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Plasticity of epithelial cell shape in response to upstream signals: A whole-organism study using transgenic *Hydra*

Friederike Anton-Erxleben, Antje Thomas, Jörg Wittlieb, Sebastian Fraune, Thomas C.G. Bosch*

Zoological Institute, Christian-Albrechts-University, Kiel, Olshausenstr. 40, Am Botanischen Garten 9, D-24098 Kiel, Germany

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Abstract

Multicellular organisms consist of a variety of cells of distinctive morphology, with the cell shapes often reproduced with astonishing accuracy between individuals and across species. The morphology of cells varies with tissues, and cell shape changes are of profound importance in many occasions of morphogenesis. To elucidate the mechanisms of cell shape determination and regulation is therefore an important issue. One of the simplest multicellular organisms is the freshwater polyp *Hydra*. Although much is known about patterning in this early branching metazoan, there is currently little understanding of how cells in *Hydra* regulate their shape in response to upstream signals. We previously reported generation of transgenic *Hydra* to trace cells and to study cell behavior *in vivo* in an animal at the basis of animal evolution. Here, we use a novel transgenic line which expresses enhanced green fluorescent protein (eGFP) specifically in the ectodermal epithelial cells to analyze the structure and shape of epithelial cells as they are recruited into specific regions along the body column and respond to upstream signals such as components of the canonical Wnt signaling pathway. As a general theme, in contrast to epithelial cells in more complex animals, ectodermal epithelial cells in *Hydra* are capable of drastic changes in structure, shape, and cell contact along the body column. The remarkable phenotypic plasticity of epithelial cells in response to positional signals allows *Hydra* to build its body with only a limited number of different cell types.

Keywords: Cnidaria; Hydra; Cell shape; Cytoskeleton; Morphogenesis

Introduction

As sister group to the bilaterians (Collins, 1998; Philippe et al., 2005), cnidarians provide information for reconstructing the early history of bilaterian developmental mechanisms. Cnidaria such as the freshwater polyp *Hydra* are characterized by the presence of a nervous system, specialized types of cell junctions, and well-organized tissues in distinct cell layers. *Hydra* has a simple body plan which is basically a tube with a head at the apical end, and a foot, or basal disc, at the other end (Fig. 1A). The body wall consists of two epithelial layers, the ectoderm and endoderm, separated by the mesoglea, a typical extracellular matrix. Each layer comprises a cell lineage. While the ectodermal and endodermal epithelial cells constitute the principal structural element, all other cell types are located in the interstices among the epithelial cells of both layers,

*Corresponding author. Tel.: +49 431 880 4169; fax: +49 431 880 4747.
E-mail address: tbosch@zoologie.uni-kiel.de (T.C.G. Bosch).

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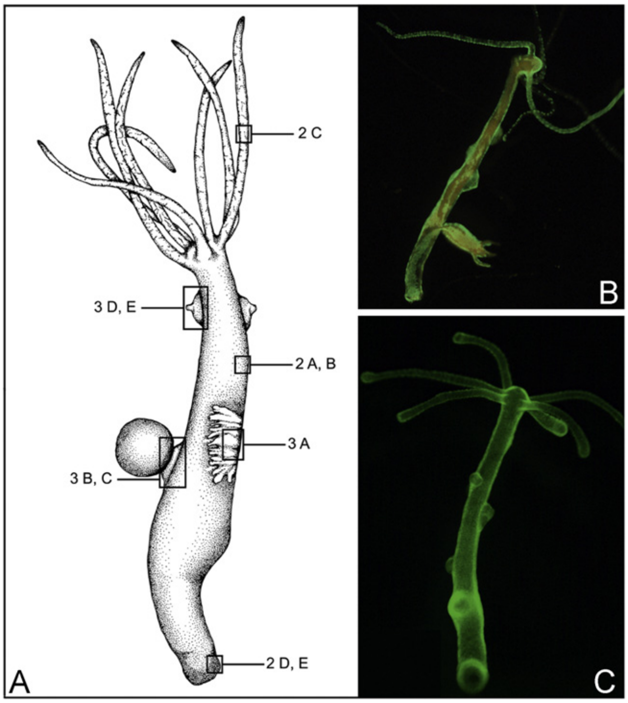

Fig. 1. (A) Schematic drawing of the freshwater polyp *Hydra vulgaris* indicating the tissue areas in which the shape of ectodermal epithelial cells was examined. Letters correspond to panels in Figs. 2 and 3. (B) Transgenic ecto-1 polyp with a mosaic expression of eGFP in some of its ectodermal epithelial cells. (C) Transgenic ecto-1 polyp expressing eGFP in all of its ectodermal epithelial cells. Fluorescence microscopy. Green: GFP fluorescence.

and are part of the interstitial cell lineage (Bosch, 2007a, b).

Hydra have a remarkable potential to regenerate lost tissue after injury (Bosch, 2007a). Cellular reactions to wounding include morphallactic differentiation with rearrangement of the actin cytoskeleton and enhanced matrix metalloproteinase activity in order to reestablish an intact epithelial sheet over the wound (Leontovich et al., 2000; Yan et al., 2000). The ability to regenerate is at least partially due to the continuous presence of stem cells with high self-renewal capacity and high phenotypic plasticity (Bosch, 2007a, b, 2008). In *Hydra*, both the epithelial cells as well as the interstitial cells in the body column continuously undergo self-renewing mitotic divisions. As a result of these tissue dynamics, cells are constantly displaced either apically onto the head, or basally onto developing buds, or onto the foot. Non-dividing differentiated cells of all three lineages are lost by displacement from the body column (Campbell, 1967). Hence, the two epithelial layers are in a steady state of production and loss of cells.

Epithelial cells in *Hydra* are multifunctional epitheliomuscular cells (West, 1978). They are interconnected by apical junctional complexes, and basal extensions containing myofilaments are their distinguishing character, as compared to epithelia in other animals. Ectodermal epithelial cells in *Hydra* are epitheliomuscular stem cells with an extensive, probably unlimited, self-renewal capacity. In response to positional signals, these cells differentiate into head-, foot-, and gonadal-specific epithelial cells. Since changing the local environment appears to cause changes in the regulatory influences these cells receive, epithelial cell differentiation in *Hydra* is a complex process with many steps sensitive to different regulatory influences. The high degree of plasticity required by this variety of processes is likely to be provided by the cytoskeleton.

Although much is known about patterning of the *Hydra* body axis (for recent reviews see Bosch, 2007a, b; Meinhardt, 2008), there is currently little understanding of how the cells regulate their shape in response to upstream signals. To characterize the critical regulatory events that control cell behavior and cell plasticity *in vivo*, we generated transgenic *Hydra* lines (Wittlieb et al., 2006; Khalturin et al., 2007; Siebert et al., 2008) which express enhanced green fluorescent protein (eGFP) in the different cell types. We have previously shown (Wittlieb et al., 2006) that endodermal epithelial cells along the body column have stem cell characteristics. We have also reported (Khalturin et al., 2007) that the Wnt pathway appears to be involved in interstitial stem cell decision making in *Hydra*. Here we demonstrate the remarkable phenotypic plasticity of ectodermal epithelial cell shape and provide direct evidence that components of the canonical Wnt signaling pathway are among the signals instructing ectodermal epithelial cells to execute different programs of terminal differentiation.

Materials and methods

Animals and culture conditions

Experiments were carried out with animals of the *Hydra vulgaris* AEP strain as described earlier (Wittlieb et al., 2006). The animals were cultured according to standard procedures at 18 °C. Sexual differentiation was induced by starving the animals for up to 2 weeks. Treatment with alsterpaullone was performed for 2 days as described by Khalturin et al., (2007).

Generation of transgenic *H. vulgaris* AEP expressing eGFP in ectodermal epithelial cells

Transgenic founder polyps overexpressing eGFP under control of ß-actin promoter were produced at the University of Kiel Transgenic Hydra Facility (http://www.uni-kiel.de/zoologie/bosch/transgenic.htm) using the hotG vector (Wittlieb et al., 2006; Khalturin et al.