Silencing the SPINK-related gene Kazal1 in hydra gland cells induces an excessive autophagy of both gland and digestive cells, leading to animal death. Moreover, during regeneration, autophagosomes are immediately detected in regenerating tips, where Kazal1 expression is lowered. When Kazal1 is completely silenced, hydra no longer survive the amputation stress (Chera S, de Rosa R, Miljkovic-Licina M, Dobretz K, Ghila L, Kaloulis K, Galliot B. Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human Spink1 pancreatic phenotype. J Cell Sci 2006; 119:846-57). These results highlight the essential digestive and cytoprotective functions played by Kazal1 in hydra. In mammals, autophagy of exocrine pancreatic cells is also induced upon SPINK1/Spink3 inactivation, whereas Spink3 is activated in injured pancreatic cells. Hence SPINKs, by preventing an excessive autophagy, appear to act as key players of the stress-induced self-preservation program. In hydra, this program is a prerequisite to the early cellular transition, whereby digestive cells of the regenerating tips transform into a head-organizer center. [...]
**Addenda**

**Autophagy and Self-Preservation**

A Step Ahead of Cell Plasticity?

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**ABSTRACT**

Silencing the SPINK-related gene Kazal1 in hydra gland cells induces an excessive autophagy of both gland and digestive cells, leading to animal death. Moreover, during regeneration, autophagosomes are immediately detected in regenerating tips, where Kazal1 expression is lowered. When Kazal1 is completely silenced, hydra no longer survive the amputation stress (Chera S, de Rosa R, Miljkovic-Licina M, Dobretz K, Ghila L, Kaloulis K, Galliot B. Silencing of the hydra serine protease inhibitor Kazal1 gene mimics the human Spink1 pancreatic phenotype. J Cell Sci 2006; 119:846-57). These results highlight the essential digestive and cytoprotective functions played by Kazal1 in hydra. In mammals, autophagy of exocrine pancreatic cells is also induced upon SPINK1/Spink3 inactivation, whereas Spink3 is activated in injured pancreatic cells. Hence SPINKs, by preventing an excessive autophagy, appear to act as key players of the stress-induced self-preservation program. In hydra, this program is a prerequisite to the early cellular transition, whereby digestive cells of the regenerating tips transform into a head-organizer center. Enhancing the self-preservation program in injured tissues might therefore be the condition for unmasking their potential cell and/or developmental plasticity.

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**HYDRA, A MODEL SYSTEM FOR DUODENAL DIGESTION AND REGENERATION**

Hydra is a freshwater cnidarian, made up of two cell layers, the ectoderm and the endoderm, separated by an extracellular matrix named mesoglea. This animal exhibits a tube shape, mostly a gastric cavity limited by a single opening at the top, the mouth, circled by a ring of tentacles, forming together the head region. At the other end, the basal disk secretes mucous to attach to the substratum (Fig. 1A). Hence cnidarian polyps display an oral-aboral polarity, with differentiated tissues and structures at the extremities, including a sophisticated neuromuscular system, but no organs as recognized in bilaterians.

The digestive function requires the cooperation of gland cells and endodermal epithelial cells (also named digestive cells) of the gastric cavity. The gland cells, packed with secretory granules full of zymogens, display a cellular organization very similar to that of the vertebrate exocrine pancreatic cells and the proteases they release in the gastric cavity participate in the enzymatic digestion. Hence, hydra gland cells are considered as “pancreatic cells.”

Beside its physiology, hydra provides a unique model to investigate cell and developmental plasticity. Upon regular feeding, hydra continuously reproduce asexually through budding; after amputation they regenerate the missing part of their body in few days; after dissociation of their tissues, they can reaggregate and regenerate. The molecular work carried out over the last 15 years, showed a tremendously high level of conservation between hydra and mammalian genes supporting the paradigmatic value of this simple animal.

**IN HOMEOSTATIC CONDITIONS, A TIGHT CONTROL OF AUTOPHAGY IS REQUIRED FOR SURVIVAL, GROWTH AND BUDDING IN HYDRA**

Among those conserved genes, Kazal1, which belongs to the SPINK (Serine Protease Inhibitor Kazal-type) gene family, is specifically expressed in gland cells. Recently, Chera et al., succeeded, for the first time in cnidarians, in producing loss-of-function cellular phenotypes by silencing gene expression in hydra fed with bacteria expressing dsRNAs. Repeated exposures to Kazal1 dsRNAs led to a progressive silencing; meanwhile hydra stopped budding and died. The cellular analysis showed strongly disorganized gland cells, with large vacuoles containing cytoplasmic organelles, together with a concomitant decrease in cell size. Interestingly, those vacuoles, identified as autophagosomes (i.e., containing...
organelles), were also observed in digestive cells, whereas the other cell types were not affected. Subsequently a massive cell death of both cell types was observed. Therefore, Kazal1, by preventing an excessive autophagy, tightly tunes the interactions between the two cell types, which, in homeostatic conditions, achieve an efficient digestive function, and consequently a sustained growth and budding rate (Fig. 1B). Interestingly this hydra Kazal1(-) cellular phenotype appears very similar to that detected in excocrine pancreas of Spink3-/- newborn mice and humans suffering from chronic pancreatitis, linked in some cases to SPINK1 mutations.8,9 In both species, a dramatic autophagy of pancreatic cells was reported. Moreover the Spink3-/- mice also show a concomitant degeneration of the duodenal and intestinal cells, those defects leading to severe growth retardation and animal death.7 This comparative analysis points to the essential cytoprotective function played by the Kazal-type serine protease inhibitors from hydra to mammals.

**Kazal1 and the Immediate Self-Preservation Program after Amputation**

In normal conditions, Kazal1 expression is strongly induced in regenerating tips immediately after amputation. Upon partial silencing, Kazal1 knocked-down hydra survived the amputation stress and regenerated their head perfectly well, without any delay. Nevertheless, the gland and digestive cells isolated from the regenerating tips already showed strong cellular alterations immediately after amputation, implying that the amputation stress dramatically speeds up the Kazal1 cellular phenotype previously observed in Kazal1(-) intact animals. Noticeably these alterations were restricted to the regenerating tip and were reversible as long as silencing was not complete. In fact, upon complete silencing, the amputation stress became lethal within the hours following bisection. Surprisingly, the Kazal1 knocked-down hydra showed an “all or nothing” regeneration phenotype: either the residual Kazal1 level sufficed for animals to survive the amputation stress, and then, regeneration was not affected; or Kazal1 expression was depleted, gland and digestive cells rapidly died from autophagy, and the amputated animals dissociated in several hours. These results uncover the molecular program at work during the very initial phase of regeneration: a high level of Kazal1 expression is required to achieve an endogenous cytoprotective function that allows the cells to recover from the amputation stress. Again a parallel can be drawn with the mammalian pancreas: Spink3 is strongly and immediately induced in the injured mouse pancreas, highlighting the self-preservation program activated upon repeated injury.10 Hence the mechanisms that prevent the cytotoxic effects of either amputation or drugs, appear to be highly evolutionarily-conserved.

**Self-Preservation Precedes and Allows the Emergence of Cell Plasticity**

An unexpected finding was that some protein(s) secreted by the gland cells, not only participate in the digestion process, but also carry out essential cytoprotective functions. After amputation, Kazal1, likely by inhibiting the proteases that are released upon bisection, definitely helps the regenerating tip and more precisely the endodermal digestive cells to survive the amputation stress. Interestingly, those cells support the early phase of the head-regeneration process: they rapidly dedifferentiate, undergo a blastema transition, and develop within the first hours following bisection, an organizer activity.2,5,11-14 Therefore, the self-preservation program, which protects the cells that will develop an organizer activity, precedes cellular plasticity. In fact such a self-preservation program might be a common and general requirement of the regeneration process per se, whatever the tissue or the species. Altogether these results suggest something new about the mechanisms driving regeneration: in many contexts, the cellular potential for plasticity, i.e., dedifferentiation, transdifferentiation, blastema transition, might be there, but masked by the excessive autophagy linked to the stress. Hence improving cytoprotection by reducing autophagy after amputation or toxic shocks might help uncover cell plasticity, and consequently promote regeneration.

**Autophagy and Self-Preservation**

Figure 1. Hydra anatomy (A) and scheme depicting the Kazal1 phenotype in gland and digestive cells (B).

**References**

