Development, Growth & Differentiation
The Japanese Society of Developmental Biologists
Develop. Growth Differ. (2010) **52**, 15–25 doi: 10.1111/j.1440-169X.2009.01143.x

Review Article

The Hydra polyp: Nothing but an active stem cell community

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Hydra is a powerful stem cell model because its potential immortality and extensive regeneration capacity is due to the presence of three distinct stem cell lineages. All three lineages conform to a well-defined spatial distribution across the whole body column of the polyp. Stem cell function in *Hydra* is controlled by extracellular cues and intrinsic genetic programs. This review focuses on the elusive stem cell niche of the epithelial layers. Based on a comparison of the differences between, and commonalities among, stem cells and stem cell niches in *Hydra* and other invertebrates and vertebrates, we propose that the whole body column of the polyp may be considered a stem cell ''niche'' in which stem cell populations are established and signals ensuring the proper balance between stem cells and progenitor cells are integrated. We show that, at over 500 million years old, *Hydra* offers an early glimpse of the regulatory potential of stem cell niches.

Key words: adult stem cell, differentiation, multipotentiality, self-renewal, stem cell niche.

The stem cell niche concept

After birth, adult stem cells reside in a special microenvironment termed the ''niche,'' which varies in nature and location depending on the tissue type. The ''niche'' is composed of the cellular components of the microenvironment surrounding stem cells as well as the signals emanating from the support cells (Li & Xie 2005). Key niche components and interactions include growth factors, cell-cell contacts, and cell-matrix adhesions. Stem cell niches in both animals and plants ensure the proper balance between stem cells and progenitor cells and integrate signals which mediate the balanced response of stem cells to the needs of organisms (Ohlstein *et al.* 2004; Li & Xie 2005; Xie & Li 2007; Zhang & Li 2008; Discher *et al.* 2009; Fuchs 2009; Abrash & Bergmann 2009).

The ''niche'' hypothesis was proposed in 1978 by Schofield to describe the physiologically limited microenvironment which supports stem cells (Schofield 1978). Schofield proposed that true hemopoietic stem cells (HSC) exist only in association with one or more other supporting cells. These microenvironmental cells were postulated to form a specific niche which, when in close association with the HSC, allows an indefinite capacity for self-renewal, while effectively inhibiting differentiation and maturation of the cells. A number of *in vitro* experiments, as well as bone marrow transplantation experiments in which the niche is first ''emptied'' through irradiation or drug treatments, supported the niche hypothesis (Brinster & Zimmermann 1994, Dexter *et al.* 1977, Moore *et al.* 1997, Rios and Williams 1990, Roecklein & Torok-Storb 1995, Sitnicka *et al.* 1996). In 2000, pioneering studies in germinal development in *Drosophila* uncovered the niche structure *in vivo* for the first time and showed that stem cell populations are established in such 'niches'(Xie & Spradling 2000). Subsequently, stem cell niches have been located and characterized in a number of invertebrates and vertebrates (for review see Li & Xie 2005). In *Drosophila*, in addition to the germarial tip in the ovary (Xie & Spradling 2000; Gilboa & Lehmann 2007; for review see Kirilly & Xie 2007), the hub, located at the tip of testis, was defined as the niche supporting germ line stem cells (GSC) in the testis (Kiger *et al.* 2001, Tulina & Matunis 2001; Gilboa 2008). In *Caenorhabditis elegans*, a distal tip cell (DTC) located at the tip of the germ line organization region was found to function as the niche in supporting GSC (Crittenden *et al.* 2002). In mammals, the location of epithelial stem cells was successfully identified in the bulge area of

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Received 20 August 2009; revised 9 September 2009; accepted 10 September 2009.

hair follicles, and that of intestinal stem cell near the crypt base (Cotsarelis *et al*. 1990, Potten *et al.* 2002). Key cellular components of the niche that regulates hematopoietic stem cells include osteoclasts, osteoblasts, endothelial cells and nerves (Li & Li 2006).

While traditionally the word ''niche'' is used to describe the stem cell location, in fact it is much more than a histological structure: the stem cell niche functions as the regulatory context in which stem cell decision making takes place. This function may represent an ancestral function.

Cellular biological aspects of stem cells in *Hydra*

The regeneration capacity of the freshwater polyp *Hydra* is proverbial (Bosch 2007). Polyps can be experimentally dissociated into single cells, then recombined into clumps (''aggregates'') that will naturally self-organize and form a normal, fully intact animal within two days (Gierer *et al.* 1972). In undisturbed animals, growth of tissue occurs uniformly throughout the body column (Campbell, 1967; David & Campbell 1972). Individual animals however, do not increase in size since growth is just balanced by loss of tissue in the form of buds in the lower gastric region and by sloughing of tissue at the ends of the tentacles and from the basal disk. This combination of uniform growth and local cell loss leads to continuous movement of tissue either up the body column into the tentacles or down the body column into the buds and basal disk. *Hydra*'s asexual mode of reproduction by budding and its constantly active patterning processes are based on the presence of three continuously dividing tissue-specific types of stem cells: ectodermal and endodermal epitheliomuscular cells and interstitial stem cells (Fig. 1). Each of the two epithelial layers is made up of a stem cell lineage, while the remaining cells are part of the interstitial stem cell lineage which resides among the epithelial cells of both layers.

The existence of interstitial stem cells within *Hydra*'s ectodermal epithelium was illustrated initially by the ability of single cells to repopulate interstitial cell free epithelial tissue (David & Murphy 1977). In a later modification of this clonal assay, elimination of host interstitial cells was achieved using a mutant strain (sf-1) containing temperature-sensitive interstitial cells as host tissue (Bosch & David 1987). Temperature resistant donor cells were added in low numbers to sf-1 such that the added cells grew as clones. Subsequently host sf-1 interstitial cells were eliminated by a temperature shift. This technique made long-term clonal culture of *Hydra* stem cells possible. The results indicated that interstitial stem cells are multipotent in the sense that individual stem cells can differentiate into somatic cells as well as into germ line cells (reviewed in Bosch 2009).

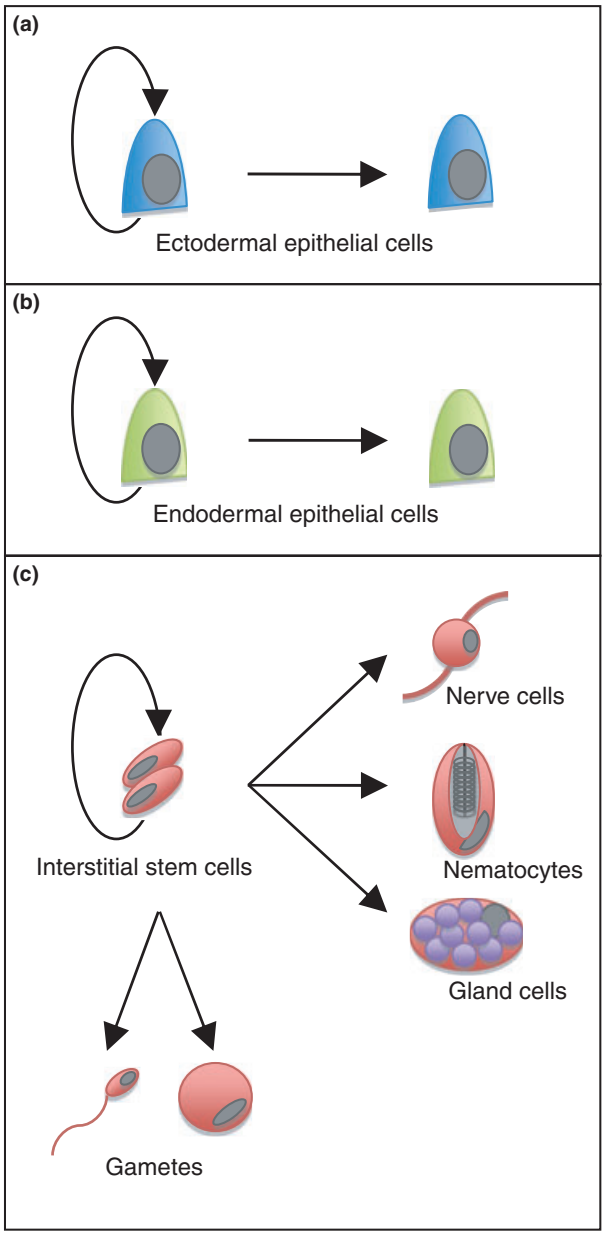

Fig. 1. The three independent stem cell lineages of *Hydra*: (a) ectodermal epithelial cells, (b) endodermal epithelial cells, and (c) interstitial stem cells.

Epithelial cells in the *Hydra* body column continuously undergo mitotic divisions (Dübel *et al.* 1987). To demonstrate their stem cell properties, we have made use of transgenic polyps and transplanted a single GFP-expressing endodermal epithelial cell into a non-transgenic polyp. By doing so we (Wittlieb *et al.* 2006) have generated polyps in which the entire ectodermal

or endodermal epithelium contains the transgene. Thus, *Hydra* epithelial cells are capable, by successive divisions, both of indefinite self-renewal and of producing different types of specialized cells such as tentacle or foot specific epithelial cells. Since there is no evidence for subpopulations of epithelial cells which cannot repopulate the host tissue, all *Hydra* epithelial cells in the gastric region must therefore be considered to be stem cells. Tracing of GFP-labeled ectodermal epithelial cells transplanted from various regions along the body column into unlabeled hosts has demonstrated that these cells have high self-renewal capacity along the whole body column (Wittlieb *et al.* 2006; Bosch 2009). This indicates that epithelial stem cells colocalize with interstitial stem cells (see Fig. 2 b). Tracing of GFP-labeled endodermal (intestinal) epithelial cells has revealed that, in addition to common genes, *Hydra* also share common cells with humans. Endodermal epithelial stem cells perform similar functions in humans and *Hydra,* lining the lumen of the small intestine in humans and the gastric cavity in *Hydra*. All absorption and most enzymatic digestion takes place on the surface of these endodermal epithelial cells. Nonetheless, our tracing results show that adult *Hydra* uniquely maintain the functionality of these endodermal epithelial stem cells indefinitely (Wittlieb *et al.* 2006; Bosch, pers. obs. 2009), a property that other systems do not have. How this is achieved in *Hydra* remains an important question.

All three stem cell types are adult stem cells, have an unlimited capacity for self-renewal, can differentiate into one or more cell types, and are an essential component of tissue homeostasis. Dividing stem cells of the interstitial lineage have a cell cycle time of 18–30 h, while stem cells of the epithelial lineages proliferate with a doubling time of about 3.5 days (David & Campbell 1972; Bosch & David 1984). Hence, cells in *Hydra* are either constantly renewing by cell division, or they are lost from the animal within a relatively short period of time. An individual cell, therefore, does not exist long in a *Hydra* body. As a result of these tissue dynamics, cells in *Hydra* are constantly displaced either apically towards the head, or basally onto developing buds, or to the foot (for review see Bosch 2007).

While there is no evidence that *Hydra* is simpler in molecular terms than vertebrates, nor that *Hydra* cells are fundamentally different from those in mice or humans, there may be a profound difference between *Hydra* and vertebrates in the differentiation potential and plasticity of their cells. Vertebrates depend on specialized cells with limited differentiation potential to perform sophisticated functions. In contrast, even adult tissue cells in *Hydra* are capable of producing and receiving positional signals continuously, and therefore have features which most cells in vertebrates have only during the short period of embryogenesis. It is this feature which makes adult *Hydra* tissue different from tissue of all other invertebrates and vertebrates. To sustain this stem cell function continuously in the adult polyp, a delicate balance between self-renewal and differentiation must be maintained. However, despite the important role that stem cells play in *Hydra*, their molecular composition has long eluded researchers.

Molecular aspects of stem cells in *Hydra*

Because Wnt signals appear to constitute the principle driving force behind developmental processes in *Hydra* (Hobmayer *et al.* 2000; Broun *et al.* 2005), we think that alterations in Wnt signaling may specifically alter interstitial cell behavior. We have summarized elsewhere that indeed signaling pathways involving Notch (Käsbauer *et al.* 2007) and glycogen synthase kinase-3 beta (GSK- 3b) (Khalturin *et al.* 2007) play a role in inducing or suppressing differentiation of stem cells in *Hydra* (Bosch 2009). GSK-3b 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-3b, *in vivo* tracing of GFP- expressing interstitial cells shows that these cells are forced to terminally differentiate into nematoblasts (Khalturin *et al.* 2007). To determine whether Wnt induced changes in interstitial cell behavior reflect cell-intrinsic activity or a response towards the changed microenvironment, we grafted tissue with GFP+ interstitial cells to unlabeled host tissue which had been treated with ALP for 48 hours preceding transplantation. We observed normal migratory activity of interstitial cells and nematoblasts into ALP treated tissue, indicating that ALP has no effect on the migratory activity of interstitial cells per se (Khalturin *et al*. 2007). In sum, extrinsic signals from the microenvironment play a major role in interstitial cell differentiation and migration, and may be mediated by the Wnt pathway. This pathway in adult *Hydra* obviously fulfils two functions, one in patterning (Hobmayer *et al* 2000; Broun *et al.* 2005) and one in interstitial cell differentiation (Khalturin *et al.* 2007). Thus, both epithelial and interstitial stem cells appear to rely heavily upon these regulatory circuits. However, just how these signaling pathways participate to preserve stemness remains elusive.

To get an initial hint as to which stem cell genes are present in *Hydra*, we made use of our local biocomputational platform ''compagen'', which contains

selected genomic and expressed sequence tag (EST) sequence datasets from sponges and cnidarians up to the lower vertebrates, and asked whether genes known to affect stem cell maintenance and differentiation in vertebrates are present in these early branching metazoans (Hemmrich & Bosch 2008). The pilot study unveiled genes closely related to the stem cell transcription factor Sox2 as the most conserved stem cell-specific genes known so far (Hemmrich & Bosch 2008). Since, to our surprise, the study provided no evidence for the presence of Nanog and Oct3/4 genes in any of the early branching metazoans, answers to questions such as ''How do stem cell genes change over evolutionary time?'' become evident. This preliminary *in silico* screening has demonstrated the existence of common stem cell identity across adult tissues of various organisms (Hemmrich & Bosch 2008), suggesting that stem cells may be identifiable through expression of certain gene subsets. Increased knowledge of the molecular definition of a stem cell should aid the field in elucidating the mechanisms involved in their regulation, an achievement that may also pave the way to better understanding why stem cells give rise to specialized cells at all. Taken together, stem cells in adult *Hydra* represent one of the most ancient stem cell systems in the animal kingdom and, therefore, provide information for reconstructing the early history of stem cell control mechanisms. Cues from the microenvironment in which these cells exist appear to be crucial for controlling their developmental potency. Where are stem cells in Hydra located?

Localization of interstitial stem cells in *Hydra*

One of the most exciting frontiers in stem cell biology is the location of stem cells. *Hydra*'s interstitial stem cells have long been thought to reside within the ectodermal epithelium, which rests upon a basement membrane (mesoglea) rich in extracellular matrix (ECM) proteins. Their spatial distribution was elucidated as early as 1980 by the elegant experiments of David and Plotnick (David & Plotnick 1980). Assaying stem cells as the concentration of cells capable of forming clones (CFU) in interstitial cell free aggregates (David & Murphy 1977), David and Plotnick inoculated aggregates with large numbers of stem cells taken from four defined regions along the body axis (head, upper gastric, lower gastric, and foot) and determined the total size of the stem cell population after a defined period of growth (4 days). In addition, David and Plotnick (1980) determined the ratio of stem cells to early committed cells in each region of *Hydra*. This ratio is a sensitive indicator of the probability of self-renewal (P_s), and was used it to estimate the growth rate of the stem cell population in specific regions of *Hydra*. Their results indicated that interstitial stem cells in *Hydra* are uniformly distributed along the body column in the gastric region, but are almost absent in the head and foot (basal disk) (Fig. 2). The concentration of CFU in the upper and lower body column was calculated as about 0.02 CFU/ epithelial cell corresponding to a total of about 2300 stem cells (David & Plotnick 1980). The much lower density of stem cells in the head and foot region, where about 0.001 CFU/epithelial cell were found, was correlated with a decrease in the ratio of stem cells to early committed cells.

Although this research had clearly documented that interstitial stem cells are distributed throughout the

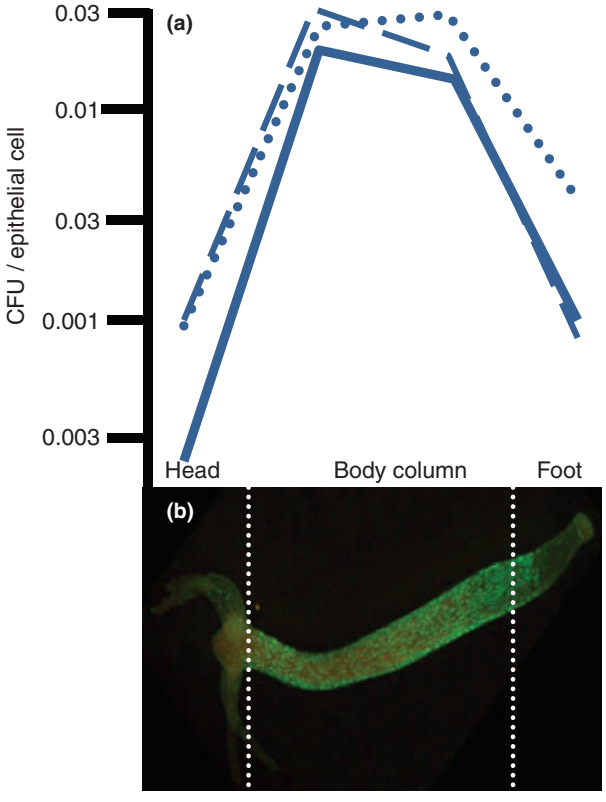

Fig. 2. Self-renewal and terminal differentiation of interstitial stem cells follow strict spatiotemporal rules. (a) Distribution of CFU along the *Hydra* body column. Interstitial cells (Is+2s) and epithelial cells were determined by maceration using pieces cut from ten *Hydra*. CFU were determined on pieces cut from 20 *Hydra* using the clonal assay described in the text. Different symbols represent results from independent experiments. (Reproduced from David and Plotnick 1980 with permission). (b) As shown by staining with monoclonal antibody C41, continuously proliferating interstitial cells in *Hydra* are restricted to the gastric region and absent from head and foot tissue.

entire body column, it remained unknown exactly where the inoculated cells were located. This ignorance was due to a lack of specific molecular markers, making it difficult technically to capture the very limited numbers of *Hydra* cells undergoing this process and to identify individual stem cells *in situ*. To resolve this problem, in 2005 our lab began investigating the properties of stem cells in *Hydra* by using transgenic technology to introduce DNA coding for a GFP molecule into the genome of *Hydra*'s epithelial and interstitial stem cells (Wittlieb *et al.* 2006; Khalturin *et al.* 2007; Siebert *et al.* 2007, Milde *et al.* 2009). Since then, using a number of different reporter gene constructs, we have generated several lines of transgenic Hydra expressing GFP under the control of stem cell gene promoters in the interstitial stem cell lineage. Stem cell specific genes such as nanos, vasa and PIWI have proved to be useful marker genes for multipotent interstitial stem cells, since they are expressed in both the interstitial stem cell lineage and the germ line (Mochizuki *et al.* 2000; Mochizuki *et al.* 2001; Bosch, pers. obs. 2008; for review see Bosch 2004). Using a nanos-GFP transgenic *Hydra* line for lineage tracing, a few months ago we made an initial step towards answering the questions of where precisely interstitial stem cells are localized in their niche and how they behave in the context of the native environment. Working out the anatomic and functional dimensions of the niche in *Hydra in vivo* is in its beginning stages. However, as shown in Figure 3, confocal microscopy in combination with novel imaging techniques has not only visualized for the first time the precise site of residence of interstitial stem cells, but also promises to reveal previously unrecognized details of the localization of interstitial stem cells within their niche environment (Anton-Erxleben, Khalturin & Bosch, unpubl.). When tracing the localization of green-fluorescent-protein-expressing (GFP+) interstitial stem cells (isc) we always found them to be localized near, or even in physical contact with, both epithelial cells and the mesoglea (Fig. 3b,c). Intriguingly, z-stacks and 3D-reconstruction revealed that in pairs of interstitial stem cells only one of the two cells is in contact with the underlying muscle fibers and mesoglea, while both of them appear to have contact with neighboring epithelial cells (Fig. 3). This observation indicates that both the mesoglea and the muscle fibers within the ectodermal epitheliomuscular cells are important constituents of the niche. The stem cell niche, therefore, appears to exhibit an asymmetric or polar structure with muscle fibers and mesoglea components located exclusively at the basal location. While these findings change the old concept of a rather uniform interstitial space, our current efforts are directed towards understanding whether, upon division, one daughter interstitial cell is maintained in the niche as a stem cell while the other daughter cell begins to proliferate and differentiate, eventually becoming a functionally mature neuron, nematocyte or gland cell. To obtain information about the dimensions of the niche, we used confocal microscopy to collect z-stack image series of the interstitial space in actin-GFP transgenic polyps with GFP-expressing ectodermal epithelial cells. The resultant high-resolution confocal images (Fig. 3d) show that the microenvironment for interstitial stem cells has a structure of three-dimensional cavernous chambers with apparently unrestricted connectedness, providing a passageway for both cells and extracellular molecules (Anton-Erxleben & Bosch, unpubl.). Taken together, the niche in *Hydra* appears to represent a complex *in vivo* milieu in which both interstitial and epithelial stem cells, as well as the mesoglea, are key components and in which stem cells and their derived lineages encounter a multitude of cues that can influence their fate (Fig. 4).

Niche interactions at invisible interfaces

Although substantial progress has been made in identifying the niche structure in *Hydra*, we have as yet gained only a rough outline of the interplay of mechanisms that coordinate proliferation and differentiation of the three stem cell lineages. Figure 4 illustrates the current view of the anatomic and functional dimensions of the niche in *Hydra*. Notably, both continuously proliferating epithelial stem cells and continuously proliferating interstitial stem cells provide the niche environment. A primary function of the niche is to anchor stem cells. Cadherin-mediated cell adhesion is required for anchoring stem cells in *Drosophila* (Song *et al.* 2002), and is important for anchoring hematopoietic stem cell in the bone marrow niche (Zhang *et al*. 2003). The role of cadherin in anchoring stem cells in *Hydra* remains to be uncovered. The simplistic view given in Figure 4 certainly paints only part of the picture, and many challenges for the future remain. Future studies in which experiments are designed to identify the sources of the various signals within the niche and how these signals are modified in response to environmental stimuli will be of great benefit. Most importantly, the fundamental question: ''What are the signals that control stem-cell proliferation and dictate whether a daughter of a stem cell shall remain a stem cell or become committed to differentiation?'' is still unanswered. The control of stem cell numbers, their commitment, and progeny generation are biological questions of great importance in *Hydra* as in any other organism.

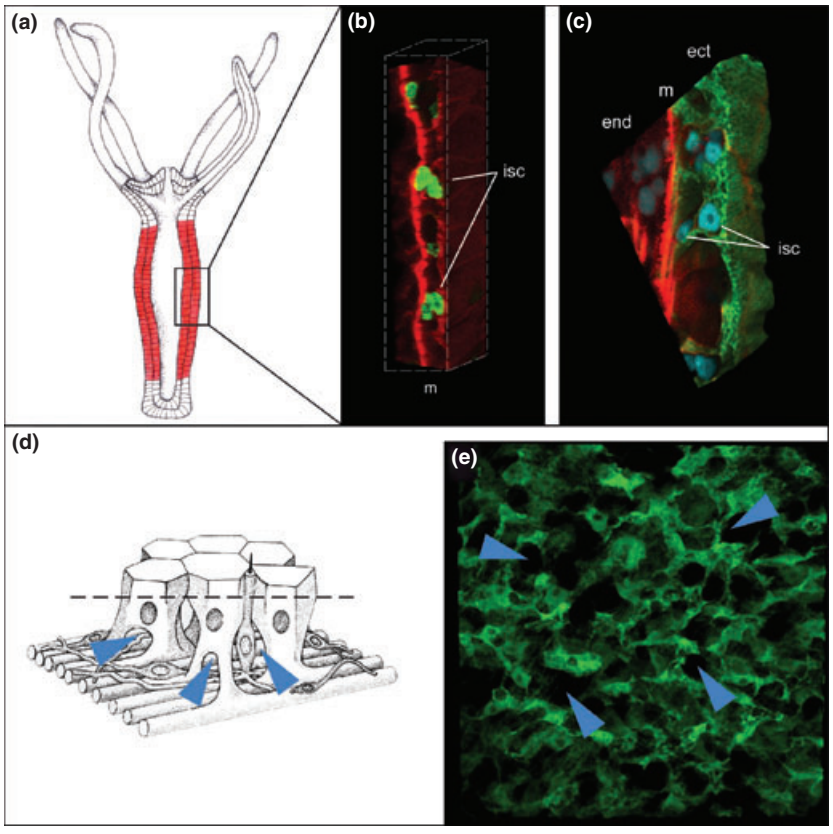

Fig. 3. *In vivo* localization of interstitial stem cells in transgenic *Hydra*. For analytical reasons, the polyps are mosaics with only part of the interstitial cell population expressing GFP. (a) Schematic diagram of a polyp. Red color indicates the localization of epithelial and interstitial stem cells with indefinite proliferation capacity. (b) High-resolution confocal microscopy (xy collage image) in combination with a nanos-GFP transgenic *Hydra* line visualizes the precise site of the residence of interstitial stem cells. Note that they are localized near, or even in physical contact with, both epithelial cells and the mesoglea and that in each pair of interstitial stem cells only one of the two cells is in contact with the mesoglea, while both of them appear to have contact with neighboring epithelial cells. (c) Actin-GFP transgenic *Hydra* line with GFP expressing ectodermal epithelial cells provides insight into the three-dimensional anatomy of the niche. (d) The interstitial space as shown schematically (figure reproduced from Brusca and Brusca [2003] with permission), indicating that interstitial cells and their derivatives such as neurons are enmeshed in a complex microenvironment. Dashed line indicates the start point of z-stock image series shown in Figure 3e. (e) Three-dimensional reconstruction of z-stack image series of the interstitial space in actin-GFP transgenic polyps with GFP-expressing ectodermal epithelial cells. The high-resolution confocal image shows that the microenvironment for interstitial stem cells is an interconnected network of cavernous chambers. Arrow heads indicate interstitial chambers. ect, ectodermal; end, endodermal; isc, interstitial stem cells; m, mesoglea.

Recognized decades ago, interstitial stem cells are distributed along the whole body column but are conspicuously absent at both ends of the single body axis. The sharp boundary between the gastric region and the head and foot region can be directly visualized in whole mounts of *Hydra* stained with an antibody specific for interstitial cells (see Fig. 2b). While the gastric region is tightly packed with interstitial cells, both head and foot regions are essentially empty except for rare, isolated interstitial cells. Notably, the boundary in the head-gastric and gastric-foot interface is very sharp. This boundary is interesting for several reasons.

First, it suggests that much of the action takes place at this head/foot-gastric cell interface, where cells receive microenvironmental cues that influence when to proliferate or differentiate. Cnidocyte, as well as neuron differentiation from multipotent interstitial stem cells, occurs exclusively in the gastric region (David & Challoner 1974; David & Gierer 1974; Shimizu & Bode 1995; reviewed in Tardent 1995; see also Nüchter *et al*. 2006). After entering the neuron differentiation pathway, about half of the neuron precursor cells migrate toward the head and foot (Heimfeld & Bode 1984; Fujisawa 1989; Teragawa & Bode 1990; 1995; Technau & Holstein 1996; Hager & David 1997). The remaining half of the neuron precursors do not migrate, but complete differentiation and are integrated into the nerve net. Thus, while the

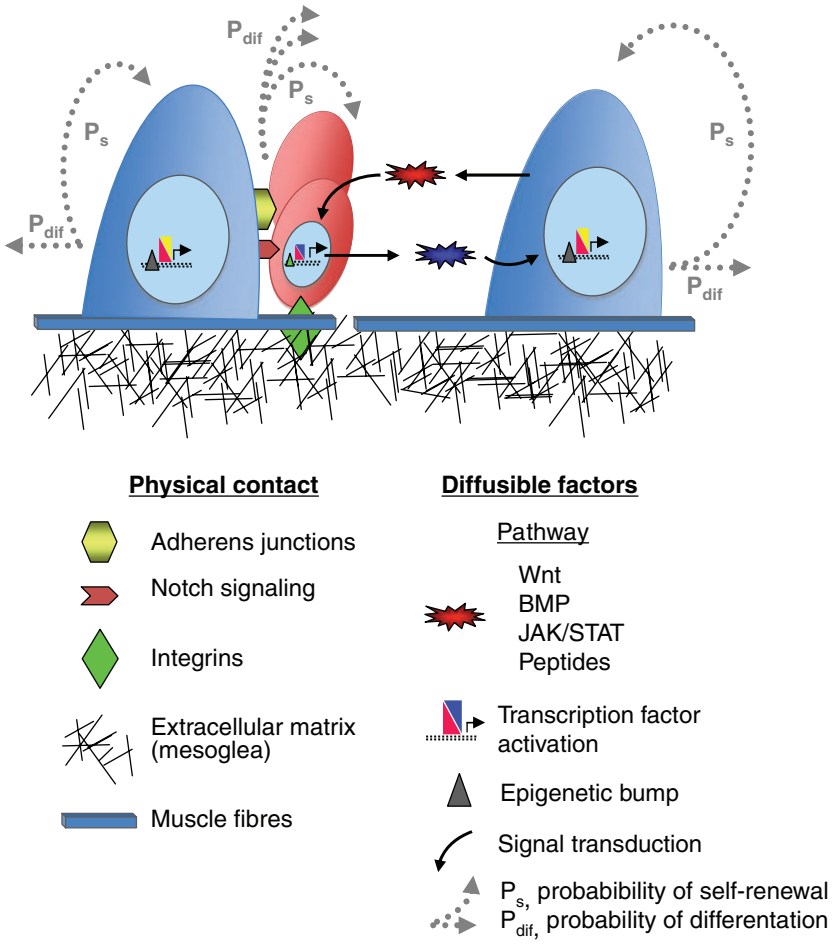

Fig. 4. Current view of the anatomic and functional dimensions of the niche in *Hydra*. The two categories of regulation of the adult stem cell by the niche are illustrated. Regulation of the stem cell requires physical interactions with its niche. These interactions include tight junctions, adherens junctions, integrins, the Notch signaling pathway, the muscle fibers and extracellular matrix proteins. Diffusible factors travel over varying distances from a cell source to instruct the stem cell, often by affecting transcription.

interstitial space between ectodermal epithelial cells in the gastric region is filled with both interstitial stem cells and differentiation products, in the head and foot region mostly terminally differentiated cells are present. The extent to which this environmental heterogeneity and the variety in external cues provided by the niche impacts stem cell decision making remains to be elucidated.

Second, the presence of a boundary raises questions of how such a boundary can be maintained in a polyp with constant tissue movement. Previous work has shown that the P_s of the stem cell population in *Hydra* is controlled by the concentration of stem cells present (Bode *et al.* 1976; David & MacWilliams 1978; Sproull & David 1979). Low stem cell concentrations cause high values of P_s; high stem cell concentrations cause low values of P_s. David and Plotnick (1980) presented a model in which the value of P_s is regulated by negative feedback from neighboring stem cells, the feedback signal is mediated by a short-range diffusible factor and the P_s of interstitial stem cells is very low in the head and foot regions.

Third, which environmental signals are controlling the boundary conditions? While working on the *Hydra* peptide project (Bosch & Fujisawa 2001; Fujisawa 2008), we discovered that epithelial cells affect interstitial cell differentiation behavior by secreting epitheliopeptides (Takahashi *et al.* 2000; Takahashi *et al.* 2009; Takahashi & Fujisawa 2009). There is additional evidence that neurons and nerve cell density affect interstitial cell proliferation and differentiation (Bosch *et al.* 1991; Takahashi *et al.* 2000). Since nerve differentiation is concentrated in the interface regions (David & Gierer 1974), the cellular composition of the niche changes drastically along the body column. Consistent with the niche concept, the simplest explanation for the sharp boundary, therefore, is to assume that localized nerve differentiation is responsible for the low value of P_s. These observations show that in *Hydra* there is extensive signaling not only between stem precursor cells and differentiated cells, but also between stem cells of the different lineages. The head-gastric interface obviously is a rich communication platform in which

soluble peptides are provided as important environmental signals.

Fourth, which molecular machinery is active in the boundary region? How are genes which will be required for executing specific differentiation steps in the head and foot region prevented from expression in stem cells in the body column? Recent studies point to the importance of unique epigenetic profiles that keep key developmental genes 'poised' in a repressed state in which activation is possible. Epigenetic regulation has emerged as an important paradigm for specifying pluripotency and lineage commitment (Azuara *et al.* 2006; Spivakov & Fisher 2007). Stem cell genes involved in differentiation are often bound by polycomb-repressive complexes 1 and 2 (PRC1 and PRC2) and are marked by histone H3 lysine 27 trimethylation. Interestingly, in *Hydra* the gene encoding polycomb protein HyEED is specifically expressed in interstitial cells and differentiating nematoblasts (Genikhovich *et al.* 2006). HyEED is not expressed at later stages of differentiation and is, therefore, absent from the head and foot regions. To explore whether epigenetic histone modifications are important for differentiation of interstitial cells, we produced polyps which, under the control of the *Hydra* actin promoter, overexpress HyEED in the interstitial cell lineage (Khalturin *et al.* 2007). Unexpectedly, the localization of the fusion protein emulates the endogenous expression of HyEED. Confocal microscopy showed HyEED-GFP expression in interstitial cells as well as in developing nematoblasts (Khalturin *et al.* 2007). To examine whether the disappearance of the GFP signal in the head and foot tissue is correlated with terminal differentiation, we monitored HyEED-GFP positive cells both in the gastric region where interstitial cells differentiate into nematocytes, as well as during regeneration when HyEED-GFP positive cells experience a rapid change in positional value (from body to head tissue). HyEED-GFP is actively degraded during the transition of a nematoblast into a mature nematocyte. Inhibition of the proteasome system resulted in the presence of HyEED-GFP+ nematocytes in head and tentacle tissue, whereas in control polyps such transgenic cells are never observed in these regions. We have proposed that nematoblasts at the head/foot–gastric interface entering head or foot territory (the region of terminal differentiation) abruptly loose the HyEED-GFP fusion protein by proteolytic degradation to facilitate terminal differentiation (Khalturin *et al.* 2007). Our overexpression construct seems to be unable to override this endogeneous control mechanism. Taken together, these observations support the view that a polycomb group (PcG)-mediated repressive histone lock prevents precocious expression of the interstitial stem cell genes which drive differentiation along the nematocyte differentiation pathway. A key challenge for the future is determining the molecular nature of this epigenetic ''bump'' by analyzing methylation profiles of regulatory sequences of interstitial cell specific genes. Such profiles will show whether the regulatory sequences of interstitial cell specific genes become methylated or not as they differentiate. Studies of this kind will also show whether, from *Hydra* to man, DNA methylation of stem cell gene promoters is an ancestral and universal step accompanying stem cell differentiation.

Conclusions: is the whole body column a stem cell niche?

Stem cell behavior in *Hydra* is regulated by coordination of environmental signals and intrinsic programs. As we have shown here, the adult stem cell niche is an important regulatory structure with impact on the homeostatic regulation of stem cells. The whole body column is composed of continuously proliferating cells and may be considered a stem cell niche. Dissection of some of the niche's biochemical and cellular components has pointed to short peptides as secreted environmental signals targeting specialized cell populations located in unique topological regions along the body column. To what extent the heterogeneity of the cellular composition of the niche in different regions of the body column affects both epithelial and interstitial stem cells in their ability to self-renew remains to be determined. Humans have numerous stem cell systems to maintain tissue homeostasis and repair function. Moreover, studies in the most extensively studied mammalian stem cell systems, such as the crypts of the small intestine, have revealed that in humans stem cells are present only in extremely small numbers (Moore & Lemischka 2006) and, due to the complexity of the niche microenvironment, difficult to study directly. By contrast, *Hydra* has only three well defined stem cell lineages, is genetically tractable and completely transparent, allowing *in vivo* imaging of stem cells at a single-cell resolution (see Fig. 3), and therefore perfect for addressing the role of the stem cell niche in stem cell differentiation in the bilaterian ancestor.

Future visions

Since its experimental tractability has been found to provide deep insight into fundamental questions, *Hydra* has rapidly expanded from being a ''curiosity'' to a ''generality''. Molecular analysis of stem cell behavior in *Hydra* has grown step by step over the last

25 years. However, increasing awareness of the shortcomings of current methods, as well as emerging technological opportunities, clearly show that the classical approaches of identifying and characterizing genes and factors controlling stem cell behavior in *Hydra* need to change. The avenues for efficient novel approaches now opening originate from a combination of ''omics'' technologies (such as genomic and proteomic analyses), bioinformatics, and novel imaging methods. For a long time molecular analyses in basal metazoan model systems were limited to ''single-'' or ''several-gene'' approaches aided by few selected draft genome-sequencing projects. Gene identification procedures are now undergoing dramatic changes with the introduction of next generation sequencing technologies. Whole transcriptome sequencing projects, RNA-seq gene-expression studies and even sequencing of new genomes are now possible in a short time at relatively low cost compared to traditional sequencing strategies. In our hands, extensive use of the Roche FLX/454 system (CD Genomics, Shirley, NY, USA) is already promising interesting insights into the evolution of distinct cell types and molecular components of the stem cell niche (Hemmrich & Bosch, unpubl.). Questions central to the understanding of tissue and cell type homeostasis such as ''how are the divisions of three distinct types of stem cells within an organism controlled?'' and ''what features do epithelial stem cells and interstitial stem cells share?'' will become approachable through using a combination of FACS and transcriptome sequencing. Moreover, the currently available functional assays have limitations and are inadequate for a model system in the twenty-first century. It will therefore become important to resolve these questions by developing efficient loss-of-function methods, genetic knock-out protocols and inducible systems to switch cellular pathways on and off and to identify key control points. Functional approaches are certainly central in deciphering the roles of specific genes and gene networks operating in *Hydra'*s stem cells. However, a dynamic understanding of the essential and molecular interactions which coordinate the specification of the different cell types and create the three-dimensional organization of the animal will also rely on the most recent advancements in microscopy and live imaging technology. Photoconvertible proteins, such as Kaede, which convert from one fluorescent state to another and change color, will become indispensible tools to selectively label and trace cells in live polyps.

''Omics'' technologies and imaging methods can compile enormous sets of information about a single *Hydra* cell. Together, these technologies not only provide researchers with novel and important information about stem cell behavior in *Hydra,* but also increasingly allow the deduction of patterns (or signatures) that are characteristic of certain cell types. By also harnessing advances in bioinformatics and *in silico* modeling, this information can be mined and then integrated with knowledge from other organisms to illuminate an early stage in the evolutionary history of the metazoans. Investigators who study various stem cell niches can benefit from the apparent similarities between them. For example, virtually every niche system utilizes specific molecules mediating physical contact (e.g. Notch) as well as mechanisms of spindle orientation, resulting in asymmetric or symmetric division of the stem cell. Diffusible factors such as Wnt also appear to regulate the stem cell in every niche. However, in different organisms and locations individual niches still have unique properties (see Fig. 4). Integrating such information and delineating these differences will be crucial for uncovering the mystery of ''stemness'' and deciphering the fundamental components controlling pluripotency and lineage commitment that underlie all stem cell systems.

Acknowledgments

We thank Judith Ittner-Bosch for critically reading the manuscript. Work in our laboratory is supported in part by grants from the Deutsche Forschungsgemeinschaft (DFG), and grants from the DFG Cluster of Excellence programs ''The Future Ocean'' and ''Inflammation at Interfaces''.

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