

Addendum

Gtdap-1 and the Role of Autophagy During Planarian Regeneration and Starvation

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Addendum to:

Gtdap-1 Promotes Autophagy and is Required for Planarian Remodeling During Regeneration and Starvation

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ABSTRACT

Planarians have been established as an ideal model organism for stem cell research and regeneration. Planarian regeneration and homeostasis require an exquisite balancing act between cell death and cell proliferation as new tissues are made (epimorphosis) and existing tissues remodeled (morphallaxis). Some of the genes and mechanisms that control cell proliferation and pattern formation are known. However, studies about cell death during remodeling are few and far between. We have studied the gene *Gtdap-1*, the planarian ortholog of human death-associated protein-1 or *DAP-1*. *DAP-1* together with *DAP-kinase* has been identified as a positive mediator of programmed cell death induced by gamma-interferon in HeLa cells. We have found that the gene functions at the interface between autophagy and cell death in the remodeling of the organism that occurs during regeneration and starvation in sexual and asexual races of planarians. Our data suggest that autophagy of existing cells may be essential to fuel the continued proliferation and differentiation of stem cells by providing the necessary energy and building blocks to neoblasts.

PLANARIAN, A MODEL SYSTEM FOR STEM CELL RESEARCH AND REGENERATION

Whereas many types of regenerative phenomena exist in animals, planarians (Fig. 1A) display an extreme capacity for regeneration, and this property has made them a classical model for studying the regeneration process.¹ The close to completed genome sequence from the species *Schmidtea mediterranea* together with the recent advances in our use of planarians as a model system for the study of stem cells opens up new avenues of exciting research.²⁻⁵

The potential importance of planarians as a model system for studies of regeneration stems from the fact that adults have a stable population of somatic stem cells called neoblasts. These stem cells are spread throughout the body, with the notable exception of the head and the pharynx, and together with their immediate progeny are approximately 15–25% of total cell number.⁶ The stem cells participate in the perpetual homeostatic turnover of all cell types in planarians and contribute to its great corporal plasticity which can be easily observed during regeneration and starvation.

Planarians can regenerate along any body axis, which allows them to reproduce asexually by fission but also to restore missing parts after injury (Fig. 1C). Even a very tiny piece will regenerate in about 7 days at 20°C all the missing structures, and within 15 days will remodel to correct proportions. Planarians are capable of continuously resizing and remodeling their bodies in response to nutrient status and thus can endure long periods of starvation, when they can shrink from an adult size to, and sometimes beyond, their initial size at hatching, regrowing back to adult size when fed (reviewed in ref. 7) (Fig. 1B).

GTDAP-1: A NEW PLAYER IN AUTOPHAGY SHOWS THAT THIS PROCESS IS ESSENTIAL FOR REMODELING DURING PLANARIAN STARVATION AND REGENERATION

Gtdap-1 is the ortholog of human death-associated protein-1 (*DAP-1*). The *DAP-1* protein was originally identified together with *DAP-kinase* (*DAP-2* or *DAPk*) as a positive mediator of PCD induced by gamma-interferon in HeLa cells.⁸ Whereas *DAPk* has recently been linked to autophagic cell death,⁹ no additional studies have been done with *DAP-1*.

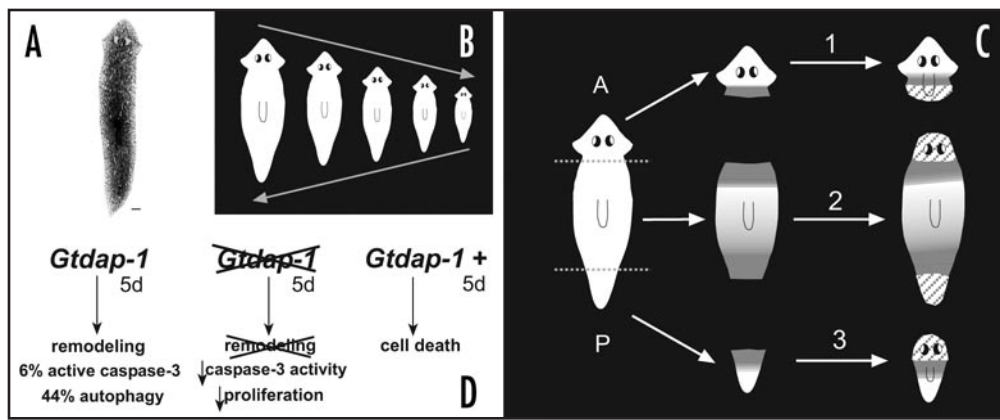


Figure 1. (A) External morphology of *Girardia tigrina*; scale bar, 500 μ m; (B) planarians are capable of continuously resizing and remodeling their bodies in response to nutrient status; (C) if a planarian is cut in three pieces, every piece will regenerate a complete organism which will have to remodel the body to adapt the proportions to the new size; A, anterior; P, posterior; in grey is the postblastema region where *Gtdap-1* is up-regulated; striped is the blastema or new tissue; (D) scheme showing the wild type phenotype, the RNAi phenotype and the gain-of-function phenotype of *Gtdap-1*; 5d indicates five days of regeneration.

Interestingly, a planarian homolog of DAP-1, *Gtdap-1* was up-regulated specifically in the areas and at the time of regeneration when planarian remodeling occurs for achieving the correct scaling of the body during starvation. But it is the localized pattern of expression in the sexual organs of the sexual race during regeneration and starvation, which clearly indicated that the gene is probably involved in remodeling, particularly the removal of non-essential structures. Early literature from the turn of the century broadly described that “the reproductive organs are resorbed” during regeneration and starvation, without providing more details^{10,11} but the choice of words suggested an energetic recycling of the gonads as an adaptive response in order to maintain the rest of the body during starvation.

The attractive possibility of linking *Gtdap-1* with any process related to remodeling other than cell proliferation convinced us to dig into the unknown function of the gene. At 5 d of regeneration when remodeling is at its peak, 44% of all cells express the gene. These cells were always phenotypically differentiated or differentiating cells rather than stem cell like. By using TEM we found that *Gtdap-1* transcript is expressed in cells with autophagic morphology and never in cells with apoptotic morphology.

Interestingly we detected that approximately 6% of the cells in a 5-day regenerating planarian were positive for cleaved caspase-3 but were never TUNEL positive and that the caspase-3 activity profile through regeneration correlates with the *Gtdap-1* expression profile. Moreover, RNAi experiments with *Gtdap-1* not only showed remodeling deficiencies, but also a decrease in both the proliferation rate of neoblasts and caspase-3 activity during regeneration. Finally, we succeeded in performing the first gain-of-function experiments in planarians,¹² based on the transgenesis technique set up in our lab.² Gain-of-function mutants of *Gtdap-1* driven in the photoreceptor cells of the planarian induced cell death in those cells. All these results suggest that although *Gtdap-1* is involved in autophagy, a small percentage of these autophagic cells will be undergoing cell death at any particular time (Fig. 1D). If this kind of cell death is autophagic cell death, and whether this occurs directly or indirectly has to be further investigated.

It has been extensively shown that regeneration in planarians is a process that involves cell proliferation and much effort has been put into the study of neoblasts.¹³⁻¹⁷ But it is clear that some kind of cell

death must also occur during the remodeling or morphallactic (i.e., the regeneration of body parts by means of reorganization rather than extensive proliferation of new cells) process. The only work related to cell death processes in planarians was done in the 1970s. By using micrographs and acid phosphatase activity analyses¹⁸⁻²⁰ it was shown that autophagy and a type of cell death which was then called cell autolysis, a non apoptotic cell death with features of autophagic PCD, seem to play a role during regeneration. But since these exquisite classical observations no further attention has been given to these exciting questions. The results of our experiments support the view that widespread autophagy is a response to nutrient deprivation but also to injury in planarians.

AUTOPHAGY ENTERS THE GAME: A NEW POSSIBLE SCENARIO FOR MORPHALLAXIS

There is an indirect relationship between *Gtdap-1* and neoblast proliferation, since lethal doses of gamma irradiation, which eliminate all neoblasts, prevents expression of *Gtdap-1*, but not low doses, which do not eliminate radiotolerant neoblasts.¹⁶ From this we conclude that proliferating cells must be present to facilitate *Gtdap-1* expression in differentiated cells. Moreover, RNAi for *Gtdap-1* decreases neoblast proliferation by 30%. This suggests that autophagy and proliferation in planarians are coupled during stress-induced events. We hypothesize that this correlation could be indirect and simply related to the balance between the “energy supply” by autophagy and “energy demand” created by production of new cells. Testing this idea will shed light on how nutritional status, regeneration, growth, degrowth and stem cell proliferation and renewal are coordinated.

During starvation in planarians while the animals decrease in size and cell number the basal proliferation rate of the remaining neoblasts stays constant, but there is a significant decrease in the number of differentiated cells in the parenchyma.²¹ We suggest that at the beginning of starvation, resources for continued proliferation will come from food present in the gastrodermal cells and from reserves present in the parenchyma cells. After their depletion, non-essential cells (such as the cells of the sexual organs) undergo autophagy. The planarian will decrease in size but will be able to keep its basal

proliferation rate. If new food is encountered, the starved cells will just produce new organelles. However, if no new food is encountered the cells will reach a point-of-no-return and start to undergo cell death.

During blastema formation in regeneration, the proliferation and differentiation of neoblasts must be a tremendously resource-demanding process. Moreover no food can be eaten until a new functional pharynx is formed. We propose that autophagy plays an essential role in fuelling this process. Fixed parenchyma cells establish intercellular gap junctions with neoblasts²² and these gap junctions are required for neoblast maintenance.²³ This may provide an explanation for how resources are supplied to neoblasts from autophagic cells. As regeneration proceeds, many cells will be superfluous or in the wrong place as symmetry and proportions are restored and many cells of different organs will undergo cell death in order to scale to the new proportions. One exciting possibility is that post-mitotic differentiating cells (neoblast-like cells) may transdetermine (process by which one cell switches the cell fate, i.e., a cell determined to become a secretory cell would change the fate to be a muscle cell) through autophagy. This would explain why 39% of all neoblast-like cells also expressed *Gtdap-1*. Our future work will investigate further whether autophagy that leads to intercellular renewal could be involved in returning potency to neoblasts.

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