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Cell type complexity in the basal metazoan *Hydra* is maintained by both stem cell based mechanisms and transdifferentiation

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Abstract

Understanding the mechanisms controlling the stability of the differentiated cell state is a fundamental problem in biology. To characterize the critical regulatory events that control stem cell behavior and cell plasticity *in vivo* in an organism at the base of animal evolution, we have generated transgenic *Hydra* lines [Wittlieb, J., Khalturin, K., Lohmann, J., Anton-Erxleben, F., Bosch, T.C.G., 2006. Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis. Proc. Natl. Acad. Sci. U. S. A. 103, 6208–6211] which express eGFP in one of the differentiated cell types. Here we present a novel line which expresses eGFP specifically in zymogen gland cells. These cells are derivatives of the interstitial stem cell lineage and have previously been found to express two Dickkopf related genes [Augustin, R., Franke, A., Khalturin, K., Kiko, R., Siebert, S. Hemmrich, G., Bosch, T.C.G., 2006. Dickkopf related genes are components of the positional value gradient in *Hydra*. Dev. Biol. 296 (1), 62–70]. In the present study we have generated transgenic *Hydra* in which eGFP expression is under control of the promoter of one of them, *HyDkk1/2/4 C*. Transgenic *Hydra* recapitulate faithfully the previously described graded activation of *HyDkk1/2/4 C* expression along the body column, indicating that the promoter contains all elements essential for spatial and temporal control mechanisms. By *in vivo* monitoring of eGFP+ gland cells, we provide direct evidence for continuous transdifferentiation of zymogen cells into granular mucous cells in the head region. We also show that in this tissue a subpopulation of mucous gland cells directly derives from interstitial stem cells. These findings indicate that both stem cell-based mechanisms and transdifferentiation are involved in normal development and maintenance of cell type complexity in *Hydra*. The results demonstrate a remarkable plasticity in the differentiation capacity of cells in an organism which diverged before the origin of bilaterian animals.

Keywords: *Hydra*; Regeneration; Transdifferentiation; HyDkk1/2/4; Stem cell; Gland cells

Introduction

It has long intrigued researchers why some animals possess remarkable powers of self-regeneration and others not. A profound difference between animals with high and low regeneration capacity may be in the differentiation potential and plasticity of the cells (Bosch, 2007a). In most organisms, cellular commitment and the differentiated state are very stably controlled and cell type identity seems to be irreversibly fixed. For renewal of differentiated tissue to occur, animals and plants rely on multipotent and/or unipotent stem cells. In the absence of stem cells, and under specialized circumstances (e.g., regeneration), new tissue can also be formed from differentiated cells via transdifferentiation, a process by which cells are able to dedifferentiate (lose the characteristics of their origin) and subsequently redifferentiate. The term was coined to distinguish the switching of a terminally differentiated cell type into another during insect development (Selman and Kafatos, 1974). For a cellular behavior to be interpreted as transdifferentiation, two conditions must be fulfilled: first, the differentiation state before and after the switch must be reliable described; and second, a direct ancestor–descendant relationship and common developmental history between the cells must be clearly demonstrated (Eguchi and Kodama, 1993).

One animal in which cell plasticity may play a major role during normal development is the freshwater polyp *Hydra*.

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There, about 20 cell types are distributed among three cell lineages. Each of the epithelial layers is made up of a cell lineage, while the remaining cells are part of the interstitial cell lineage which resides among the epithelial cells of both layers. Interstitial stem cells in *Hydra* are abundant throughout the gastric region; in the head and foot region, they are present in very low numbers (David and Plotnick, 1980). Multipotent interstitial stem cells give rise to neurons, cnidocytes and gametes in a position dependent manner (Bosch and David, 1987; reviewed in Bosch, 2007b). These stem cells give also rise to gland cells. Gland cells are found in the endodermal layer with zymogen gland cells (ZMG) in the gastric region and mucous gland cells (MGC) in the head. The head comprises two types of MCGs, granular MGCs (gMGC) and spumous MGCs (sMGC) (Semal-van Gansen, 1954). Labeling experiments and measurement of nuclear DNA content showed (Schmidt and David, 1986; Bode et al., 1987) that the ZMG population contains transiently amplifying cells. Whereas the origin of ZMGs previously could be demonstrated unambiguously to lie in the interstitial cell population (Schmidt and David, 1986; Bode et al., 1987), the origin of the MGCs in the head remained elusive. Rose and Burnett (1970) and Dübel (1989) both indicated that hypostomal MGCs may be derivatives of head-specific interstitial stem cells but did not provide quantitative experimental data to support this notion.

In adult *Hydra* in contrast to most other animals, cell proliferation takes place continuously in both of the cell layers along the body column with all epithelial cells in both cell layers being in the mitotic state (Dübel et al., 1987, reviewed in Bosch, 2007a). Despite this dynamic situation and continuous displacement of cells, in each polyp the overall axial distribution of the cell systems remains constant. Previous observations have revealed two strategies that underlie the maintenance of the tissue organization: differentiation from multipotent stem cells (Bosch and David, 1987) as well as phenotype conversion (Bode, 1992). The latter appears to be particularly evident in the plasticity of the differentiated state of the nerve net as immunocytochemical studies have revealed that neurons displaced from region to region appear to undergo changes in phenotype (Bode, 1992; Technau and Holstein, 1996). A direct lineage relationship between the different neurons, however, so far could not be established.

The development of transgenic *Hydra* (Wittlieb et al., 2006; Khalturin et al., 2007) provides an opportunity to address these points of uncertainty and to re-investigate the origin and differentiation plasticity of *Hydra* cells *in vivo*. The structural organization of *Hydra* makes it possible to trace individual labeled cells and to analyze the reconstitution of the cell type repertoire and homeostasis in the context of regeneration. Here, we re-addressed the issue of gland cell differentiation by generating transgenic polyps expressing eGFP specifically in these cells. Tracing individually labeled ZMGs allowed us (i) to show that continuous changes in position along the single body axis are accompanied by continuous changes in gene expression and morphology and (ii) to define a distinct ancestor–descendant relationship between ZMGs in the gastric region and gMGC in the head. In addition, we (iii) observed that part of the mucous gland cell population in the head is directly derived from interstitial cells. The *in vivo* observations, therefore, show that in *Hydra* both stem cell-based mechanisms and transdifferentiation are required for maintaining the different types of gland cells found along the body axis. The study describes the first transgenic *Hydra* line expressing a reporter gene under control of a cell-type-specific promoter. It reveals differentiation in *Hydra* to be a surprisingly dynamic process and, to our knowledge, provide the strongest evidence to date that transdifferentiation *in vivo* plays a major role in maintaining cell complexity.

Materials and methods

Animals and culture conditions

Experiments were carried out with *Hydra vulgaris* (AEP strain). *HyDKK1/2/4 C* 5′-flanking sequence was amplified using gDNA isolated from *Hydra magnipapillata*. The animals where cultured according to standard procedures at 18 °C.

Transgenic polyps

Founder transgenic animals bearing a gland cell-specific transgene were produced at the University of Kiel Transgenic *Hydra* Facility (http://www.uni-kiel.de/zoologie/bosch/transgenic.htm) as previously described (Wittlieb et al., 2006). Briefly a fragment of 1093 bp comprising 1027 bp *HyDKK1/2/4 C* 5′ flanking region and 66 bp coding for the predicted *HyDKK1/2/4 C* signal peptide and three subsequent residues was amplified from *H. magnipapillata* gDNA using Platinum High Fidelity polymerase (Invitrogene) and primers (Dlp_pr *Xba*I ACT CTA GAC GTA CAC GTT CAC ACG TCC TAT AG and Dlp_pr Not 1 CAG CGG CCG CCC AGA ATA TTC CTT CGC TGC AAA C). The fragment was cloned into the modification of Hot G eGFP expression vector using *Xba*I and a newly inserted *Not*I cutting site. The resulting transfection construct was sequenced, plasmid DNA was purified using Quiagen MidiPrep Kit and injected into *H. vulgaris* (AEP) embryos. Founder transgenic animals bearing the HyDKK1/2/4 C::eGFP construct started to hatch 14 days after microinjection. Out of 66 embryos injected with the construct, one stable transgenic line was generated expressing eGFP exclusively in a fraction of zymogen gland cells. Initial founder transgenic animals were further expanded into a mass culture by clonal propagation via budding. As a control we used transgenics which express eGFP selectively in the interstitial stem cell lineage driven by 1386 bp of the *H. vulgaris* actin 5′ flanking region (Fig. 1B; Khalturin et al., 2007).

Regeneration experiments

In all regeneration experiments, animals were bisected within the maximum of eGFP/HyDKK1/2/4 C expressing cells shortly below the boundary of expression (see Fig. 3A). Regenerating animals were not fed unless otherwise stated and fixed to different time points for further analysis.

In situ hybridization

In situ hybridizations were adapted from previous works (Grens et al., 1996; Philipp et al., 1995). Double in situ hybridizations were performed using DIG- and Biotin-labeled RNA probes simultaneously. Probes were prepared according to the manufacturer's instructions (Roche) using sequence specific primer combinations as follows: Dlp F: CAG TGG GCA ACC TGA ATA CC – Dlp R: CCA CTT CCG GAG TTG TCA AC, GFP_F(29): GAGTTGTCCCAATTCTTGTTG – GFP_R(711): GTA TAG TTC ATC CAT GCC ATG, HyTSR1 (22): FW CTG TTT GCC TCA ATG CTG AC – HyTSR1 (949) R: TAC CAC CAA ATG CAG GTT TGG. For better penetration of probe, antibody and substrate animals were cut longitudinal after fixation.