

# Trembley's polyps go transgenic

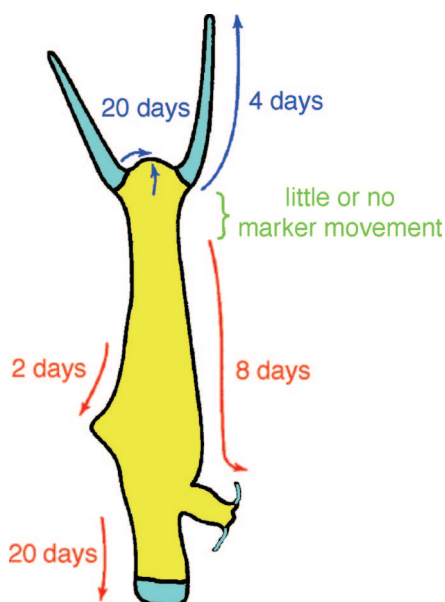
Robert E. Steele\*

Department of Biological Chemistry and the Developmental Biology Center, 240D Medical Sciences I, University of California, Irvine, CA 92697-1700

In 1744, a book entitled *Mémoires, Pour Servir à l'Histoire d'un Genre de Polypes d'Eau Douce, à Bras en Forme de Cornes* (Memoirs Concerning the Natural History of a Type of Freshwater Polyp with Arms Shaped Like Horns) was published in The Netherlands (1). The author of the book was Abraham Trembley, a tutor in the household of Count Bentinck; the freshwater polyp was *Hydra*. In the more than 250 years since the appearance of Trembley's work, *Hydra* has been an important system for studies of pattern formation (2), regeneration (3), and stem cells (4). However, with the increasingly powerful genetic and molecular tools available in model systems such as *Drosophila*, mouse, zebrafish, and *Caenorhabditis elegans*, interest in and support for *Hydra* research have lagged. With the publication of the work of Wittlieb *et al.* (5) in a recent issue of PNAS, that situation seems about to change.

In a scientifically and aesthetically beautiful piece of work, Wittlieb *et al.* (5) have succeeded in producing the first stably transgenic *Hydra*. Efforts in this regard have been made sporadically over the 20 years since the first *Hydra* genes were cloned (6, 7), but none have been successful. Transient transfection methods were developed for *Hydra* (8–10), and these have provided useful tools for the localization of proteins (11, 12). However, none of these methods has been used successfully to produce a phenotype. The need for a method to make transgenic *Hydra* became especially pressing with the development of genomics resources for this organism. A National Science Foundation-funded large-scale *Hydra* EST Project ([www.hydrabase.org](http://www.hydrabase.org)) and a smaller-scale EST project at the National Institute of Genetics in Japan were recently completed. In late 2004, a National Human Genome Research Institute-funded *Hydra* genome project was begun at the J. Craig Venter Institute. An assembled draft genome sequence should appear later this year. The inability to produce transgenic *Hydra* placed a serious impediment in the path of researchers wanting to exploit these genomics resources for functional studies.

The method developed by Wittlieb *et al.* (5) is surprisingly straightforward. Supercoiled plasmid DNA containing a GFP gene driven by a *Hydra* actin gene



**Fig. 1.** Tissue dynamics and cell division in the adult *Hydra* polyp. The portion of the polyp colored yellow indicates where epithelial cells are mitotically active. In the parts of the polyp colored turquoise, cells are arrested in the G<sub>2</sub> phase of the cell cycle. An early-stage bud is shown on the left side of the polyp, and a later-stage bud is shown on the right. The arrows indicate results from the study by Campbell (14), in which displacement of marked regions of the ectodermal epithelial layer was examined. The arrows indicate the starting and ending positions of the marked tissue. The number of days required for the displacement is indicated next to the arrow. [Reprinted with permission from ref. 21 (Copyright 2002, Elsevier).]

promoter was injected into blastomeres of two- to eight-cell *Hydra* embryos. There was no need to remove vector sequences, as is done for mouse embryo injections, and the DNA did not need to be exceptionally pure (i.e., no cesium chloride gradient centrifugation was needed). From a sample of 65 injected embryos, Wittlieb *et al.* (5) obtained eight polyps (12%) that contained stably integrated transgenes. This yield of transgenic animals is of the same order of magnitude as that seen with mouse embryos. Southern blot analysis suggests that multiple copies of the transgenes were integrated and that they may have integrated at multiple sites in the genome. More-detailed studies of the state of the transgenes will be needed to determine whether this is the case.

As with transgenic mice, the initial *Hydra* polyps obtained from micro-

injected embryos are mosaic. Unlike the mouse, it is not necessary to carry out breedings to produce nonmosaic animals. The tissue dynamics of the adult *Hydra* polyp (Fig. 1) make it possible to obtain fully transgenic animals simply by asexual propagation. The adult polyp consists of two concentric epithelial layers (endoderm and ectoderm). At the oral end of the polyp is the mouth opening and a ring of tentacles (that together constitute the head). At the aboral end is a disk of adhesive cells that constitutes the foot. The epithelial cells in the body column (the yellow part of the polyp in Fig. 1) are mitotic, whereas the epithelial cells of the tentacles and the foot are arrested in G<sub>2</sub> of the cell cycle (indicated by turquoise in Fig. 1) (13). As new cells are produced by mitosis in the body column, cells are displaced into asexual buds and sloughed from the ends of the tentacles and the center of the foot (14). Thus the pool of transgenic cells in a mosaic polyp expands by cell division and moves into buds. As this process progresses, one eventually ends up with some polyps that are no longer mosaic for the transgene-containing cell lineage.

An additional interesting feature of *Hydra* that comes into play in the formation of transgenic animals is the fact that the adult polyp is composed of three distinct cell lineages, the ectodermal epithelial cell lineage, the endodermal epithelial cell lineage, and the interstitial cell lineage. The first two lineages form the epithelial layers of the polyp. Epithelial cells are in a differentiated state that allows them to form these two layers and, in addition, they are stem cells that give rise to new body column tissue. The interstitial cell lineage contains multipotent stem cells that give rise to nerve cells, gametes, nematocytes (the stinger cells used to capture prey), and mucus and gland cells (4). These three lineages do not interconvert in the adult polyp. As a result of this lack of interconversion, Wittlieb *et al.* (5) were able to obtain polyps in which only one of the three lineages was transgenic. They obtained animals with transgenic

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\*E-mail: [resteele@uci.edu](mailto:resteele@uci.edu).

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