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Review

Why polyps regenerate and we don't: Towards a cellular and molecular framework for *Hydra* regeneration

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Abstract

The basis for Hydra's enormous regeneration capacity is the "stem cellness" of its epithelium which continuously undergoes self-renewing mitotic divisions and also has the option to follow differentiation pathways. Now, emerging molecular tools have shed light on the molecular processes controlling these pathways. In this review I discuss how the modular tissue architecture may allow continuous replacement of cells in *Hydra*. I also describe the discovery and regulation of factors controlling the transition from self-renewing epithelial stem cells to differentiated cells.

Keywords: Regeneration; Stem cells; Epithelium; Peptides; Cnidaria; Hydra

Introduction

If we have had or will have a finger cut off, we cannot restore it. If, however, we dissociate an intact *Hydra* into single cells, a perfect polyp will reconstitute itself from the pellet of centrifuged cells within the next few days. What is the difference between "us" and "them"? Why possess some animals remarkable powers of self-regeneration and others not? *Hydra* is the superstar of regeneration since more than 200 years. In the 1740s, the Swiss scientist Abraham Trembley (1744) discovered that freshwater polyps could regenerate their heads and feet and – if cut into a few pieces – all of them would regenerate to form new individuals (Lenhoff and Lenhoff, 1988). Scientists have long wondered how *Hydra* regenerates so well. *Hydra's* regeneration capacity and the underlying mechanism responsible for specification of positional information has inspired (and is still inspiring) computational biologists to demonstrate that mathematical equations can be applied to explain morphogenetic events in animals (for review see Meinhardt and Gierer, 2000; Meinhardt, 2002, 2004a,b; Crampin et al., 2002; Marciniak-Czochra, 2006). *Hydra* also presents excellent opportunities for understanding how gradients of morphogens could be set up and maintained to control local developmental processes (Wolpert et al., 1972, 1974). By application of quantitative cellular techniques much has been learned about *Hydra*'s cell populations, and the mechanisms controlling pluripotency, lineage commitment, and position dependent cell differentiation (for reviews see Bode, 1996; Bosch, 2006). But precisely how in *Hydra* the regenerating tissue is reorganized, how positional information is encoded at the molecular level, and how cells respond to diffusible positional signals (or "morphogens") remained largely mysterious. An impressive accumulation of gene sequences, novel tools and the development of genomic resources over the past few years has brought a new perspective on *Hydra*'s regeneration capacity. A National Science Foundation-funded large-scale *Hydra* EST Project (www.hydrabase.org) resulted in 170,000 ESTs. A National Human Genome Research Institute-funded *Hydra* genome project at the J. Craig Venter Institute currently provides 6x coverage of the *Hydra magnipapillata* genome with an assembled draft genome sequence appearing later this year. *Hydra* became also amenable to reverse genetics through RNAi experiments, further expanding the capabilities of this model organism (Lohmann et al., 1999; Takahashi et al., 2005; Cardenas and Salgado, 2003; Chera et al., 2006; Amimoto et al., 2006). Finally, transgenic *Hydra* (Wittlieb et

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al., 2006; Steele, 2006) now pave the way for many important scientific and technological applications making resources and methods available to fully explore the biological opportunities that the polyp provides. *Hydra*'s unique advantages as model for morphological and molecular studies of regeneration include (i) the optical transparency of the two tissue layers allowing the direct visualization of individual cells by means of GFP fluorescence and facilitating *in vivo* tracking of cells within the intact organism; (ii) the rapid growth rate with a population doubling time of 3.5 days; and (iii) the mass-culturing of clonally derived animals. Since in *Hydra* the epithelial cells are key players in regeneration, I will focus here on epithelial stem cells. We will first follow them at the site of regeneration and then discuss the mechanisms by which they are thought to become morphologically and molecularly distinct from their neighbours in the head and foot region.

Regeneration in *Hydra* occurs by morphallaxis

Hydra is made up of two cell layers – the ectoderm and endoderm – separated by a thin extracellular matrix (ECM) called the mesoglea (Fig. 1). The polar body plan has a head and tentacles on one end and a foot on the opposite end of a hollow column (Fig. 1A). The cells either belong to the ectodermal or endodermal epithelial cell lineage, or to the interstitial cell lineage. Epithelial cells are epitheliomuscular cells covering the outside of the animal or lining the gastric cavity. Interstitial cells are mostly localized in the interstitial space between ectodermal epithelial cells and differentiate into nerve cells, cnidocytes, gland cells, and – during sexual differentiation – into gametes

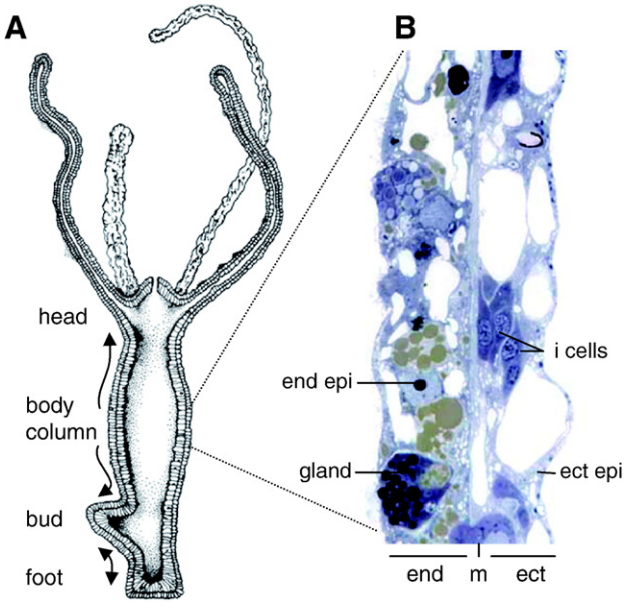

Fig. 1. The freshwater polyp Hydra. (A) Schematic longitudinal cross section indicating the simple epithelial organization. Arrows indicate the direction of tissue displacement. (B) Photograph of a section of part of the epithelial lining of the body column, showing the diploblastic organization. Note how interstitial cells and gland cells are interspersed between ectodermal and endodermal epithelial cells, respectively. End, endoderm; ect, ectoderm; m, mesoglea; Photograph courtesy of Dr. Friederike Anton-Erxleben (Kiel).

(Bosch and David, 1987; Bosch, 2006). Any isolated fragment of the *Hydra* body which is larger than a few hundred epithelial cells can regenerate into a miniature version of the animal (Fig. 2A). Even aggregates of dissociated cells (Fig. 2B) will regenerate into viable polyps (Noda, 1971; Gierer et al., 1972; Technau et al., 2000). This ability for self-organization is due to the continuous production of cells and signal factors in the adult tissue. Regenerating tissue pieces cut from the gastric regions show a directional property called polarity (Fig. 2A). Such pieces regenerate a head in the apical end of the isolated fragment. A foot is always regenerated at the basal end of such a piece. Polarity is thought to be based on gradients of molecules whose concentration provides positional information (Wolpert et al., 1974; MacWilliams, 1983a,b). The commitment, for example, of the apical tissue to undergo head formation is made a few hours after cutting, long before any head-like structure is visible (MacWilliams, 1983a,b). Thus, regeneration in *Hydra* represents a beautiful experimental system for the study of *de novo* pattern formation and points to an important process of patterning in multicellular organisms: visible patterns are preceded by prepatterns or morphogenetic fields.

In the early 20th century, Thomas Hunt Morgan coined the terms morphallaxis and epimorphosis to describe the two major types of regeneration which can be observed in various animal groups (Morgan, 1901). Morphallaxis refers to the type of regeneration that occurs in the absence of cellular proliferation and involves the transformation of existing body parts or tissues into newly organized structures. Epimorphosis refers to regeneration that requires active cellular proliferation. In planarians as in some vertebrates such as salamanders, both the generation of new tissue at the wound site via cell proliferation (blastema formation) and morphallaxis are needed for complete regeneration (Brockes et al., 2001; Agata, 2003; Reddien and Sánchez Alvarado, 2004; Sanchez Alvarado, 2006). There, cells near the site of the injury lose their specialized properties and revert to a primordial state in a process called de-differentiation. It is thought that those stem cells then multiply rapidly and redifferentiate to form the tissue needed to rebuild the limb or organ (Brockes and Kumar, 2005; Slack, 2006).

In the marine hydrozoan *Podocoryne*, some cells under certain conditions can de-differentiate or trans-differentiate (Schmid and Reber-Muller, 1995; Reber-Muller et al., 2006). Early regenerative processes in *Hydra*, however, always occur in the absence of DNA synthesis as a morphallactic process in which cells from the gastric region differentiate into head or foot specific cells (Cummings and Bode, 1984). Pulse labeling experiments have demonstrated that the number of labeled cells in regenerating tissue declines sharply at the site of cutting during the first 12 h (Holstein et al., 1991) pointing to the release of factor(s) which inhibit mitosis. My lab has reinvestigated the issue of cell proliferation at the injury site by using transgenic polyps and *in vivo* tracking of GFP expressing endodermal epithelial cells in regenerating tissue (Wittlieb et al., 2006). We have shown (Fig. 2C; Wittlieb et al., 2006) that at the tip of the regenerating tissue there is no localized cell proliferation of endodermal epithelial cells. These