

Hydra and the evolution of stem cells

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Hydra are remarkable because they are immortal. Much of immortality can be ascribed to the asexual mode of reproduction by budding, which requires a tissue consisting of stem cells with continuous self-renewal capacity. Emerging novel technologies and the availability of genomic resources enable for the first time to analyse these cells *in vivo*. Stem cell differentiation in Hydra is governed through the coordinated actions of conserved signaling pathways. Studies of stem cells in Hydra, therefore, promise critical insights of general relevance into stem cell biology including cellular senescence, lineage programming and reprogramming, the role of extrinsic signals in fate determination and tissue homeostasis, and the evolutionary origin of these cells. With these new facts as a backdrop, this review traces the history of studying stem cells in Hydra and offers a view of what the future may hold.

Keywords: hydra; stem cells; evo-devo

Promises and conflicts from the past: Sex in a single polyp

To many people, Hydra is an enigma in evolution. It is one of the very few examples of animals which appear to be truly immortal. The animal broke onto the scene as early as in the eighteenth century (Fig. 1A), when a Swiss naturalist and private scholar, Abraham Trembley, used Hydra's extensive regenerative capacity to demonstrate⁽¹⁾ to his disciples that—against prevailing belief—not everything in nature is “pre-formed” but that living creatures and structures can form *de novo* simply following nature's laws. About 160 years later, during 1906–1908, Ethel Browne, an American Graduate student at Columbia University, performed under the guidance of T. H. Morgan a series of experiments in Hydra in which she demonstrated for the first time that a transplant (the hypostome) could induce a secondary axis of polarity in a host.⁽²⁾ A similar process in amphibian embryos was later named “organization” by Spemann and Mangold.⁽³⁾ Despite Spemann's knowledge of Browne's work in Hydra, it was never referred to in any of Spemann's publications.⁽⁴⁾

About 70 years after Browne's remarkable study, another feature of Hydra became uncovered: The asexual mode of

budding (Fig. 1B) and constantly active axial patterning processes are based on the presence of continuously dividing cells.⁽⁵⁾ Diverse cell biological methods and the availability of mutants enabled to reveal the lineages and corresponding cell cycle characteristics.^(6–10) Early statistical cloning experiments by Charles David at Albert Einstein College of Medicine in New York⁽¹¹⁾ demonstrated that these cells have a remarkable potential to self-renew and differentiate following strict spatiotemporal rules. However, when in the early 1980s Charles David accepted a position at the Zoological Institute of the University of Munich, one troubling question was still open: Are these cells truly totipotent in the sense that they can differentiate into both somatic and germ line cells or are there germ-line-restricted stem cells in Hydra as in most other animals?

About a century ago, August Weismann observed in hydrozoans⁽¹²⁾ that germ cells are derivatives of “common embryonic tissue cells” found in a given part (“Keimstätte”) of the tissue. Based on this observation he concluded⁽¹³⁾ that only certain groups of predetermined cells can differentiate into gametes, and published his doctrine of “the continuity of the germ-line”. In the meantime the separation of immortal germ cells from the somatic tissue has been documented in a wide diversity of metazoan taxa.^(14,15) But despite the fact that all Hydra species have been observed⁽¹⁶⁾ to go through occasional sexual phases (Fig. 1C), the situation of germ line cells in Hydra was unclear.

Task of my thesis project in the Zoological Institute of the University of Munich was to find that out. Although, I principally used the already established⁽¹¹⁾ dissociation-reaggregation procedure for cloning stem cells in Hydra, progress was slow. Only after turning to mutant strains of the Japanese species *Hydra magnipapillata* I finally succeeded in getting aggregates containing viable interstitial stem cells carrying various genetic markers. In summer 1985, following the fate of aggregates containing single interstitial stem cells, we observed for the first time that these aggregates not only had developed into polyps undergoing sexual differentiation but also had testis with immobile sperm. Since the stem cell donor strain was *H. magnipapillata* strain ms-1 (male-sterile 1, because of immobile sperm), this observation indicated that interstitial cells in a single Hydra polyp can do both: differentiate into somatic cells and—under appropriate environmental stimuli—differentiate into germ cells (Fig. 2C). In his relaxed style of dealing with scientific highlights, my thesis adviser's comment was simply: “Wow,

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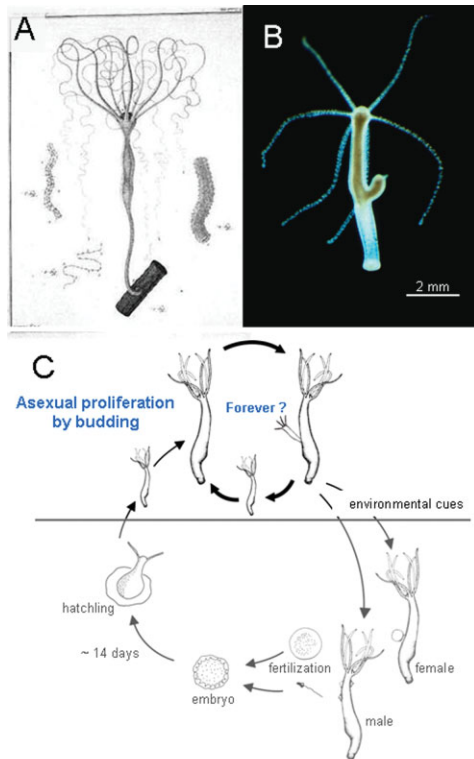


Figure 1. The freshwater polyp Hydra. **(A)** A hydra as depicted in Trembley's 1744 book. **(B)** *H. oligactis* polyp with a bud. **(C)** Hydra have adopted a life cycle in which proliferation and population growth occurs exclusively asexually by budding.

sex in a single polyp." The observation was presented at the first international workshop on hydroid development in September 1985 and published soon after.^(17,18) The results provided direct experimental support for a proposal made by Buss and Green⁽¹⁹⁾ (see also Ref. 20) that asexual proliferation by budding ("ramet production") requires the presence of an actively dividing multipotent cell line capable of differentiating into somatic as well as germ cells.

The trouble was that, in the absence of true genetic approaches and relevant genetic mutations, reports on Hydra research based on similar experimental strategies were often confronted with rival conclusions casting doubts on, e.g., the multipotency of interstitial stem cells,^(21–23) the role of cell density in growth regulation,^(24–26) or the migratory activity of interstitial cells.^(27–29) This was disturbing. Apparently due to limitations in existing techniques, fully understanding of the spatiotemporal mechanisms controlling patterning and cell differentiation in Hydra was a goal hard to achieve in the early 1990s. Hydra researchers, therefore, suffered from diminished attention in that decade, as mainstream opinion at that time held that one had to understand only mouse, fly and worm—and then development would be understood.

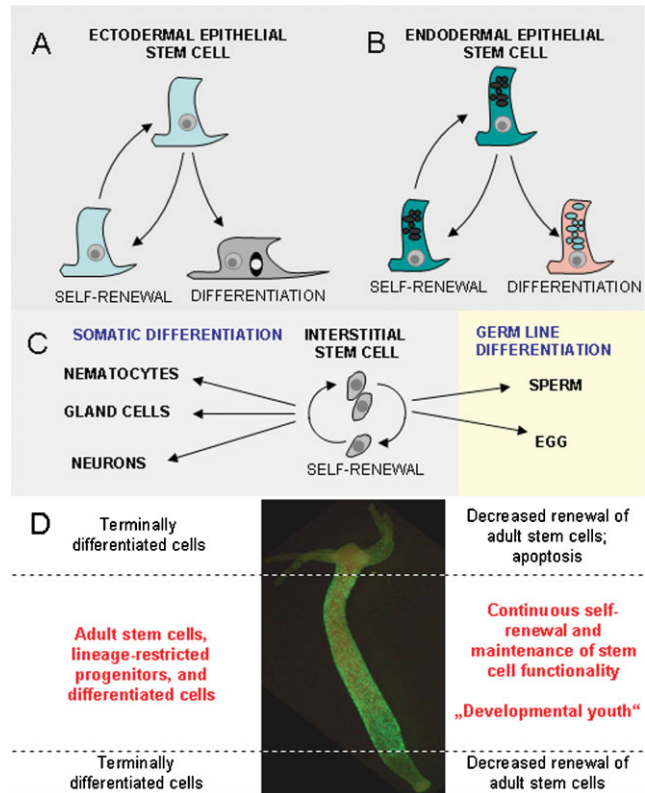


Figure 2. Hydra has three independent stem cell lineages: ectodermal epithelial cells, endodermal epithelial cells, and interstitial stem cells. **(A)** Ectodermal epithelial cells. **(B)** Endodermal epithelial cells. **(C)** Interstitial stem cells. **(D)** Self-renewal and terminal differentiation follow strict spatiotemporal rules. Continuously proliferating interstitial cells in Hydra stained with monoclonal antibody C41 are restricted to the gastric region and are absent in head and foot tissue.

It needed the advent of "omics" and transgenesis to bring Hydra and their stem cells back to the main stage. Now, the availability of novel molecular tools and rich genomic resources including the *H. magnipapillata* genome sequence returned these cells to a key position in evo-devo research by promising answers to questions such as: How do stem cells in basal metazoan differ from stem cells in Bilateria? Are certain features common to all stem cells? How did stem cells evolve?

Transgenic stem cells enable *in vivo* tracing in an animal made up of only three distinct stem cell lineages

Tissue function and maintenance in hydra is based on three tissue-specific types of stem cells: ectodermal and endodermal epitheliomuscular cells and interstitial stem cells (Fig. 2). All three stem cell types have unlimited self-renewal capacity and can differentiate into one or more cell types. Cues from the microenvironment in which these cells exist

appear to be crucial for controlling their developmental potency. In the last few years, we and a few other dedicated enthusiasts of Hydra research happily seized the opportunity to revisit these cells and the long-standing questions with emerging methodologies. Among them, we developed a method for the generation of transgenic stem cells using embryo microinjection. It was not an easy task. Efforts dated back to the time I was a postdoc in the laboratories of Hans Bode and Robert Steele at the University of California, Irvine, in the mid-1980s. Success came only 20 years later at the University of Kiel when we abandoned commercially available expression vectors, made our own Hydra-specific reporter gene constructs with Hydra promoters and Hydra terminator sequences flanking the reporter genes, and employed embryo microinjection making use of a previously not available strain of *Hydra vulgaris* which undergoes sexual reproduction and thus, generates embryos continuously throughout the year.

The yield of transgenic animals obtained by this method is of the same order of magnitude as that seen with mouse embryos. And similar to transgenic mice, the founder polyps obtained from microinjected embryos are mosaic (Fig. 3A). Unlike the mouse, however, it is not necessary to carry out breedings to produce non-mosaic animals. Due to the tissue dynamics of the adult Hydra polyp, the pool of transgenic cells

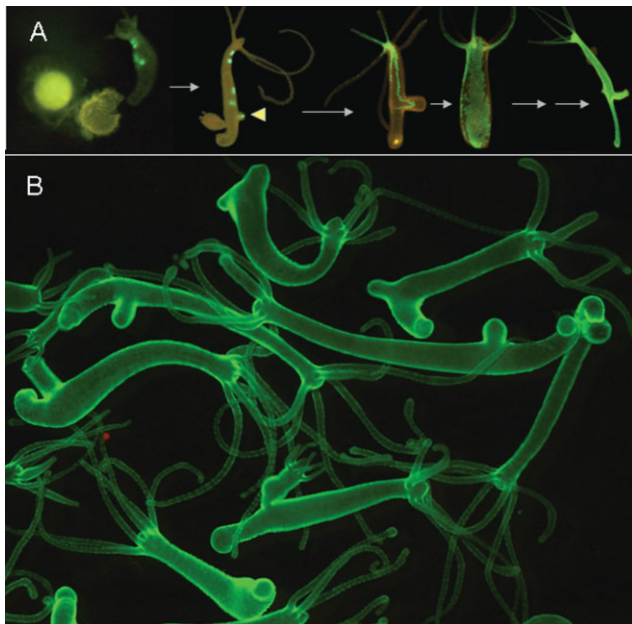


Figure 3. Generation of fully transgenic polyps from mosaic founder polyps. **(A)** Generation of transgenic *H. vulgaris* (AEP) line endo-2 from a polyp with a mosaic expression of EGFP in some of its endodermal epithelial cells to a transgenic endo-2 polyp expressing EGFP in all of its endodermal epithelial cells. (modified from Ref. 30) **(B)** Continuously growing mass culture of transgenic polyps expressing EGFP in all of their ectodermal epithelial cells.

in a mosaic polyp expands by cell division and moves into buds (see arrowhead in Fig. 3A). As this process progresses, one eventually ends up with some polyps which have the transgene in all cells of the particular cell lineage (Fig. 3A and B). The findings appeared in the Proceedings of the National Academy of Sciences in 2006⁽³⁰⁾ and culminated in imaging of transgenic interstitial stem cells carrying either the dsRED or the eGFP reporter gene at a single-cell resolution (Fig. 4).⁽³¹⁾

Transgenic interstitial cells expressing the GFP or dsRED reporter gene under control of the actin promoter (Fig. 4B and C) have enabled to track these cells and their derivatives *in vivo* under normal and experimentally altered conditions.⁽³¹⁾ Moreover, by *in vivo* monitoring of transgenic gland cells (which are differentiation products of interstitial stem cells), we observed to our great surprise that zymogen cells continuously transdifferentiate into granular mucous cells in the head region.⁽³²⁾ These findings demonstrate that derivatives of the interstitial cell lineage exhibit a remarkable plasticity in terms of their differentiation capacity, and that, beside stem cell-based mechanisms, transdifferentiation is involved in normal development and maintenance of cell type complexity in Hydra.⁽³²⁾ Together with preliminary observations in sponges (Noriko Funayama, pers. communication) which indicate cellular reprogramming to be one of the key factors contributing to tissue complexity, this might indicate that in organisms which diverged before the origin of bilaterian animals, commitment, and differentiation might be less stable events than in more complex metazoans.

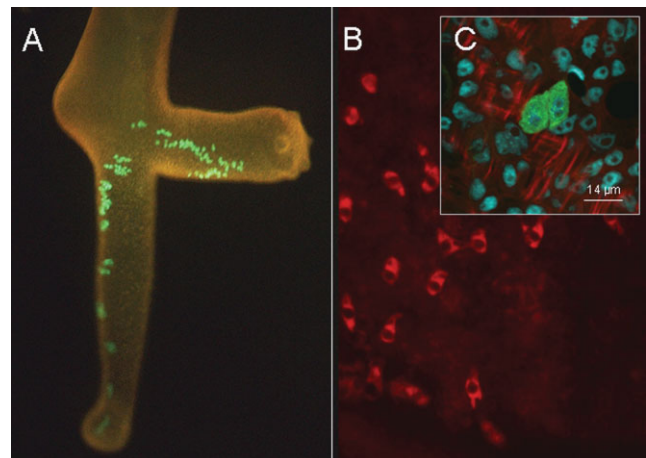


Figure 4. Translucent polyps allow *in vivo* tracking of transgenic cells. **(A)** Whole-mount fluorescence analysis of transgenic endodermal epithelial cells expressing reporter gene eGFP. **(B)** Whole-mount fluorescence analysis of dsRED expression in interstitial stem cells. **(C)** Confocal analysis of a pair of undifferentiated eGFP+ interstitial cells. Cell nuclei were counterstained with DAPI. Rhodamine-phalloidin was used to stain the muscle fibres. Photograph by F. Anton-Erxleben.

Tracing of GFP-labeled transgenic ectodermal epithelial cells showed⁽³³⁾ that in contrast to epithelial cells in more complex animals, in *Hydra* these cells have the potency for drastic changes in structure, shape, and cell contact along the body column. Among the signals instructing ectodermal epithelial cells to execute different programs of terminal differentiation are components of the canonical Wnt signaling pathway.⁽³³⁾ It seems likely that 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.

Interestingly, cells of the three co-existing stem cell lineages (Fig. 2) have never been observed to be able to transform from one lineage into the other. Do common regulatory networks exist between the three stem cell lineages? Key components regulating self-renewal of stem cells in vertebrates and invertebrates and being involved in epigenetic regulation are the Polycomb group (PcG) family genes.⁽³⁴⁾ The unexpected observation that in *Hydra* two members of the PcG family of genes, PRC2 components Eed and Ezh, are expressed in interstitial stem cells but not in epithelial stem cells^(31,35) may indicate that lineage-specific regulatory mechanisms control the transition into the various somatic cell types. In the long run, *Hydra*, therefore, appears to be an excellent model for studying how the divisions of three distinct types of stem cells within an organism are controlled—a question central to the understanding of tissue and cell type homeostasis.

The molecular mechanisms and signal pathways governing stem cells in *Hydra* have recently been reviewed and summarized elsewhere.^(36,37) In brief, signaling pathways involving Notch and glycogen synthase kinase-3 beta (GSK-3 β) play a role in inducing or suppressing differentiation of stem cells in *Hydra*. GSK-3 β is a cytoplasmic enzyme controlling degradation of β -catenin, a critical regulator of pattern formation and cell differentiation. When Wnt signaling is experimentally activated by the addition of alsterpaullone (ALP), a drug which specifically inhibits GSK-3 β , *in vivo* tracing of GFP-expressing interstitial cells shows that these cells are forced to terminally differentiate into nematoblasts.⁽³¹⁾ Therefore, in adult *Hydra* this pathway obviously fulfils two functions, one in patterning^(38–40) and one in interstitial cell differentiation.⁽³¹⁾

Similar observations have been reported⁽⁴¹⁾ in the colonial hydroid *H. echinata*, where short-term blocking of GSK-3 results in a significant increase in nematocytes and nerve cell numbers, indicating that the canonical Wnt cascade is one of the factors controlling the recruitment of stem cell derivatives in Cnidaria, similar to the role that Wnt signaling plays in stem cells in vertebrates. Furthermore, during interstitial cell differentiation in *Hydra*, Notch activity is required by differentiated nematocytes and appears to be a key component in the acquisition of nematocyte fate.^(31,42)

Suppression of Notch signaling causes immediate death of differentiating nematoblasts. Inhibition of Notch does neither affect neurons nor interstitial cells. Taken together, since Wnt and Notch pathways are involved in the control of stem cell behavior in *Hydra*, similar key signaling pathways appear to orchestrate stem cell behavior throughout the animal kingdom from *Hydra* to man. Moreover, *in vivo* monitoring of transgenic polyps which overexpress HyEED in the interstitial cell lineage under the control of the *Hydra* actin promoter⁽³¹⁾ indicated that remodeling of chromatin structure is involved in interstitial cell differentiation. Determining how these epigenetic features relate to the transcriptional signatures of stem cells, and whether they are also important in other types of stem cells in *Hydra*, is a key challenge for the future.

“Omics” shed light on the evolution of stem cells

Novel computational tools and the development of genomic resources such as large-scale EST projects as well as genome projects with assembled draft genome sequences have brought a new perspective on the developmental capacities of *Hydra* and related basal metazoan animals. To facilitate access and analysis of genomic and transcriptomic data in these organisms, we have established a local bio-computational platform, compagen.⁽⁴³⁾ The sequence databases at compagen contain selected raw genomic and expressed sequence tag (EST) sequence datasets from sponges and cnidarians up to the lower vertebrates. In addition to the public datasets, compagen also provides already processed data like assembled EST datasets or predicted peptides.

We have recently made use of the database for studying the evolutionary origin of stem cell genes and asking whether genes known to affect stem cell maintenance and differentiation in vertebrates are present in these early branching metazoans. The pilot study unveiled⁽⁴³⁾ Sox2 as one of the most conserved stem cell-specific genes known so far. Cnidarians and bilaterians diverged about 560 million years ago; yet the detection of Sox2 in early branching metazoans suggests that similar regulators of stem cell potency may be present in both groups. Since the study, to our surprise, provided no evidence for the presence of nanog and Oct3/4 genes in any of the early branching metazoans,⁽⁴³⁾ answers to questions such as “How do stem cell genes change over evolutionary time?” become evident.

It is obvious that the evolution of multicellularity necessitated the development of strict controls to keep cellular proliferation and the interaction between the various stem cell lineages in a steady state. The interpretation, however, remains complicated until we have extensively characterized

these cells and found a way to define the regulatory mechanisms that control their differentiation. Complementing the candidate approach described above, we therefore currently use massively parallel pyrosequencing of EST libraries made of the various cell lineages in Hydra (Hemrich, Rosenstiel, and Bosch, unpubl.). The lineages can be purified by FACS since we have produced transgenics carrying the GFP reporter gene in each of the two epithelial cell lines, the entire interstitial stem cell lineage, and derivatives of interstitial stem cells such as gland cells and nematocytes. The global expression profile of the isolated Hydra cells promises, for the time being, to provide a molecular signature of the different cell types in Hydra, to expand our understanding of the biological processes that are active in them, and to unveil ancient mechanisms that control tissue homeostasis and cell proliferation.

Tissue homeostasis in Hydra: a delicate balance, an experimentally tractable niche, and an important unanswered question

One of the intriguing features of Hydra is that its tissue is in a dynamic state, constantly undergoing renewal as a result of continuous growth and differentiation of epithelial and interstitial cells. For example, while in mammals neurogenesis is highly restricted in adulthood, in Hydra there is continuous differentiation of interstitial cells into neurons. Since epithelial cells are also produced constantly, neuron density must be maintained at a constant level as well. Pioneering studies in *Drosophila* germinal development⁽⁴⁴⁾ have shown that stem cell populations are established in 'niches'. Stem cell niches ensure the proper balance between stem cells and progenitor cells and integrate signals that mediate the balanced response of stem cells to the needs of organisms.⁽⁴⁵⁾

Working out *in vivo* the anatomic and functional dimensions of the niche in Hydra is in its beginning. When we recently generated polyps which express GFP under control of the Hydra nanos promoter in their interstitial stem cells (Puchert and Khalturin et al., in prep), we were surprised to detect only little if any migratory activity of these multipotent stem cells. In control transgenics containing interstitial cells expressing GFP under the actin promoter, numerous migratory cells were observed and found to be neuron and nematoblast precursors. We conclude from these observations that interstitial stem cells prefer to stay at home. To visualize the precise site of their residence (referred to as niche⁽³¹⁾) we performed scanning electron microscopy. The interstitial stem cell niche in Hydra is shown in Fig. 5A and B.

From the point of view of an individual interstitial stem cell, what are the molecular components of this niche? Briefly,

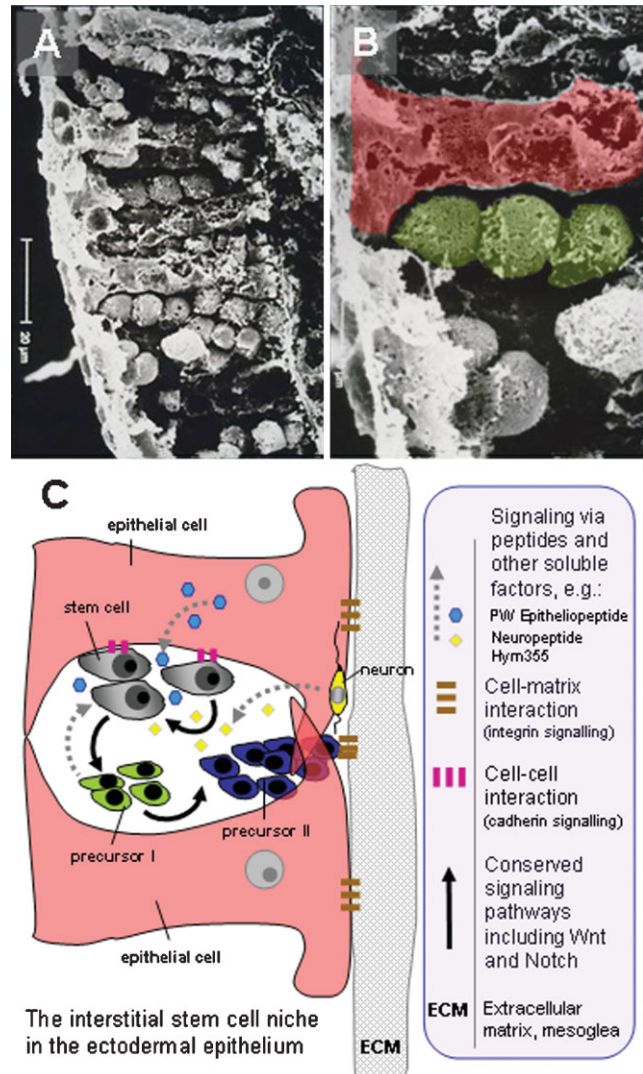


Figure 5. The architecture of the interstitial stem cell niche in the ectodermal epithelium. Interstitial cells are found adjacent to ectodermal epithelial cells and affected by epitheliopeptides. Interstitial stem cells are also found adjacent to the mesoglea (extracellular matrix). **(A)** Scanning electron microscopic view of a longitudinal section through the ectoderm. Modified from Ref. 31. **(B)** Scanning electron micrograph showing interstitial cells within their niche. Note the close contact of interstitial cells (green) to an ectodermal epithelial cell (red). Modified from Ref. 31. **(C)** Schematic view of interstitial stem cell niche in the ectodermal epithelium. Similar to tissue-specific stem cells in vertebrates, multipotency in Hydra appears to be governed by extrinsic cues from the microenvironment and through the coordinated actions of a number of conserved signaling pathways such as the Wnt and Notch pathway that interpret soluble signals. The epitheliopeptide Hym33H (AALPW) regulates the differentiation of interstitial cells into neurons.⁽⁴⁶⁾ Neuron differentiation is also affected by peptides secreted from neuronal precursors.⁽⁴⁶⁾ Interstitial cell density also influences stem cell function and survival. It is likely but not yet proven that also pathways that interpret cell–cell interactions and spatial cues, including integrin and cadherin signaling, are involved.

there is evidence^(46,47) that epithelial cells affect interstitial cell differentiation behavior by secreting epithelipeptides. There is also experimental evidence that derivatives of the interstitial cell lineage such as neurons affect interstitial cell differentiation,⁽⁴⁶⁾ and that nerve cell density influences interstitial cell proliferation.⁽⁴⁸⁾ Putative components of the spatiotemporal dialog between interstitial stem cells and niche cells may also include integrin and cadherin signaling (Fig. 5C). To uncover the niche characteristics, we currently use transgenesis to introduce alterations in the niche by gain-of-function and loss-of-function experiments. For example, does overexpression of PW epithelipeptides in transgenic polyps affect nerve cell differentiation? Such insights will be crucial for the detailed understanding of the role the microenvironment plays in stem cell decision making.

The niche holds one more important question which needs to be addressed in the next few years: how is the size of a polyp controlled? The niche obviously ensures the proper balance between stem cells and progenitor cells available for tissue homeostasis and proportion regulation. Earlier observations indicated^(49,50) that polyps can regulate the relative sizes of the various structures during regeneration, and that (not yet defined) growth control mechanisms take care that the several populations of cells in the continually growing Hydra polyps remain in constant proportion to one another over hundreds of asexual generations. Moreover, polyps are always of distinct and species-specific size. Size is obviously encoded in the genes and depends on cell number and cell size. Polyps are, however, not simply the sum of cell size and cell number; rather, there appear to be rules that control polyp size. What these rules are and how growth is controlled remains an unsolved mystery. Determining the mechanisms underlying these unknown integration systems will provide important insight into the mechanism not only of tissue homeostasis and pattern formation in Hydra but also of multicellular organogenesis in general.

Principles of stem cell control in other cnidarians

Stem cells have also been studied and visualized in two phylogenetically distant cnidarians, the hydrozoan jellyfish *Clytia hemisphaerica* and the colonial hydroid *Hydractinia echinata*. In *H. echinata*, interstitial stem cells are found predominantly in the stolon compartment of the colony.⁽⁵¹⁾ In elegant experiments using *Hydractinia* colonies depleted of their interstitial cells, Müller and coworkers repopulated them with allogeneic interstitial cells from a histocompatible donor. Since donors and recipients differed in phenotypes, following repopulation from the donor revealed that the phenotype of the recipient was reverted to that of the donor. Most interestingly, introducing BrdU-labeled donor cells showed

that epithelial cells in *Hydractinia*, in conspicuous contrast to epithelial cells in hydra (see Fig. 2), can derive from interstitial cells, suggesting that interstitial stem cells in colonial hydroids are totipotent.⁽⁵¹⁾

Recent research in the hydrozoan *Clytia hemisphaerica*⁽⁵²⁾ has provided a novel model system to dissect how stem cell-intrinsic factors are integrated with signaling events in the microenvironment of the “stem cell niche”, in order to maintain tissue homeostasis. In *Clytia*, nematoblasts are numerous in the interstices between ectodermal epithelial cells within the “tentacle bulbs”, spherical outgrowths on the bell margin of medusa from which the tentacles grow. Nematogenesis progresses from the base to the tip of the bulb. Spatial progression of nematoblast stages along the bulb axis is correlated with differential gene expression with most of the cells at the base of the tentacle bulb expressing the *Clytia* Piwi homologue but not a differentiation marker.⁽⁵²⁾ Since the Piwi gene is a widely conserved stem cell marker throughout multicellular eukaryotes,^(53,54) these cells might be considered as a population of stem cells. It seems likely that, similar to Hydra (see Fig. 5), the molecular signals exchanged between the stem cells and the other cells within the microenvironment are key factors in stem cell control. Finding and identifying these signals in *Hydra* and *Clytia* and comparing them to signals identified in invertebrates such as *Drosophila*⁽⁵⁵⁾ and planarians^(56,57) will become instrumental in formulating basic concepts of stem cell biology.

A presumed enigma: Life in the absence of senescence

To us, as practically to all the creatures around us, apply the words in one of Bach's masterpieces: “Here we do not have an eternal staying”. Aging and death are integral and intrinsic to the evolution of life. As first described in human fibroblasts nearly 50 years ago,^(58–60) mammalian cells all have a limited proliferative lifespan and a finite number of population doubling, termed the Hayflick number. Senescence defined as progressive declines of physiological functions, leading to an increase in the mortality rate as a function of time, has been found in all metazoans where careful studies have been carried out. However, although it is often postulated that stem and progenitor cell depletion or dysfunction might contribute to senescence, the biochemical basis behind remained elusive. Immortal Hydra may provide an answer since, as I will outline below, lack of senescence in Hydra is simply due to the fact that the body is constantly renewed from continuously self-renewing stem cells.

Analyzing the mortality patterns and reproductive rates of four groups of individuals of *H. vulgaris* for a period of four years, Daniel Martinez at the Pomona College in Claremont, California, could find no evidence for aging and no apparent

signs of decline in reproductive rates.⁽⁶¹⁾ Hydra, therefore, has been suggested not to undergo senescence, and being biologically immortal.^(61,62) This view has recently been challenged by Yoshida et al.,⁽⁶³⁾ who searched for signs of aging in sexually differentiated *H. oligactis*. Since after sexual reproduction Yoshida et al.⁽⁶³⁾ found a significant decline in the capacities for food capture, contractile movements, and reproduction, as well as an exponential increase in the mortality rate of the population, they proposed that the degenerative process in *H. oligactis* following sexual reproduction represents the aging process.

These data, however, have to be taken cum grano salis for two reasons. First, these degeneration processes are observed only under laboratory conditions and might simply be the consequence of the excessive sexual production activity described by the late Pierre Tardent⁽⁶⁴⁾: “Particularly in males, gamete production is so intense that we can speak in terms of a “gametic crisis” (“crise gametique,” see Ref. 65) leading to a complete exhaustion and death of the animals.” Second, there are several species of closely related Hydra, e.g., *H. magnipapillata* and *H. vulgaris*, which do not undergo degeneration after sexual reproduction but continue to proliferate and grow indefinitely. Since it seems unlikely that there are immortal Hydra species and mortal ones, degeneration in *H. oligactis* might simply be based on the fact that in this species environmental signals cause multipotent stem cells to shift their differentiation program exclusively to germ cell differentiation.

In vivo tracking of individual GFP-expressing cells showed unquestionably that epithelial cells⁽³⁰⁾ as well as interstitial cells⁽³¹⁾ in Hydra located throughout the gastric region are continuously proliferating and differentiating (see also Fig. 3). Offspring of individual cells were tracked for several years without obtaining evidence for apparent signs of decline in proliferation rates. The apparent lack of cellular senescence results in an adult polyp which has an indefinite proliferative lifespan, and adds support to the study by D. Martinez.⁽⁶⁰⁾ However, although obviously still a matter of debate and for the non-specialist a not-understood issue (e.g., see Ref. 66), the biological reason behind that seemingly unique potential to escape mortality and senescence is simple: the animals have adopted a life cycle in which proliferation and population growth occur exclusively asexually by budding (Fig. 1C). This asexual mode of reproduction demands that each individual polyp maintains continuously proliferating cells. If it would not, the species would lose in number of offspring and would, sooner or later, be outcompeted by other species that did. Thus, there is strong selective constraint to equip the adult polyp's tissue with cells which are capable of continuous proliferation and differentiation. A polyp's life in the absence of senescence is, therefore, not “an evolutionary scandal” (*sensu* John Maynard Smith⁽⁶⁷⁾), but the consequence of natural selection and strong evolutionary constraint.

The implications of studying a tissue packed with stem cells which lack cellular senescence are of course manifold and important. Intriguingly, a recently postulated attractive theory is that aging-related phenomena might be, at least in part, due to the decline in the number or function of tissue stem cells.^(68,69) An example in support of this theory is the recent demonstration that progeria is caused by a problem of adult stem cells.⁽⁷⁰⁾ Earlier studies in support of this idea have shown that the proliferative and regenerative capacity of human hematopoietic stem cells diminishes with age (for review see Ref. 69). Thus, since Hydra maintain the number and functionality of stem cells indefinitely, methodological developments tempt us to propose that insight into these cells will help us to learn what causes the decline in stem cell numbers and function in more complex animals. Approaching aging and senescence from this comparative point of view is a primary goal of evolutionary medicine.

Why polyps matter: The power of being slightly different

Hydra's efficient regeneration capacity and stem cells reflect a rather unique and very dynamic developmental system controlled by distinct signaling pathways and instructions. The imminent availability of methods for functional analyses of genes and the massive advances in molecular technology that are presently taking place, make Hydra a powerful and also intellectually attractive system for studying stem cells. Admittedly, Hydra is simple. And it may not have changed dramatically over the last few hundred million years. It certainly did not evolve in something complicated and, for example, neither developed a third germ layer, a centralized nervous system, nor specialized muscle cells. However, evolution is not always going as fast as it could and Hydra, far from being a flop, is rather a success and by all means informative. Take into consideration, for example, that before therapies using human stem cells can be approved, researchers will have to answer one key question: where do the cells go when they are injected into the body? Since Hydra are completely transparent and allow *in vivo* imaging of stem cells at a single-cell resolution (Fig. 4), it promises to contribute to work out the yet hidden basic principles.

Taken together, the answer to the question “Why polyps matter?” is straightforward: Hydra is experimentally tractable and provides deep insight into fundamental questions. Organisms become models when they support sustainable opportunities with uncompromising experimental rigor and ease of use. With the molecular dissection of the components controlling stem cell behavior in Hydra, the stage is set for lower metazoan biologists to uncover the mystery of “stemness” and deciphering the fundamental components controlling pluripotency and lineage commitment that underlie

all stem cell systems. As I have outlined elsewhere,⁽³⁷⁾ now is the time to make use of the growing possibilities of comparative stem cell biology—and to take stem cells in Hydra to new heights.

Conclusion

The extensive capacity of cnidarians to regenerate is due to the presence of stem cells which continuously transit through the proliferation/differentiation switch and rapidly respond to signals from the cellular environment. Efforts using the nearly unlimited potential for tissue manipulation combined with functional transgenesis show the emerging importance of Wnt and Notch signaling in controlling stem cell behavior at the base of animal evolution. Although, more data are needed to support clear evolutionary scenarios, stem cells in Hydra and other cnidarians promise insights into signaling pathways and strategies involved in stem cell differentiation in the Bilaterian ancestor.

Acknowledgments: My sincere thanks are to the members of my laboratory: Friederike Anton-Erxleben, René Augustin, Sebastian Fraune, Georg Hemmrich, Konstantin Khalturin, and Jörg Wittlieb. I thank JSB and LH741 for helping to shape the manuscript and acknowledge continuous support from the Deutsche Forschungsgemeinschaft (DFG). Judith Ittner-Bosch read the manuscript, and her support has been invaluable.

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