature of 17°C. Since this process is so rapid, it occurs in an extremely narrow piece of old tissue, because the blastema is hardly evident after 24 h of regeneration. All these classic patterning experiments are now reinforced by the expression patterns described for neural cell differentiation markers (reviewed in [8]). As such, planarians are becoming a good model system for the study of re-patterning. Studies carried out over the past 2 years have identified the role of

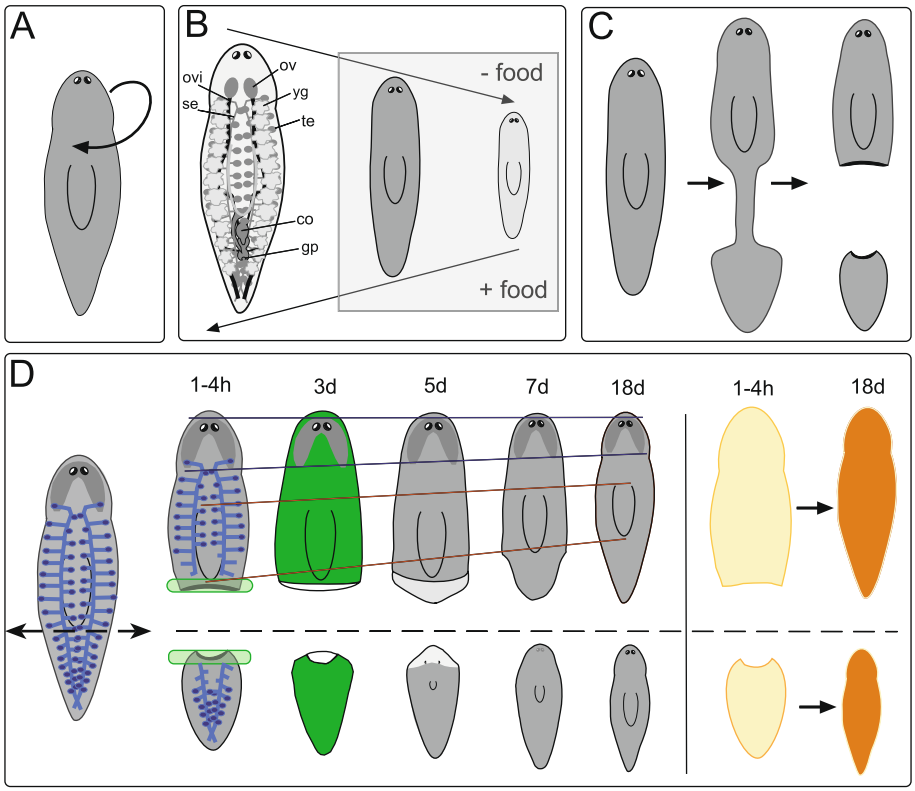

Fig. 2 Cell death in planarians. **a** Cell death happens as part of the normal cell turnover in the adult planarian. **b** During food shortage, planarians suffer a process of degrowth. Degrowing involves cell death; in the case of the sexual race, first the reproductive system undergoes cell death. The order of disappearance of the gonads is not clear yet. The process of body degrowth is resumed in case of food availability. The *grey square* indicates the process of body degrowing/growing of the asexual strain. *co* copulatory apparatus, *gp* genital pore, *ov* ovaries, *ovi* oviduct, *se* seminal duct, *te* testis, *yg* yolk glands. **c** *Schmidtea mediterranea* asexual planarians reproduce by transversal fission at the post-pharyngeal level. Fission involves cell death. However, it is not known either if there is activation of cell death in preparation of cell fission or if cell death occurs only during the separation of both pieces. The resulting planarian pieces will regenerate two complete planarians. **d** Regeneration involves cell death. If a sexual planarian (for simplification only the brain, the pharynx and the testes are shown) is cut (*dotted double arrow*) at the post-pharyngeal level, the resulting fragments will regenerate a perfectly scale planarian in about 3 weeks. It is known that a burst of cell death happens in a restricted area of about 100 μm at the wound site from 1–4 h after amputation (transparent box); another cell death peak, although more systemic happens at 3 d after amputation. In addition, the reproductive system undergoes cell death during regeneration (represented by the testes); however, the timing and the order of disappearance of the gonads is not known yet. Cell death plays also a role in the remodelling of all planarian organs during regeneration; for instance the brain and the pharynx will be adjusted to the new planarian size (*marked by two parallel lines*). Finally, the whole planarian parenchyma is also extremely remodelled during regeneration (see comparison of the shapes of the bodies for 1–4 h regenerating planarian and 18 d regeneration). *h* hours, *d* days

evolutionary conserved signalling pathways in the re-establishment and maintenance of anterior–posterior (A–P), dorsal–ventral (D–V), and medial–lateral (M–L) polarity, as well as significantly advancing our understanding of early events during planarian regeneration (reviewed in [51]). As a result, several elements of the Wnt pathway have been functionally characterized and for instance, the loss of β-catenin [52–54] or the ligands of Wnt [55] produces "posterior heads" or hypercephalized organisms. By contrast, gain of Wnt signalling, as achieved by interfering with elements of the destruction complex such as APC, produces the reciprocal phenotype "anterior tails" [52]. Bone morphogenetic protein (BMP) signalling has also been demonstrated to regulate planarian dorso-ventral and midline patterning. Indeed, the disruption of several elements of the BMP pathway produces ventralization phenotypes as well as indented blastemas, which are probably due to disrupted midline re-patterning [56–58].

Besides the re-patterning of the blastema remodelling of the old tissue also occurs to adjust it to the new proportions. Remodelling is a very complex process, requiring the perfect orchestration of cell proliferation, cell differentiation, autophagy and apoptosis. How all these processes are regulated is not yet fully understood. Indeed, remodelling has been studied by analysing the changes in the expression of developmental genes, such as the *Hox* genes, during regeneration. These studies indicated that within 2 days, positional values are reset to adapt the whole body to the

new situation (reviewed in [4]). Indeed, the analysis of Gt-POU-1protein C6 epitope during regeneration, a protein expressed in some nerve cells located at the anterior third of the body, also revealed that body size is quickly readapted. During tail regeneration the whole head fragment silences the expression of this epitope and it re-appears in the new anterior third region while it is lost from the rest of old tissue [59].

Despite all the interest in these processes, cell death has become the forgotten issue in planarian biology, although we will attempt to redress this situation in this review. We will start by defining the temporal and spatial moments that cell death would be expected to play an important role. Subsequently, we will present the information currently available about cell death in two different sections, one dealing with apoptosis and the other autophagy. Although other types of cell death may exist in planarians, such as necrosis, we shall not consider these here.

Hypothetical points of death in planarians

Adult tissue homeostasis

Well-fed planarians that have reached their maximum species specific size [60] can maintain their body size (Fig. 2). However, there is also a constantly proliferating population of neoblasts in such adult organisms and differentiation also occurs [33]. This implies that cell loss occurs continuously to maintain the homeostasis of the planarian body plan, thereby enabling them to maintain their size and cell number. Thus, cell death in adult tissues would be essential to contribute to the turnover of old or damaged tissues or organs, to selectively remove unfit populations of proliferating cells and perhaps, to respond to generally common environmental stresses such as changes in temperature, water pH, etc.

Growth and degrowth

Under conditions of food shortage, planarians suffer a process of body shrinking or degrowth (Fig. 2). Although they can reach a minimum size of less than one millimetre, they remain physiologically normal and they keep themselves perfectly proportioned and in scale throughout this process. This event is completely reversible and as such, they start the inverse process of growth when fed. It is known that degrowth primarily results from a change in cell number, rather than a change in cell size [61, 62]. Furthermore, there is no decrease in the number of proliferating neoblasts in response to starvation, as demonstrated in three experiments. In the first of these the mitotic index appeared to remain constant when calculated at different starvation points in planarians that were about 10 mm long at the end of the experiment [20]. In the second experiment, the mitotic index appeared to increase slightly when calculated at different starvation points in planarians that were about 10 mm long at the beginning of the experiment [20]. These results were in agreement with the observation that smaller planarians have a higher mitotic index that bigger ones [20]. The third study concluded that the number of PCNA positive cells (a marker that labels neoblasts) remains unaltered after 1 month starvation [30]. Therefore, cell death must explain the shrinkage of planarians during starvation.

Nevertheless, a massive and random increase in cell death alone could not explain degrowth because this would not preserve the perfect proportions of the planarians during periods of starvation. Therefore, while large-scale cell death of unnecessary cells or tissues may occur, more finely regulated cell death must be perfectly integrated with developmental signals. For instance, such processes will trim organ size to adapt them to the new body size. Indeed, the gonads are organs that are unnecessary to sexual strains during starvation and in fact, as we explained earlier, sexual planarians "re-absorb" their gonads during starvation. For the animal it is not worthwhile keeping the reproductive organs in a context where survival is the main priority and thus, they may undergo cell death and then readily grow back when starvation ends. Similar events might be active during the growth process where bulk cell death may not be necessary but finely regulated cell death would serve to proportion the different organs in the animal.

Repair and regeneration

From the point of view of cell death, regeneration may represent a complex process that unifies many different mechanisms of cell death (Fig. 2). Just after amputation, it is very likely that cell death events are activated as a response to the stress induced, a kind of cell death that may be more accidental than programmed. Nevertheless, the mitotic peaks that follow wound closure could again be coupled to some kind of cell death that would select the fitter neoblasts to contribute to the blastema. At the blastema, the re-patterning that occurs is integrated into the old tissue by remodelling, and the old organs have to definitively re-organize themselves to adjust to the new positional values that reflect the new size of the planarian. In this process, cell death is expected to play a very important role. In addition, other types of injury may activate other mechanisms of death, for example a small puncture, since the healing mechanism would depend more on remodelling processes than on the formation of a blastema.

Asexual reproduction

We can consider that there is one precise moment when programmed cell death may be essential for asexual reproduction to take place (Fig. 2). The reproduction of the asexual strain occurs by transverse fission and for instance, in *Schmidtea mediterranea* it occurs at the post-pharyngeal level. In this case, the tail of the animal attaches to the substrate while the head pulls away, generating two fragments that will regenerate two complete planarians. The specific point of division at the post-pharyngeal level could be where cell death is activated during asexual reproduction. Among other factors, fission can be stimulated by low population density, high temperature and large size, while it is inhibited at high population densities. Moreover, fission predominantly occurs in the dark and it is stimulated by head amputation [63]. Thus, all these environmental factors might contribute to the activation of signalling pathways ending with the activation of cell death at this precise site.

Autophagy: cell death and cell protection in planarians

Macroautophagy or autophagy is an evolutionary conserved mechanism that occurs continuously to maintain the homeostasis of all tissues. This cellular process involves the formation of double-membrane vacuoles, called autophagosomes, which sequester material from proteins to whole organelles. The content of these autophagosomes is then ultimately degraded following fusion to the lysosomal compartment. Autophagy was first discovered in yeast where more than 30 autophagy-related genes have been identified (*ATG1-ATG32*), and where autophagy has always been crucial to survival during starvation. Indeed, yeast cells will self-digest their own components in order to survive until the situation gets better ([64], and reviewed in [65]). Many of these *ATG* genes have also been found in higher eukaryotes, including mammals (reviewed in [66, 67]), and it is now clear that autophagy is important for many physiological and pathological processes in higher eukaryotes, such as cancer and neurodegenerative diseases [68]. This process is required for normal development [69–71] and surprisingly it participates in the clearance of apoptotic cells during embryogenesis [72]. During development it is usually associated with the degradation of large amounts of unnecessary tissues, for instance during the metamorphosis of many insects like *Drosophila* [73, 74]. Moreover, in adults autophagy seems to be involved in extending the life span and in protecting cells from the stress response, such as starvation [75]. Autophagic degradation of cellular constituents can efficiently recycle essential nutrients to sustain basic biological processes. Autophagy is also used as a defence mechanism to clear intracellular microbes, misfolded proteins and damaged organelles [76, 77].

Autophagy can be activated as an adaptation to stress that suppresses apoptosis, whereas in other settings autophagy may be an alternative way of dying. Therefore, the general consensus is currently that autophagy acts both in cell survival and in cell death. It is generally thought that autophagy must be tightly regulated so that is induced when needed, whereas otherwise it is maintained at a basal level. If the levels are less than basal, cells lose this "protection" and can die, usually by apoptosis, whereas when the levels are too high the cell may undergo autophagic cell death. In contrast to apoptosis, autophagic cell death is characterized by the early degradation of organelles by autophagic vesicles and the cell's own lysosomal system, while the cytoskeleton and nucleus are preserved until very late stages. In addition, it is very typical to observe a large number of double-membrane vacuoles. The molecular mechanisms underlying autophagic cell death are very complex since they are often linked to the apoptotic machinery. This complex relationship will be commented later in the "Apoptosis" section.

In planarians, some very old observations hinted at the importance of autophagy during regeneration. The fact that regeneration is slower under conditions of starvation and in detriment to the lack of a normal well sized blastema, suggested that planarians regenerate by remodelling existing tissue [43]. This remodelling process could happen perfectly by the activation of autophagy. In addition, the "regression" of the testes, ovaries, copulatory organs and yolk glands by sexual planarians during starvation, regeneration or after low-doses X-rays (see Introduction [78]) that was observed could be due to the removal of these extensive tissues by autophagy (Fig. 2). The first evidence of autophagy in planarians came from the work done by Bowen, Ryder and collaborators in the species *Polycelis tenuis*. By transmission electron microscopy (TEM) and biochemical studies of acid phosphatase activity, they observed autophagy at the end of the first week of starvation and autolysis during the following days (autophagic cell death: [79, 80]). During regeneration they also detected some autolysis [81], although little attention was subsequently paid to these exciting observations.

At the molecular level, we recently demonstrated the existence of autophagy and cell death in the planarian species *Girardia tigrina* through the study of a novel gene, *Gtdap-1* [82–85], the homologue of *DAP-1* (death-associated protein-1) in humans. The DAP-1 protein was originally identified, along with the protein DAP-kinase (DAP-2 or DAPk), as a positive mediator of programmed cell death induced by gamma-interferon in HeLa cells [86]. While DAPk has been well studied in other organisms, the role of

DAP-1 is less well understood. Therefore, we examined the role of the homologue of *DAP-1* in planarians, *Gtdap-1*, opening a new perspective on neoblast dynamics that is related to the promotion of autophagy in planarians. *Gtdap-1* expression is up-regulated specifically in the areas and at the time that remodelling occurs during regeneration and that takes place to achieve the correct scaling of the body. At day 5 of regeneration, when remodelling is at its peak, 44% of all cells express this gene and 39% of all neoblast-like cells or differentiating neoblasts also express *Gtdap-1*. Through TEM, we found that the *Gtdap-1* transcript is expressed in cells with autophagy morphology and never in cells with apoptotic morphology (Fig. 3). Interestingly, we detected that around 6% of *Gtdap-1* positive cells in a 5 day regenerating planarian contained cleaved caspase-3 but they were never labelled by TUNEL. Indeed, the profile of caspase-3 activity during regeneration correlates with *Gtdap-1* expression. Moreover, RNA interference (RNAi) for *Gtdap-1* decreases caspase-3 activity at 3 days of regeneration, reverting its activity to basal levels. Together, we established a link between the gene and cell death that was in accordance with the fact that gain-of-function mutants for *Gtdap-1* under the control of three artificial Pax6 dimeric binding sites (3xP3, [39]) produced areas of cell death in the cephalic region. Inhibiting *Gtdap-1* by RNAi also revealed remodelling defects and produced slower regeneration, which we attribute to a reduction in autophagy and autophagic programmed cell death, as well as a 30% decrease in neoblast proliferation. This suggests that autophagy and proliferation are coupled in stress-induced events that occur in planarians. We hypothesize that this correlation could be indirect and simply related to the balance between the "energy supply" offered by autophagy and "energy demand" created by the need to produce new cells. Accordingly, autophagy defects have been linked to cell proliferation in a human disease, cancer. However, the role of autophagy in cancer is not clear yet. Autophagy can act in tumour suppression by removing damaged organelles and reducing chromosome instability. At the same time, autophagy can act as a cytoprotective mechanism that helps cancer cells resist anti-cancer treatments and survive in conditions of low nutrient supply [68, 87–89]. Finally, we corroborated the earlier observations regarding the regression of the gonads following starvation and we found that *Gtdap-1* was expressed in the ovaries, testes and copulatory apparatus only under such adverse conditions.

The results of our experiments support the view that as well as a response to nutrient deprivation, widespread autophagy is also a response to injury in planarians, mainly in the process of body remodelling. During blastema formation, proliferation and differentiation of neoblasts must be a tremendously resource-demanding process. Moreover, no food can be eaten until a new functional central nervous system (CNS) and pharynx is formed. Thus, we propose that autophagy plays an essential role in fuelling this process and we also hypothesise that many neoblast-like cells that are in the process of differentiating during the scaling up of the organism can undergo transdetermination through autophagy.

Interestingly, we have never observed any expression of this gene in organs such as the pharynx, gut or brain ganglia. These organs of the old tissue have to be remodelled or scaled during the regenerative processes. We think that the kind of autophagy that is linked to *Gtdap-1* could be involved in the deletion of entire unnecessary structures, like gonads, or of just the mislocated cells in the parenchyma. Other types of cell death like apoptosis might play a role in the finer trimming of different organs.

We cannot rule out that dying cells expressing *Gtdap-1* are phagocytised before DNA is fragmented, as has commonly been observed (reviewed in [90]) and which would explain why we never find cells that are both *Gtdap-1*/TUNEL positive or cleaved caspase-3/TUNEL positive.

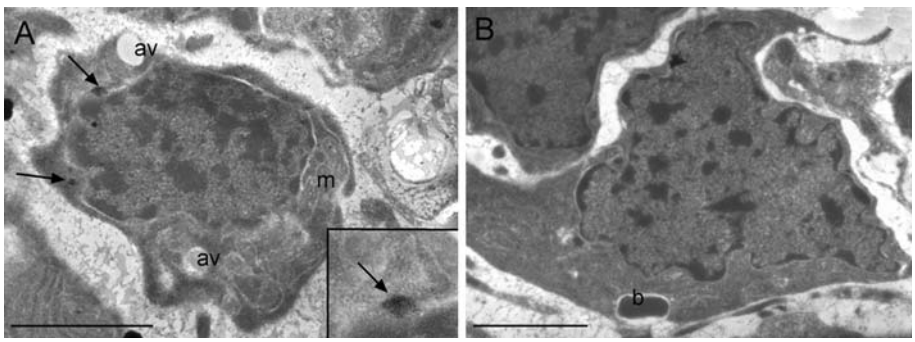

Fig. 3 Micrographs from in situ hybridization for TEM to detect *Gtdap-1* positive cells at the postblastema level of 5d-regenerating sexual *Girardia tigrina* species. **a** Cell undergoing autophagy where two visible autophagic vesicles (*AV*) can be observed. The gold particles (*arrows*) indicate that the cell is positive for *Gtdap-1* (see high magnification at the right bottom). **b** Differentiated cell negative for *Gtdap-1*, since no gold particles can be observed. *m* mitochondria, *b* phagocytic (bacterium) inclusion, *scale bars* indicate 2 μm

This means that despite displaying the morphological characteristics of autophagy, the kind of cell death in which *Gtdap-1* is involved could involve the apoptotic machinery, as occurs in *Drosophila* salivary glands (reviewed in [91]).

Autophagy also participates in cytoprotection and basal autophagy might be essential for neurons since these cells do not divide and thus, they cannot dilute the amount of waste they accumulate (e.g., protein aggregates). In fact, in human neurodegenerative diseases, mutations affecting the structure of neuron-specific proteins lead to the generation of constitutively misfolded proteins. This applies, for example, to alpha-synuclein in familial Parkinson's disease [92] or to huntingtin in Huntington's disease [93, 94]. These mutated proteins slowly accumulate in specific subpopulations of neurons and cause a progressive, age-dependent functional decline that is linked to pathological cell loss. Autophagy is strongly up-regulated by protein aggregates and oxidative stress, as well as damage affecting distinct cytoplasmic organelles [87]. Indeed, neuron specific knock-out of the autophagy genes *Atg5* or *Atg7* causes neurodegeneration in mice [95, 96] and moreover, cell death is activated when *Atg5* is specifically knocked out in T-cells [97]. Depletion of *beclin-1* during *C. elegans* development increases apoptosis and the presence of apoptotic cell corpses [98]. Together, these studies suggest that the pharmacological induction of autophagy may constitute a valuable strategy for the prevention and even the treatment of neurodegenerative diseases in humans [99]. In *Hydra* we find another example of autophagy as a cytoprotective mechanism [100] since an increase in autophagy has been observed after RNAi experiments of the gene *Kazal1*. This increase of autophagy led to cell death and subsequently animal death. *Kazal1* is expressed in *Hydra* gland cells and during regeneration this gene is immediately activated in regenerating tips. The conclusion from these studies is that the control of the level of autophagy is essential for cytoprotection and cell survival in a homeostatic and regenerative context. It is very likely that autophagy also plays a similar role in planarians, at least during the regeneration of the CNS. Studies on the planarian *atg* genes should reveal this role, although an indirect example of the possible role of autophagy in cytoprotection in the regenerating planarian brain has been seen when the *GSK3* genes were studied [101]. GSK3 is a protein that acts in the Wnt signalling pathway and it is also regulated by the PI3 K-AKT-Tor pathway, one of the main regulators of autophagy (reviewed in [102]). In fact, it has recently been shown that inhibiting *GSK-3b* in mammalian cell cultures can lead to the activation of mTOR, thereby impairing autophagy [103]. Interestingly, *GSK3* genes in planarians are expressed in the CNS of adult and regenerating planarians. Furthermore, inhibition of *GSK3*'s in planarians by the drug azakenpaullone interferes with the regeneration of brain ganglia. It has not been shown if autophagy is impaired after *GSK3*'s are inhibited or if apoptosis is one of the causes of the neural degeneration observed. Whether *GSK3* fulfils a cytoprotective role in the planarian CNS, at least in part by activating autophagy, remains to be tested.

Apoptosis

Apoptosis is the best described mechanism involved in programmed cell death in Metazoans. Apoptosis is important for adult tissue homeostasis, development, cell selection and repair or regeneration after damage or amputation. Apoptosis occurs as part of the constant turnover of cells and for example, skin [104] or gut cells [105] that have a high turnover rate, rely heavily on apoptosis. During development, apoptosis is necessary for the morphogenesis of many organs or tissues. For instance, apoptosis is utilized to sculpt the tetrapod limb, including the septation of the digits [106, 107], while embryonic cavities are formed by apoptosis of epiblast cells in the developing mouse embryo [108]. In addition, apoptosis plays an essential role in notochord development during the formation of the antero-posterior axis in *Xenopus* embryos [109].

However, the functional relationship between the different cell death processes is very complex, especially that of apoptosis and autophagy [110, 111]. Cross-talk between apoptosis and autophagy has been described on an increasing number of occasions [112]. Through in vitro cell cultures studies we know that apoptosis and autophagy can respond to very similar stimuli. Indeed, in some contexts where apoptotic components may be inhibited, the cell can compensate by activating autophagy and dying by this mechanism [113]. Autophagy is usually rapidly induced to promote survival, for instance HeLa cells cultured in the absence of nutrients, and inhibition of autophagy very early after the starvation induces cell death by apoptosis. However, if autophagy is inhibited later cell death acquires a mixed morphology between autophagy and apoptosis [114, 115]. Similarly, a mixed autophagic and apoptotic phenotype can be detected in some developmental contexts. Thus, it is not straightforward to define which kind of cell death occurs in a given developmental process. Indeed, in a cell with a morphologically apoptotic appearance, the autophagic components may be the primary elements activating the cell death process. For instance, overexpression of ectopic *ATG1* in *Drosophila* causes cell death with an apoptotic morphology but that is blocked by inhibiting *ATG3* or *ATG8a* [116]. Autophagic and apoptotic morphologies may also be evident at different times in the same cells. As such, cell death in the *Drosophila*

salivary glands is initiated in cells with an autophagic morphology, which is then followed by apoptotic DNA fragmentation. In this case, the inhibition of the caspase *drice* can partially prevent cell death [117] as also happens during the degeneration of larval organs during metamorphosis of other holometabolous insects [74]. Another very interesting example indicates that autophagy can also occur downstream of apoptosis. The last stage of apoptosis is the elimination of apoptotic cells by phagocytic cells. Very recently, autophagy has been associated with the exposure of phosphatidylserine by dying cells and in the secretion of signals to attract phagocytes that eliminate apoptotic cells during cavitation in mouse embryos [72].

The only comprehensive study on cell death in adult planarian tissue and during planarian degrowth associated with starvation does not mention apoptosis [79, 80]. However, these are classical studies from the end of the 70 s and beginning of the 80 s when the molecular techniques that could be applied to planarians were very limited. Recently, some papers demonstrated cell death in adult planarians by using TUNEL (TdT-mediated dUTP nick-end labelling) to detect DNA fragmentation [82, 118–121]. The most recent of these papers [120] developed a whole-mount TUNEL procedure to follow apoptosis in entire animals, which is a great advance compared to studies on dissociated cells or tissue sections. This technique allows for a quick, automated, and quantitative analysis of cell death in the entire animal. They were able to show a basal level of cell death in adults and an increase in TUNEL positive cells after 5 weeks of starvation. The mechanism of cell death, apoptosis or autophagy, still remains to be investigated. Finally, in the same paper they found a gene homologous to the human antiapoptotic gene *BCL-2*. They found that *Smed-bcl2-1* is required for cell survival in adult planarians.

Thus, the complexity of programmed cell death and the still missing links between the different types of cell death represent an important area for future studies. In addition, apoptosis may serve as a mechanism that contributes to a selection process. For example, it may act to select cells with the most appropriate receptors, as occurs during *T* cell selection in the immune system or as a selective mechanism for the correct connections in the nervous system. In addition, "cell competition" processes exemplify this role in selection [122–126]. It is well established that tissue development and homeostasis are regulated by the coordination of cell death and cell proliferation in multicellular organisms. Cell competition is the selective process that may be critical in this coordination. During cell competition the faster growing cells in a tissue eliminate adjacent slower growing cells by inducing cell death. Although much effort has been invested to define the molecular mechanism of cell competition, this still remains somewhat controversial [127–130]. Currently, cell competition is thought to play a role in the removal of viable but developmentally abnormal cells, which, for instance, do not interpret developmental cues correctly. It would be a mechanism to remove viable cells that are not developmentally adapted to the growing tissue [131]. There are several examples where cell competition may be acting in vertebrates. It has been suggested that cell competition may be a factor in tumour progression in circumstances in which tumour cells are able to outcompete normal cells [128, 132, 133]. Moreover, mouse cells heterozygous for a mutation defective in a riboprotein gene show decreased proliferation and are out-competed by wild-type cells [134]. Cell competition appears to play a role in rat liver reconstitution by transplanted stem cells [135]. Finally, cell competition may be playing a role in the maintenance of stem cell populations in their niche. That seems to be the case for the *Drosophila* gonad where both somatic stem cells and germline stem cells compete to occupy a given niche, although in this case the losing cells are displaced from the niche rather than killed [136, 137]. Thus, cell competition may represent a general phenomenon implicated in tissue homeostasis.

So far there is no evidence of cell competition in planarians, although they do possess a stable population of stem cells and the existence of a neoblast niche has been hypothesized [138]. Thus, it is likely that there is some cell competition in planarians and some research efforts are now focusing on this issue [138]. Cell competition may be occurring during normal planarian homeostasis as a selective process to favour only those genetically and metabolically fit neoblasts present in the niche. In addition, the post-blastema may represent another possible site for cell competition during regeneration. The post-blastema is the area just beneath the blastema or new tissue. This is the area where the most proliferation occurs and it will contribute to form the blastema. Hence, there is likely to be selective pressure on neoblasts in this domain since many stress signals converge at the stump.

In multicellular organisms, activation of apoptosis can trigger compensatory proliferation in surrounding cells to maintain tissue homeostasis. In other words, additional divisions of the remaining cells can compensate for the excessive cell loss in a developing tissue. Compensatory proliferation has mainly been described in experimental models where cells are killed by physical damage, surgery, or by an external factor such as irradiation [132, 139, 140]. For example, irradiation-induced cell death in the *Drosophila* wing imaginal disc is followed by compensatory cell proliferation, which results in an adult wing of nearly normal size [141]. The use of toxins to induce ectopic cell death in wing discs shows that the cells adjacent to the apoptotic cells undergo compensatory proliferation [142].

Currently, there is a big controversy about the possibility of apoptotic cells being responsible of inducing compensatory proliferation of neighbouring cells by secreting mitogens [132, 139, 140, 143, 144].

Nevertheless, compensatory proliferation seems to be a common process that also happens during regeneration in vertebrates and invertebrates. For instance, it happens during liver regeneration in vertebrates [145–147], although whether apoptosis drives this compensatory proliferation is still unknown. This question has been addressed recently and it was found that compensatory proliferation does in fact occur during regeneration of the invertebrate cnidarian *Hydra vulgaris* [148]. Strikingly, this process only occurs in anterior regeneration after mid-gastric bisection and indeed, apoptosis is both necessary and sufficient to induce Wnt3 secretion and head regeneration. Like the vertebrate liver, *Hydra* regenerate via morphallaxis while planarians do so by a combined process that implies epimorphosis and morphallaxis. In fact, wild type *Hydra* even regenerate when mitosis is inhibited [149], which distinguishes them from planarians. So far the only example of epimorphic regeneration in which apoptosis induces compensatory proliferation in blastema formation comes from *Drosophila* disc regeneration, which has been shown to be dependent on the caspase *dronc* [150].

There are only two works that show cell death with some features of apoptosis occurring during regeneration. The first uses TEM to investigate apoptosis in the first 3 h after amputation, observing that gland cells and fixed parenchymal cells became rounded in the parenchyma immediately below the wound surface, entering what is suggested as an apoptotic process. During the first 24 h, phagocytosis of cell debris and of these rounded cells is also observed [81, 151]. This agrees with the most recent discussed above about the developing of whole-mount TUNEL; there the authors also followed cell death during regeneration [120]. Very interestingly they found that amputation triggers two waves of cell death: a localised response near anterior and posterior wounds (100 microns) that peaks at 1–4 h after amputation and a latter more systemic response that peaks at 3 days after amputation (Fig. 2). Because these cell death peaks correspond to the mitotic peaks it would be interesting to test if planarian blastema formation, or any of the mitotic peaks observed during regeneration, depend on apoptosis. In fact the authors suggest that possibility since they have shown that initiation of the cell death responses dos not require neoblasts. However, incubating regenerating planarians with BOC-D-FMK, a pan-caspase inhibitor, does not seem to affect anterior or posterior planarian regeneration (González-Estévez C, de la Rosa EJ and Saló E, unpublished data), while Z-VAD-fmk, another pan-caspase inhibitor, inhibits anterior regeneration in *Hydra*. Finally, the work also shows that the systemic increase in cell death at 3 days after amputation promotes remodelling of uninjured organs such as the pharynx.

Moreover, Salvetti and co-workers [152] very recently suggested that compensatory proliferation may occur when planarians are irradiated with low doses of X-rays. Irradiation at lethal doses of X-rays specifically destroys planarian neoblasts [153], whereas non-lethal doses permit the organism to recover [152]. These authors show that non-lethal doses activate cell proliferation, which allows the neoblast system to be reconstituted and thus, the entire planarian. While 5 Gy irradiation (non-lethal doses) produces a general decrease in the number of neoblasts in the parenchyma of the animal, 7 days after this irradiation compensatory proliferation is activated on the ventral side of the animal, specifically in the areas surrounding the ventral nerve cords (VNC) and brain ganglia. This effect is followed by an increase in the number of proliferating neoblasts in the parenchyma, even higher than that in non-treated planarians, and the subsequent recovery of the animal. One possible explanation is that after low dose irradiation, the cell cycle of neoblasts is arrested due to DNA damage and/or they undergo apoptosis. In fact neoblasts undergo apoptosis after lethal doses of X-rays [30, 154] and they are then phagocytosed by reticular cells [30]. Previous research has shown the influence of the nervous system on planarian cell proliferation and/or regeneration [8, 155–157]. One possibility is that a neural-induced cell proliferation would compensate for the missing neoblasts by activating proliferation in the radioresistant cells. Another possibility is that the apoptotic cells liberate mitogens to stimulate proliferation of the radioresistant cells. The features that confer resistance to these cells are unknown but they may be committed/differentiating cells, or those in a particular stage of the cell cycle, which would explain why the cells are more radiotolerant.

Conclusions and future perspectives

The planarian field has advanced considerably in recent years, with many new tools becoming available, such as the sequencing of the genome of *Schmidtea mediterranea*, and many important discoveries about neoblast biology and re-patterning. However, it is still necessary for us to advance further in this field particularly in terms of our understanding of apoptosis and autophagy in planarians, and their association with regeneration and homeostasis. In order to make such advances it is essential to establish more techniques for the detection of apoptosis and autophagy in planarians that will complement the existing ones.

Moreover, a comprehensive study of the principal genes involved in apoptosis and autophagy, as well as their main developmental or metabolic regulators, would clarify some of the information missing regarding regeneration and remodelling.

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References

1. Philippe H, Lartillot N, Brinkmann H (2005) Multigene analyses of bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. Mol Biol Evol 22:1246–1253
2. Hyman L (1951) The Invertebrates: Platyhelminthes and Rhynchocoela— The Acoelomate Bilateria. McGraw-Hill Book Company Inc, New York
3. Baguñà J, Saló E, Auladell C (1989) Regeneration and pattern formation in planarians III. Evidence that neoblasts are totipotent stem cells and the source of blastema cells. Development 107:77–86
4. Saló E (2006) The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). Bioessays 28:546–559
5. Rossi L, Salvetti A, Batistoni R, Deri P, Gremigni V (2008) Planarians, a tale of stem cells. Cell Mol Life Sci 65:16–23
6. Sanchez Alvarado A (2006) Planarian regeneration: its end is its beginning. Cell 124:241–245
7. Reddien PW, Sanchez Alvarado A (2004) Fundamentals of planarian regeneration. Annu Rev Cell Dev Biol 20:725–757
8. Cebria F (2007) Regenerating the central nervous system: how easy for planarians!. Dev Genes Evol 217:733–748
9. Umesono Y, Agata K (2009) Evolution and regeneration of the planarian central nervous system. Dev Growth Differ 51:185–195
10. Curtis W (1902) The life history, the normal fission, and the reproductive organs of *Planaria maculata*. Proc Boston Soc Nat Hist 30:515–559
11. Newmark PA, Wang Y, Chong T (2008) Germ cell specification and regeneration in planarians. Cold Spring Harb Symp Quant Biol 73:573–581
12. Sato K, Shibata N, Orii H et al (2006) Identification and origin of the germline stem cells as revealed by the expression of nanos-related gene in planarians. Dev Growth Differ 48:615–628
13. Handberg-Thorsager M, Fernandez E, Salo E (2008) Stem cells and regeneration in planarians. Front Biosci 13:6374–6394
14. Wang Y, Zayas RM, Guo T, Newmark PA (2007) Nanos function is essential for development and regeneration of planarian germ cells. Proc Natl Acad Sci USA 104:5901–5906
15. Morgan TH (1902) Growth and regeneration in *Planaria lugubris*. Arch Entw Mech Org 13:179–212
16. Fedecka-Bruner B (1967) Differentiation of the male gonads in the planarian, *Dugesia lugubris*, during regeneration. C R Seances Soc Biol Fil 161:21–23
17. Berninger J (1911) Über die Einwirkung des Hungers auf Planarien. Zool Jahrb 30:181–216
18. Benazzi M, Gremigni V (1982) Developmental biology of triclad turbellarians (Planaria). In: Harrison FW, Cowden RR (eds) Developmental biologogy of freshwater invertebrates. Liss, New York, pp 151–211
19. Romero R, Baguñà J (1991) Quantitative cellular analysis of growth and reproduction in freshwater planarians (Turbellaria, Tricladida). I. A cellular description of the intact organism. Invertebr Reprod Dev 19:157–165
20. Baguñà J (1976) Mitosis in the intact and regenerating planarian *Dugesia mediterranea* n. sp. I. Mitotic studies during growth, feeding and starvation. J Exp Zool 195:53–64
21. Baguñà J (1976) Mitosis in the intact and regenerating planarian *Dugesia mediterranea* n. sp. II. Mitotic studies during regeneration and a possible mechanism of blastema formation. J Exp Zool 195:65–80
22. Morita M, Best JB (1984) Electron microscopic studies of planarian regeneration. IV. Cell division of neoblasts in *Dugesia dorotocephala*. J Exp Zool 229:425–436
23. Tiras K, Sakharova NY (1984) The vital computer morphometry of planarian regenration. Ontogenez 15:42–48
24. Reddien PW, Oviedo NJ, Jennings JR, Jenkin JC, Sanchez Alvarado A (2005) SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. Science 310:1327–1330
25. Salvetti A, Rossi L, Deri P, Batistoni R (2000) An MCM2-related gene is expressed in proliferating cells of intact and regenerating planarians. Dev Dyn 218:603–614
26. Salvetti A, Lena A, Rossi L et al (2002) Characterization of DeY1, a novel Y-box gene specifically expressed in differentiating male germ cells of planarians. Gene Expr Patterns 2:195–200
27. Salvetti A, Rossi L, Lena A et al (2005) *DjPum*, a homologue of *Drosophila Pumilio*, is essential to planarian stem cell maintenance. Development 132:1863–1874
28. Guo T, Peters AH, Newmark PA (2006) A Bruno-like gene is required for stem cell maintenance in planarians. Dev Cell 11:159–169
29. Solana J, Lasko P, Romero R (2009) Spoltud-1 is a chromatoid body component required for planarian long-term stem cell self-renewal. Dev Biol 328:410–421
30. Orii H, Sakurai T, Watanabe K (2005) Distribution of the stem cells (neoblasts) in the planarian *Dugesia japonica*. Dev Genes Evol 215:143–157
31. Newmark PA, Sanchez Alvarado A (2000) Bromodeoxyuridine specifically labels the regenerative stem cells of planarians. Dev Biol 220:142–153
32. Hayashi T, Asami M, Higuchi S, Shibata N, Agata K (2006) Isolation of planarian X-ray-sensitive stem cells by fluorescence-activated cell sorting. Dev Growth Differ 48:371–380
33. Eisenhoffer GT, Kang H, Sanchez Alvarado A (2008) Molecular analysis of stem cells and their descendants during cell turnover and regeneration in the planarian Schmidtea mediterranea. Cell Stem Cell 3:327–339
34. Conte M, Deri P, Isolani ME, Mannini L, Batistoni R (2009) A mortalin-like gene is crucial for planarian stem cell viability. Dev Biol 334:109–118
35. Newmark PA, Reddien PW, Cebria F, Sanchez Alvarado A (2003) Ingestion of bacterially expressed double-stranded RNA inhibits gene expression in planarians. Proc Natl Acad Sci USA 100(Suppl 1):11861–11865
36. Reddien PW, Bermange AL, Murfitt KJ, Jennings JR, Sanchez Alvarado A (2005) Identification of genes needed for

regeneration, stem cell function, and tissue homeostasis by systematic gene perturbation in planaria. Dev Cell 8:635–649
37. Zayas RM, Hernández A, Habermann B, Wang Y, Stary JM, Newmark PA (2005) The planarian *Schmidtea mediterranea* as a model for epigenetic germ cell specification: analysis of ESTs from the hermaphroditic strain. Proc Natl Acad Sci USA 102:18491–18496
38. Sanchez Alvarado A, Newmark PA, Robb SM, Juste R (2002) The Schmidtea mediterranea database as a molecular resource for studying platyhelminthes, stem cells and regeneration. Development 129:5659–5665
39. Gonzalez-Estevez C, Momose T, Gehring WJ, Salo E (2003) Transgenic planarian lines obtained by electroporation using transposon-derived vectors and an eye-specific GFP marker. Proc Natl Acad Sci USA 100:14046–14051
40. Gonzalez-Estevez C, Arseni V, Thambyrajah RS, Felix DA, Aboobaker AA (2009) Diverse miRNA spatial expression patterns suggest important roles in homeostasis and regeneration in planarians. Int J Dev Biol 53:493–505
41. Sánchez Alvarado A, Reddien PW, Newmark P, Nusbaum C (2003) Proposal for the sequencing of a new target genome: white paper for a planarian genome project. NHGRI
42. Robb SM, Ross E, Alvarado AS (2008) SmedGD: the Schmidtea mediterranea genome database. Nucleic Acids Res 36:D599–D606
43. Morgan TH (1901) Regeneration. Macmillan, New York
44. Pedersen KJ (1976) Scanning electron microscopical observations on epidermal wound healing in the planarain *Dugesia tigrina*. Wilhelm Roux Arch Dev Biol 179:251–273
45. Chandebois R (1980) The dynamics of wound closure and its role in the programming of planarian regeneration. II. Distalization. Dev Growth Differ 22:693–704
46. Saló E, Baguñà J (1984) Regeneration and pattern formation in planarians. I. The pattern of mitosis in anterior and posterior regeneration in *Dugesia* (G) *tigrina*, and a new proposal for blastema formation. J Embryol Exp Morphol 83:63–80
47. Saló E, Baguñà J (1989) Regeneration and pattern formation in planarians. II. Local origin and role of cell movements in blastema formation. Development 107:69–76
48. Gremigni V, Miceli C (1980) Cytophotometric evidence of cell "transdifferentiation" in planarian regeneration. Wilhelm Roux's Arch 188:107–113
49. Gremigni V, Nigro M, Puccinelli I (1982) Evidence of male germ cell redifferentiation into female germ cells in planarian regeneration. J Embryol Exp Morphol 70:29–36
50. Baguñà J, Saló E, Romero R, Garcia-Fernàndez J, Bueno D (1994) Regeneration and pattern formation in planarians: cells, molecules and genes. Zool Sci 11:781–795
51. Forsthoefel DJ, Newmark PA (2009) Emerging patterns in planarian regeneration. Curr Opin Genet Dev 19:412–420
52. Gurley KA, Rink JC, Sanchez Alvarado A (2008) Beta-catenin defines head versus tail identity during planarian regeneration and homeostasis. Science 319:323–327
53. Petersen CP, Reddien PW (2008) Smed-betacatenin-1 is required for anteroposterior blastema polarity in planarian regeneration. Science 319:327–330
54. Iglesias M, Gomez-Skarmeta JL, Salo E, Adell T (2008) Silencing of Smed-betacatenin1 generates radial-like hypercephalized planarians. Development 135:1215–1221
55. Adell T, Salo E, Boutros M, Bartscherer K (2009) Smed-Evi/Wntless is required for beta-catenin-dependent and -independent processes during planarian regeneration. Development 136:905–910
56. Orii H, Watanabe K (2007) Bone morphogenetic protein is required for dorso-ventral patterning in the planarian *Dugesia japonica*. Dev Growth Differ 49:345–349
57. Reddien PW, Bermange AL, Kicza AM, Sanchez Alvarado A (2007) BMP signaling regulates the dorsal planarian midline and is needed for asymmetric regeneration. Development 134:4043–4051
58. Molina MD, Salo E, Cebria F (2007) The BMP pathway is essential for re-specification and maintenance of the dorsoventral axis in regenerating and intact planarians. Dev Biol 311:79–94
59. Muñoz-Mármol AM, Casali A, Miralles A et al (1998) Characterization of platyhelminth POU domain genes: ubiquitous and specific anterior nerve cell expression of different epitopes of GtPOU-1. Mech Dev 76:127–140
60. Calow P (1981) Growth in lower invertebrates. Comp Anim Nutr 4:53–76
61. Baguñà J, Romero R (1981) Quantitative analysis of cell types during growth, degrowth and regeneration in the planarians *Dugesia mediterranea* and *Dugesia tigrina*. Hydrobiologia 84:181–194
62. Oviedo NJ, Newmark PA, Sanchez Alvarado A (2003) Allometric scaling and proportion regulation in the freshwater planarian Schmidtea mediterranea. Dev Dyn 226:326–333
63. Best JB, Goodman AB, Pigon A (1969) Fissioning in planarians: control by the brain. Science 164:565–566
64. Kanki T, Wang K, Cao Y, Baba M, Klionsky DJ (2009) Atg32 is a mitochondrial protein that confers selectivity during mitophagy. Dev Cell 17:98–109
65. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10:458–467
66. Yoshimori T, Noda T (2008) Toward unraveling membrane biogenesis in mammalian autophagy. Curr Opin Cell Biol 20:401–407
67. Kourtis N, Tavernarakis N (2009) Autophagy and cell death in model organisms. Cell Death Differ 16:21–30
68. Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451:1069–1075
69. Fimia GM, Stoykova A, Romagnoli A et al (2007) Ambra1 regulates autophagy and development of the nervous system. Nature 447:1121–1125
70. Tsukamoto S, Kuma A, Murakami M, Kishi C, Yamamoto A, Mizushima N (2008) Autophagy is essential for preimplantation development of mouse embryos. Science 321:117–120
71. Tsukamoto S, Kuma A, Mizushima N (2008) The role of autophagy during the oocyte-to-embryo transition. Autophagy 4:1076–1078
72. Qu X, Zou Z, Sun Q et al (2007) Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 128:931–946
73. Penaloza C, Lin L, Lockshin RA, Zakeri Z (2006) Cell death in development: shaping the embryo. Histochem Cell Biol 126:149–158
74. Tettamanti G, Salo E, Gonzalez-Estevez C, Felix DA, Grimaldi A, de Eguileor M (2008) Autophagy in invertebrates: insights into development, regeneration and body remodeling. Curr Pharm Des 14:116–125
75. Vellai T (2009) Autophagy genes and ageing. Cell Death Differ 16:94–102
76. Levine B, Deretic V (2007) Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol 7:767–777
77. Rubinsztein DC (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. Nature 443:780–786
78. Calow P (1978) Life cycles: and evolutionary approach to the physiology of reproduction, development and ageing. Chapman and Hall, London
79. Bowen I, Ryder T (1974) Cell autolysis and deletion in the planarian *Polycelis tenuis* Iijima. Cell Tissue Res 154:265–271

80. Bowen ID, Ryder T, Dark C (1976) The effects of starvation on the planarian worm *Polycelis tenuis* Iijima. Cell Tissue Res 169:193–209
81. Bowen ID, den Hollander JE, Lewis GH (1982) Cell death and acid phosphatase activity in the regenerating planarian Polycelis tenuis Iijima. Differentiation 21:160–167
82. Gonzalez-Estevez C, Felix DA, Aboobaker AA, Salo E (2007) Gtdap-1 promotes autophagy and is required for planarian remodeling during regeneration and starvation. Proc Natl Acad Sci USA 104:13373–13378
83. Gonzalez-Estevez C, Felix DA, Aboobaker AA, Salo E (2007) Gtdap-1 and the role of autophagy during planarian regeneration and starvation. Autophagy 3:640–642
84. Gonzalez-Estevez C (2008) Autophagy in freshwater planarians. Methods Enzymol 451:439–465
85. Gonzalez-Estevez C (2009) Autophagy meets planarians. Autophagy 5:290–297
86. Deiss LP, Feinstein E, Berissi H, Cohen O, Kimchi A (1995) Identification of a novel serine/threonine kinase and a novel 15-kD protein as potential mediators of the gamma interferon-induced cell death. Genes Dev 9:15–30
87. Levine B, Kroemer G (2008) Autophagy in the pathogenesis of disease. Cell 132:27–42
88. Liang XH, Jackson S, Seaman M et al (1999) Induction of autophagy and inhibition of tumorigenesis by beclin 1. Nature 402:672–676
89. Degenhardt K, Mathew R, Beaudoin B et al (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10:51–64
90. Krieser RJ, White K (2002) Engulfment mechanism of apoptotic cells. Curr Opin Cell Biol 14:734–738
91. McPhee CK, Baehrecke EH (2009) Autophagy in Drosophila melanogaster. Biochim Biophys Acta 1793:1452–1460
92. Pan T, Kondo S, Zhu W, Xie W, Jankovic J, Le W (2008) Neuroprotection of rapamycin in lactacystin-induced neurodegeneration via autophagy enhancement. Neurobiol Dis 32:16–25
93. Iwata A, Christianson JC, Bucci M et al (2005) Increased susceptibility of cytoplasmic over nuclear polyglutamine aggregates to autophagic degradation. Proc Natl Acad Sci USA 102:13135–13140
94. Sarkar S, Perlstein EO, Imarisio S et al (2007) Small molecules enhance autophagy and reduce toxicity in huntington's disease models. Nat Chem Biol 3:331–338
95. Hara T, Nakamura K, Matsui M et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441:885–889
96. Komatsu M, Waguri S, Chiba T et al (2006) Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature 441:880–884
97. Pua HH, Dzhagalov I, Chuck M, Mizushima N, He YW (2007) A critical role for the autophagy gene Atg5 in *T* cell survival and proliferation. J Exp Med 204:25–31
98. Takacs-Vellai K, Vellai T, Puoti A et al (2005) Inactivation of the autophagy gene bec-1 triggers apoptotic cell death in C. elegans. Curr Biol 15:1513–1517
99. Williams A, Jahreiss L, Sarkar S et al (2006) Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol 76:89–101
100. Chera S, de Rosa R, Miljkovic-Licina M et al (2006) Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human SPINK1 pancreatic phenotype. J Cell Sci 119:846–857
101. Adell T, Marsal M, Salo E (2008) Planarian GSK3s are involved in neural regeneration. Dev Genes Evol 218:89–103
102. Rayasam GV, Tulasi VK, Sodhi R, Davis JA, Ray A (2009) Glycogen synthase kinase 3: more than a namesake. Br J Pharmacol 156:885–898
103. Inoki K, Ouyang H, Zhu T et al (2006) TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell 126:955–968
104. Yen TH, Wright NA (2006) The gastrointestinal tract stem cell niche. Stem Cell Rev 2:203–212
105. Lippens S, Hoste E, Vandenabeele P, Agostinis P, Declercq W (2009) Cell death in the skin. Apoptosis 14:549–569
106. Mori C, Nakamura N, Kimura S, Irie H, Takigawa T, Shiota K (1995) Programmed cell death in the interdigital tissue of the fetal mouse limb is apoptosis with DNA fragmentation. Anat Rec 242:103–110
107. Zuzarte-Luis V, Hurle JM (2005) Programmed cell death in the embryonic vertebrate limb. Semin Cell Dev Biol 16:261–269
108. Coucouvanis E, Martin GR (1995) Signals for death and survival: a two-step mechanism for cavitation in the vertebrate embryo. Cell 83:279–287
109. Malikova MA, Van Stry M, Symes K (2007) Apoptosis regulates notochord development in Xenopus. Dev Biol 311:434–448
110. Levine B, Yuan J (2005) Autophagy in cell death: an innocent convict? J Clin Invest 115:2679–2688
111. Baehrecke EH (2005) Autophagy: dual roles in life and death? Nat Rev Mol Cell Biol 6:505–510
112. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G (2007) Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 8:741–752
113. Shimizu S, Kanaseki T, Mizushima N et al (2004) Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 6:1221–1228
114. Gonzalez-Polo RA, Boya P, Pauleau AL et al (2005) The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 118:3091–3102
115. Boya P, Gonzalez-Polo RA, Casares N et al (2005) Inhibition of macroautophagy triggers apoptosis. Mol Cell Biol 25:1025–1040
116. Scott RC, Juhasz G, Neufeld TP (2007) Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol 17:1–11
117. Martin DN, Baehrecke EH (2004) Caspases function in autophagic programmed cell death in Drosophila. Development 131:275–284
118. Bueno D, Fernandez-Rodriguez J, Cardona A, Hernandez-Hernandez V, Romero R (2002) A novel invertebrate trophic factor related to invertebrate neurotrophins is involved in planarian body regional survival and asexual reproduction. Dev Biol 252:188–201
119. Hwang JS, Kobayashi C, Agata K, Ikeo K, Gojobori T (2004) Detection of apoptosis during planarian regeneration by the expression of apoptosis-related genes and TUNEL assay. Gene 333:15–25
120. Pellettieri J, Fitzgerald P, Watanabe S, Mancuso J, Green DR, Alvarado AS (2009) Cell death and tissue remodeling in planarian regeneration. Dev Biol. doi:10.1016/j.ydbio.2009.09.015
121. Inoue T, Hayashi T, Takechi K, Agata K (2007) Clathrin-mediated endocytic signals are required for the regeneration of, as well as homeostasis in, the planarian CNS. Development 134:1679–1689
122. Lambertsson A (1998) The minute genes in Drosophila and their molecular functions. Adv Genet 38:69–134
123. Morata G, Ripoll P (1975) Minutes: mutants of drosophila autonomously affecting cell division rate. Dev Biol 42:211–221
124. Simpson P (1979) Parameters of cell competition in the compartments of the wing disc of Drosophila. Dev Biol 69:182–193
125. Simpson P, Morata G (1981) Differential mitotic rates and patterns of growth in compartments in the Drosophila wing. Dev Biol 85:299–308
126. Bryant PJ, Simpson P (1984) Intrinsic and extrinsic control of growth in developing organs. Q Rev Biol 59:387–415

127. Moreno E, Basler K, Morata G (2002) Cells compete for decapentaplegic survival factor to prevent apoptosis in Drosophila wing development. Nature 416:755–759
128. Moreno E, Basler K (2004) dMyc transforms cells into supercompetitors. Cell 117:117–129
129. Li W, Baker NE (2007) Engulfment is required for cell competition. Cell 129:1215–1225
130. de la Cova C, Abril M, Bellosta P, Gallant P, Johnston LA (2004) Drosophila myc regulates organ size by inducing cell competition. Cell 117:107–116
131. Martin FA, Herrera SC, Morata G (2009) Cell competition, growth and size control in the Drosophila wing imaginal disc. Development 136:3747–3756
132. Perez-Garijo A, Martin FA, Morata G (2004) Caspase inhibition during apoptosis causes abnormal signalling and developmental aberrations in Drosophila. Development 131:5591–5598
133. Moreno E (2008) Is cell competition relevant to cancer? Nat Rev Cancer 8:141–147
134. Oliver ER, Saunders TL, Tarle SA, Glaser T (2004) Ribosomal protein L24 defect in belly spot and tail (Bst), a mouse minute. Development 131:3907–3920
135. Oertel M, Menthena A, Dabeva MD, Shafritz DA (2006) Cell competition leads to a high level of normal liver reconstitution by transplanted fetal liver stem/progenitor cells. Gastroenterology 130:507–520 (Quiz 590)
136. Jin Z, Kirilly D, Weng C et al (2008) Differentiation-defective stem cells outcompete normal stem cells for niche occupancy in the Drosophila ovary. Cell Stem Cell 2:39–49
137. Nystul T, Spradling A (2007) An epithelial niche in the Drosophila ovary undergoes long-range stem cell replacement. Cell Stem Cell 1:277–285
138. Pellettieri J, Sanchez Alvarado A (2007) Cell turnover and adult tissue homeostasis: from humans to planarians. Annu Rev Genet 41:83–105
139. Huh JR, Guo M, Hay BA (2004) Compensatory proliferation induced by cell death in the Drosophila wing disc requires activity of the apical cell death caspase Dronc in a nonapoptotic role. Curr Biol 14:1262–1266
140. Ryoo HD, Gorenc T, Steller H (2004) Apoptotic cells can induce compensatory cell proliferation through the JNK and the Wingless signaling pathways. Dev Cell 7:491–501
141. James AA, Bryant PJ (1981) A quantitative study of cell death and mitotic inhibition in gamma-irradiated imaginal wing discs of Drosophila melanogaster. Radiat Res 87:552–564
142. Milan M, Campuzano S, Garcia-Bellido A (1997) Developmental parameters of cell death in the wing disc of Drosophila. Proc Natl Acad Sci USA 94:5691–5696
143. Fan Y, Bergmann A (2008) Distinct mechanisms of apoptosis-induced compensatory proliferation in proliferating and differentiating tissues in the Drosophila eye. Dev Cell 14:399–410
144. Perez-Garijo A, Shlevkov E, Morata G (2009) The role of Dpp and Wg in compensatory proliferation and in the formation of hyperplastic overgrowths caused by apoptotic cells in the Drosophila wing disc. Development 136:1169–1177
145. Taub R (2004) Liver regeneration: from myth to mechanism. Nat Rev Mol Cell Biol 5:836–847
146. Michalopoulos GK (2007) Liver regeneration. J Cell Physiol 213:286–300
147. Kan NG, Junghans D, Izpisua Belmonte JC (2009) Compensatory growth mechanisms regulated by BMP and FGF signaling mediate liver regeneration in zebrafish after partial hepatectomy. FASEB J 23:3516–3525
148. Chera S, Ghila L, Dobretz K et al (2009) Apoptotic cells provide an unexpected source of Wnt3 signaling to drive hydra head regeneration. Dev Cell 17:279–289
149. Park HD, Ortmeyer AB, Blankenbaker DP (1970) Cell division during regeneration in Hydra. Nature 227:617–619
150. Wells BS, Yoshida E, Johnston LA (2006) Compensatory proliferation in Drosophila imaginal discs requires Dronc-dependent p53 activity. Curr Biol 16:1606–1615
151. Hori I (1991) Role of fixed parenchyma cells in blastema formation of the planarian *Dugesia japonica*. Int J Dev Biol 35:101–108
152. Salvetti A, Rossi L, Bonuccelli L et al (2009) Adult stem cell plasticity: neoblast repopulation in non-lethally irradiated planarians. Dev Biol 328:305–314
153. Wolff E, Dubois F (1948) Sur la migration des cellules de régénération chez les planaires. Rev Swisse Zool 55:218–227
154. Rossi L, Salvetti A, Lena A et al (2006) DjPiwi-1, a member of the PAZ-Piwi gene family, defines a subpopulation of planarian stem cells. Dev Genes Evol 216:335–346
155. Baguñà J, Saló E, Romero R (1989) Effects of activators and antagonists of the neuropeptides substance P and substance K on cell proliferation in planarians. Int J Dev Biol 33:261–264
156. Bautz A, Schilt J (1986) Somatostatin-like peptide and regeneration capacities in planarians. Gen Comp Endocrinol 64:267–272
157. Saló E, Baguñà J (1985) Proximal and distal transformation during intercalary regeneration in the planarian *Dugesia (S) mediterranea*. Roux's Arch Dev Biol 194:364–368