Symmetry Breaking in Stem Cells of the Basal Metazoan Hydra

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Abstract

Among the earliest diverging animal phyla are the Cnidaria. Cnidaria were not only first in evolution having a tissue layer construction and a nervous system but also have cells of remarkable plasticity in their differentiation capacity. How a cell chooses to proliferate or to differentiate is an important issue in stem cell biology and as critical to human stem cells as it is to any other stem cell. Here I revise the key properties of stem cells in the freshwater polyp *Hydra* with special emphasis on the nature of signals that control the growth and differentiation of these cells.

1 Stem Cells and the Need to Have Comparative Data from Ancestral Metazoans

Stem cells have the unique ability to undergo self-renewing mitotic divisions in which one or both progeny retain stem cell identity and the capacity to replicate almost indefinitely. The daughters of stem cell divisions also have the option to follow a differentiation pathway. The balance between self-renewal and differentiation must be strictly regulated to maintain the stem cell pool and to generate the required supply of fully differentiated cells. Understanding how stem cells are regulated is crucial in learning how tissues are formed and maintained. The self-renewing ability is regulated both by an intracellular mechanism and by intercellular signalling. Cell-autonomous mechanisms governing asymmetric cell divisions in invertebrates have been elucidated in a few stem cell models such as neuroblasts and germ-line stem cells (GSC) in *Drosophila* (Deng and Lin 1997; Lin and Schagat 1997), whereas the role of extrinsic signalling in controlling asymmetric cell divisions has been implicated in several systems (Morrison et al. 1997). External stimuli that alter self-renewal of several classes of stem cells and affect asymmetric divisions include cytokines, matrix proteins, hormones, and local interactions between stem cells and their neighboring

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cells. These extracellular signals may then influence the cell cycle machinery or the cytoskeletal organization of stem cells for their formation and/or divisional asymmetry. Asymmetric cell divisions also involve marked differences in gene expression as well as extraordinary genome modifications. For example, in *C. elegans* and *Drosophila* transcriptional quiescence in early germ cells is thought to be essential for the establishment of distinct germ-line and somatic fates (Seydoux 1996; Bashirullah et al. 1998). Genome modifications associated with asymmetric divisions are of importance in blood stem cells (Akashi et al. 2003) and also play crucial roles in switching of mating types in yeast, programmed chromosome breakage and chromatin elimination in *Ascaris*, and the development of a transcriptionally inert germline micronucleus in spirotrichous ciliates. Studies in insects and worms have shown that just a few signalling pathways generate most of the cellular and morphological diversity during the development of individual organisms (Pires-DaSilva and Sommer 2003). This similarity in signalling pathways between disparate animal phyla has provided convincing evidence for the monophyletic origin of metazoa. Insects, worms and vertebrates all derive from the "triploblast" or "Bilateria" clade of metazoans. Since several animal phyla diverged, however, before the origin of this clade, the discovery of shared signalling cascades tells us little about their origin and original roles, until we have comparative data from more basal animals. The aim of this chapter, therefore, is to review our understanding of the pathways that regulate proliferation, self-renewal and differentiation of stem cells in the basal metazoan *Hydra*.

2
At the Origin of Metazoan Evolution: Placozoa, Porifera and Cnidaria

Since the nineteenth century, *Trichoplax adhaerens* has been considered the most simple metazoan and proposed to be a model organism for the transition from single cellular protists to multicellular metazoa (e.g. Metschnikoff 1883; Collins 1998; Schierwater and Kuhn 1998). *Trichoplax* is composed of a ciliated epithelium that is differentiated dorsally and ventrally and contains just four distinct somatic cell types (Grell 1971). Its particular morphology, characterized by an extreme form of simplicity, has justified the creation of an own phylum, the Placozoa (Grell 1971). This simplicity together with molecular data from the *Trichoplax* mitochondrial genome (Dellaporta et al. 2006) provide convincing support for the phylogenetic placement of the phylum Placozoa at the root of the Metazoa. With regard to asymmetric cell divisions, with only one transcription factor of the Antp superclass gene, Trox-2, cloned so far, very little is known about the molecules involved leaving the molecular machinery controlling *Trichoplax* asymmetric cell divisions sitting in the dark.

Porifera (sponges) most likely evolved from simple unicellular flagellates, the choanoflagellates (Leys and Ereskovsky 2006). Porifera have six to ten different cell types including ciliated choanocytes that drive water through canals and chambers. There is a great deal of cell mobility and reversal of cell differentiation (Ruppert and Barnes 1994) and many sponges have remarkable powers of regeneration. Sponges have signal transduction pathways including receptor tyrosine kinases and protein kinase C, that are the basis for physiological processes in higher metazoa (Müller 2001). This and other observations indicate that sponges share a common ancestor with all other metazoa and that multicellularity is associated with the presence of an extracellular matrix, cell adhesion molecules and membrane associated receptors. Little is known, however, about the signals and interactions required for differentiation of sponge-specific features such as the choanocytes (Leys and Ereskovsky 2006). So far only a few homologues of homeobox transcription factors have been isolated from sponges (Müller 2001). Although these observations providing clear evidence supporting the monophyletic origin of the Metazoa, molecular techniques are still in their infancy in the Porifera (Leys and Ereskovsky 2006) and the function of developmental control genes remains to be elucidated. However, with a genome project currently underway to sequence the ceractinomorph demosponge *Reniera* sp. at the Joint Genome Institute, molecular analysis of poriferan development should make significant progress in the next few years and also offer insight into the molecular machinery needed for asymmetric cell divisions in this most basal metazoan phylum.

Among the basal metazoa, Cnidaria, the sister group of the Bilateria, are the first in evolution that have a defined body plan, stem cells, a nervous system, and a tissue layer construction. Cnidarians are diploblastic consisting of two epithelia, the ectoderm and the endoderm surrounding a gastric cavity. Cnidarians such as the freshwater polyp *Hydra* have a long history as model systems in developmental biology because of their remarkable capacity to regenerate missing body parts. Most spectacularly, *Hydra* polyps when dissociated into a suspension of single cells and pelleted into aggregates by centrifugation will organize themselves into complete polyps within a few days (Gierer et al. 1972). This ability for self-organization is at least partially due to the continuous presence of stem cells with high self-renewal capacity and high phenotypic plasticity in adult tissue. Multipotent interstitial stem cells have been identified in *Hydra* by in vivo cloning (Bosch and David 1987). Interestingly, totipotent stem cells have also been identified recently in the colonial hydrozoan *Hydractinia echinata* (Müller et al. 2004) by repopulating interstitial cell free colonies with allogeneic interstitial cells and demonstrating that the resulting phenotype was reverted to that of the donor colony. As I have outlined elsewhere (Bosch 2006), the stem cell-ness of the *Hydra* tissue is not only sufficient to explain Hydra's unprecedented regeneration capacity, but also allows *Hydra* its unique life cycle in which proliferation occurs mostly asexual by budding.

3
Key Properties of Epithelial and Interstitial Stem Cells in *Hydra*

In *Hydra*, there are about 20 cell types distributed among 3 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 reside among the epithelial cells of both layers. Both the epithelial cells as well as the interstitial cells in the body column continuously undergo self-renewing mitotic divisions (Dübel et al. 1987). To prove that the epithelial cells indeed have stem cell properties, we recently (Wittlieb et al. 2006) have made use of transgenic polyps and transplanted a single GFP-expressing endodermal epithelial cell into a nontransgenic polyp. By doing so we have generated polyps in which the entire ectodermal or endodermal epithelium contains the transgene (Wittlieb et al. 2006). Supporting earlier observations, such in vivo tracking of GFP labelled epithelial cells also showed that there is continuous tissue displacement towards the extremities. Displacement of ectodermal epithelial cells into the tentacles results in differentiation of battery cells. Displacement of epithelial cells towards the lower body regions results in budding and – at the boundary between the peduncle and the basal disk – in differentiation of epithelial cells into basal disk cells which begin to secret mucus just after passing through that boundary. This remarkable phenotypic plasticity of epithelial cells in response to positional signals allows *Hydra* to build complex structures such as the tentacles with only a limited number of different cell types.

Located primarily between the ectodermal epithelial cells throughout the gastric region there are the interstitial stem cells. The main evidence for their stem cell properties comes from in vivo cloning experiments which have shown (see Fig. 1) that these cells are multipotent and capable of somatic and germ line differentiation (David and Murphy 1977; Bosch and David 1987). Multipotent interstitial stem cells in *Hydra* give rise to neurons (see Fig. 2), secretory cells and gametes in a position dependent manner (Bosch and David 1987). These stem cells also give rise to cnidocytes, which are unique to and characteristic of all cnidarians. Interstitial stem cells in *Hydra* are found throughout the gastric region; they are absent, however, in the head and foot region (David and Plotnick 1980). In numerous cloning experiments no stem clones were found containing only one differentiated type of somatic cells. Thus, there is no evidence for extensively proliferating subpopulations of somatic intermediates in Hydra (Fig. 1).

Cnidocyte differentiation occurs exclusively in the gastric region as a highly complex, multistep process (reviewed in Tardent 1995). Cnidocytes differentiate in clusters of 8–32 cells in the body region (David and Challoner 1974). Cells within clusters remain interconnected by cytoplasmic

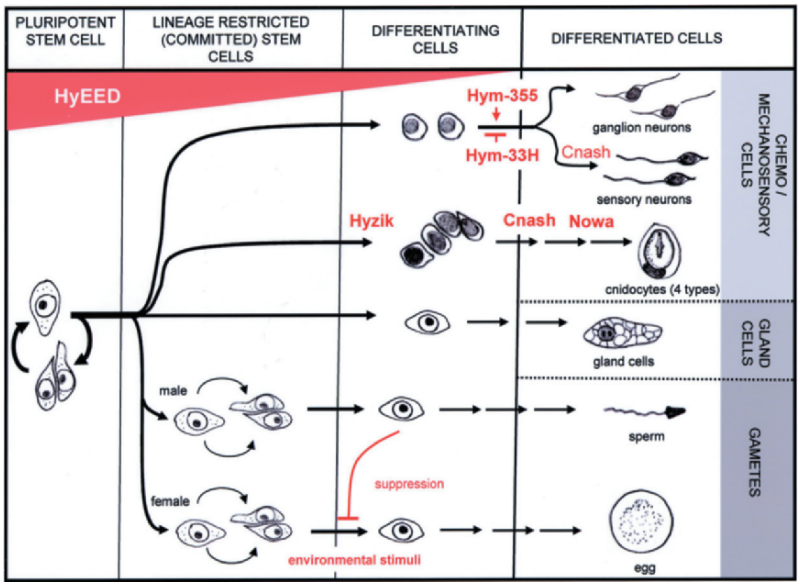

Fig. 1. A model for the differentiation of interstitial stem cells in *Hydra*. Factors affecting steps of differentiation are shown in red. For details and references see text

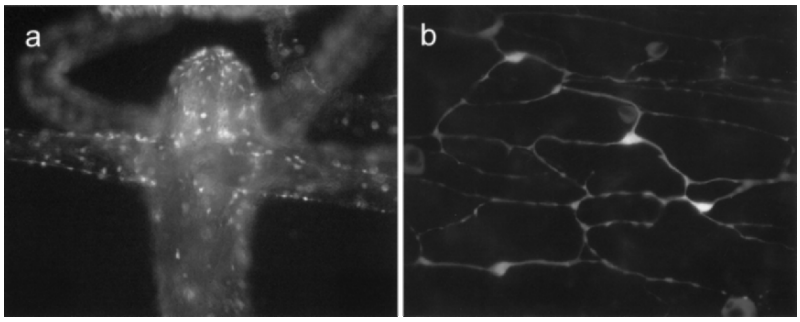

Fig. 2. **a,b** Transgenic Hydra polyps are paving the way for in vivo imaging to analyze nerve cell differentiation at the basis of metazoan evolution: **a** *Hydra vulgaris* (AEP) polyp expressing GFP exclusively in the interstitial cell lineage which includes the nerve precursors; **b** GFP expressing ganglion neurons

bridges. During differentiation (Shimizu and Bode 1995) each nematoblast produces a nematocyst capsule inside a secretory vesicle. Following capsule differentiation, the clusters of differentiating cnidocytes break up into single cells that migrate towards the tentacles and become mounted in specialized tentacle epithelial cells, termed battery cells (David and Gierer 1974).

Neuron differentiation from multipotent interstitial stem cells also occurs exclusively in the gastric region (David and Gierer 1974). After entering the neuron differentiation pathway, about a half of the neuron precursor cells migrate toward the head and foot (Heimfeld and Bode 1984; Fujisawa 1989; Teragawa and Bode 1990, 1995; Technau and Holstein 1996; Hager and David 1997). The remaining half of the neuron precursors do not migrate, but complete differentiation and are integrated into the nerve net (Fig. 2).

Little is known about gland cell differentiation from interstitial stem cells. Previous studies have described gland cells in *Hydra* as secretory cells distributed gradually along the body column and used for extracellular digestion of prey (Schmidt and David 1986; Bode et al. 1987). Recent studies based on the expression pattern of several genes (Augustin et al. 2006; Guder et al. 2006) suggest that gland cells are distributed along the whole body column down to the basal disc. The previously reported graded distribution of gland cells with a maximum density in the subhypostomal region (Schmidt and David 1986, Bode et al. 1987) appears to reflect the previously used analytical methods which were mostly histological observations.

In the gamete differentiation pathway, unipotent subpopulations of interstitial cells have been isolated (Fig. 1). They are capable of extensive proliferation but committed to either spermatogenesis (Littlefield 1985; Nishimiya-Fujisawa and Sugiyama 1993) or oogenesis (Littlefield 1991; Nishimiya-Fujisawa and Sugiyama 1995). These unipotent stem cells are present in asexually proliferating polyps in low numbers and dividing at a slower rate than their multipotent precursor cells (Holstein and David 1990). In response to environmental stimuli, these cells start to proliferate rapidly and differentiate into gametes. Interstitial cells are not only the precursors for gametes but also themselves responsible for sex determination (Littlefield 1984; Campbell 1985). Surprisingly, somatic components (e.g. epithelial cells) do not play a role in determining the sexual phenotype. In addition, male interstitial cells of *Hydra* suppress the ability of female stem cells to differentiate eggs thereby causing "masculinization" of females (Sugiyama and Sugimoto 1985; Littlefield 1994). They, however, do not interfere with the ability of female stem cells to proliferate and produce somatic cells (Bosch and David 1986). The molecular nature of this suppression is completely unkown.

Figure 1 summarizes these findings and illustrates the current view of the differentiation and proliferation potential of interstitial stem cells in *Hydra*. Transplantation experiments with genetically marked interstitial cell clones indicated that interstitial stem cells in *Hydra* display very little migratory activity and grow as contiguous patches (Bosch and David 1990). The growth of interstitial cells in clonal patches has an important implications since it affects the distribution of stem cells to daughter polyps and, for example, leads to male polyps which occasionally give rise to female polyps (Bosch and David 1987). Taken together, the differentiation

pattern of interstitial stem cells exhibits a strong dependence on position along the body axis indicating that these cells are capable to interpret positional information to differentiate according to their genetic program. Stem cells in *Hydra*, therefore, point to the importance of spatial organization and thus extrinsic influences (Wolpert 1988). Can we define these extrinsic influences in molecular terms?

4
Hydra Interstitial Stem Cells and their Niches

A specialized microenvironment, the stem cell niche, is one of the factors regulating stem cell maintenance and the crucial choice between self renewal and the initiation of differentiation (Spradling et al. 2001; Moore and Lemischka 2006). Although the niche concept was introduced already in 1978 (Schofield 1978), only recently it became clear that stem cells in vertebrates as in invertebrates require paracrine signals from the surrounding microenvironmental cells to maintain their identity and self-renewal capacity (Moore and Lemischka 2006). The best understood examples of regulation of stem-cell renewal by the niche are to be found in the female and male germ-line stem cells of *Drosophila* (Yamashita et al. 2005). In *Drosophila* female germ line, where the regulatory circuitry from the signal to the key target in the stem cell has been worked out, the main role of the signal from the niche is to block expression of genes that trigger the onset of differentiation (Chen and McKearin 2003; Xie and Spradling 2000). In *Hydra*, the interstitial cells grow and differentiate in the interstices between ectodermal epithelial cells. As I will describe below, the interstitial stem cell population in *Hydra* is strongly influenced by its complex microenvironment.

4.1
Key Elements that Specify Self-renewal and Control Differentiation of Interstitial Stem Cells

The major cellular components of the microenvironment in which *Hydra* interstitial cells grow and make their decisions are epithelial cells and cells of the interstitial cell lineage themselves. The mesoglea may present an additional important acellular component of the microenvironment. A number of transplantation experiments by which stem cells have been introduced into a variety of different host tissues have revealed two environmental parameters as particularly important. The first parameter – nerve cell density in host tissue – positively influences interstitial cell proliferation. The second parameter – interstitial cell density – negatively influences proliferation. Growth of interstitial cells is faster in tissue with reduced interstitial cell numbers than in normal tissue (Yaross and Bode 1978; Sproull and David 1979; Holstein

and David 1990). Thus, stem cell proliferation in *Hydra* is controlled by a feed-back signal from interstitial cells and their derivatives: decreasing the number of stem cells causes an increase in the self renewal probability and leads to recovery of normal stem cell levels (Sproull and David 1979). Conversely, increasing the number of stem cells decreases the self-renewal probability. Although there is evidence suggesting that the feed-back signal is of short range (David and MacWilliams 1978; Sproull and David 1979; Bosch et al. 1991), the nature of the signal(s) by which interstitial cells measure their density is unknown. It may be a diffusible molecule produced by stem cells or directly mediated by cell-cell contact. Interestingly, feedback regulation by stem cell density does not occur when stem cells are transferred in genetically unrelated tissue (David et al. 1991). Since under these conditions the stem cells behave as if the genetically distinct cells are not present, the signalling involved is species specific and not conserved.

4.2 Paracrine Signalling and Feedback Loops During Neuron Differentiation

There is accumulating evidence that the nervous system of *Hydra* (see Fig. 2) is much more complex than previously conceived. It is composed of many subpopulations which are characterized by the expression of different neuropeptides, genes, and antigens (Grimmelikhujizen et al. 1982a, b; Dunne et al. 1985; Koizumi et al. 1988; Hobmayer et al. 1990a, b; Yum et al. 1998; Darmer et al. 1998; Takahashi et al. 2000; Hansen et al. 2000; Hayakawa et al. 2004). Due to the dynamic state of the tissue which is constantly undergoing renewal as a result of continuous growth and differentiation, the nerve net is also in a steady state of production and loss of neurons. To compensate for the loss and to maintain the homeostasis, neurons in *Hydra* arise continuously by differentiation from multipotent interstitial stem cells (David and Gierer 1974). Neuron differentiation from interstitial stem cells involves several sequential events: commitment to differentiation, migration of committed precursors to the site of differentiation, final mitosis and terminal differentiation as neurons. Essential part of the signalling system involved in maintaining the neuron population are peptides (Bosch and Fujisawa 2001; Bosch 2003). Through a systematic screening of peptide signalling molecules from *Hydra* we have discovered two groups of peptides that affect neuron differentiation (Takahashi et al. 1997, 2000). Neuropeptide Hym-355 positively regulates neuron differentiation, while PW peptides such as Hym-33H that share a common C-terminal motif of Leu or Ile-Pro-Trp negatively regulate neuron differentiation (Takahashi et al. 1997, 2000). Pulse-labelling experiments indicate (Fujisawa, personal communication) that PW peptide inhibit early stages of neuron differentiation by inhibiting commitment or migration of precursor

cells. By indirect immunofluorescence staining using anti-Hym-33H antiserum, Fujisawa and colleagues recently could localize the Hym-33H PW peptide in the ectodermal epithelial cells (Fujisawa, personal communication). Thus, PW peptides appear to be part of the microenvironmental factors which directly affect neuron differentiation – underlining the importance of niche cells as source for molecules that activate pathways controlling and specifying stem cell fate. Since both groups of peptides, Hym355 and Hym-33H, counteract each other, we could incorporate the actions of these molecules into a feedback model to explain the homeostasis of a neuron population (Takahashi et al. 2000). An emerging theme is the balance between Hym-355 and PW peptides that determines the differentiation of stem cells to neurons. The model also proposes that neurons interact with epithelial cells via a short-range signal or direct cell-cell communication and induce the latter cells to deliver PW peptides. In any case, the observations provide convincing support for the niche concept and for an intensive spatio-temporal dialog occurring between interstitial stem and niche cells in *Hydra*.

5
The Molecular Regulation of Neuronal Differentiation Involves bHLH Class Transcription Factors

Proneural genes that encode the bHLH class transcription factors play key roles in the formation of the nervous system in both invertebrates and vertebrates. In *Hydra*, Grens et al. (1995) have isolated the bHLH transcription factor gene *achaete-scute* homolog *Cnash*. In bilaterians, *achaete-scute* is involved neurogenesis. The *Hydra* CnASH protein was found to form heterodimers with the *Drosophila* bHLH protein Daughterless and to bind specifically to consensus achaete–scute DNA-binding sites (Grens et al. 1995). Expression of CnASH in ac–sc double mutants of *Drosophila* can rescue the mutant phenotype. Interestingly, *Cnash* originally was thought (Grens et al. 1995) not to be expressed in nerve cells or nerve cell precursors, but exclusively in cell clusters that give rise to cnidocytes , consistent with the idea that cnidocytes are a neuronal cell type. Recently, however, Hayakawa et al. (2004) reported the involvement of CnASH in the differentiation pathway of sensory neurons in the tentacles. The authors observed that in addition to differentiating cnidocytes in the body column, sensory neurons at early stages of differentiation in the tentacles also express CnASH. Since CnASH-positive neurons are distributed from the base to the tip of tentacles, the gene may also be involved in maintenance of the differentiated state. Thus, proneural genes of the achaete–scute (ac–sc) family are involved in neurogenesis in animals which were first in evolution to develop a nervous system.

6
Neural Effector Genes Influence Cnidocyte Differentiation

Cnidocytes which are unique to and characteristic of all cnidarians (David and Gierer 1974; Holstein 1981; Tardent 1995) derive from the same pluripotent interstitial stem cell population as nerve cells (Bosch and David 1987) and in *Hydra* are present as four different types: stenoteles, holotrichous isorhizas, atrichous isorhizas and desmonemes. Although extensive studies at the biochemical (Kurz et al. 1991; Koch et al. 1998; Engel et al. 2001, 2002; Szczepanek et al. 2002) and ultrastructural (Mariscal 1974; Holstein 1981; Holstein et al. 1994) level have revealed the morphogenesis of cnidocyte capsules, little is known about the factors controlling cnidocyte differentiation.

Lindgens et al. (2004) reported the identification of a gene, *Hyzic*, which is expressed in the early cnidocyte differentiation pathway, starting at the level of interstitial stem cells. *Hyzic* is a homolog of the Zn-finger transcription factor gene *zic/odd-paired*, which acts as an early neural effector gene in vertebrates. *Hyzic* expression is restricted to the proliferative stages of cnidoblasts and, up to now, the earliest marker of cnidocyte differentiation. The above mentioned CnASH-positive cells (Grens et al. 1995) are preceded by a differentiation stage expressing *Hyzic* (Lindgens et al. 2004). Since Hyzic also acts before Nowa (Engel et al. 2002), another early cnidocyte differentiation marker gene encoding a major wall protein of the cnidocyte capsule (Ozbek et al. 2004), Lindgens et al. (2004) concluded that Hyzic may determine stem cells to differentiate into cnidocytes . The seemingly puzzling fact that in *Hydra* CnASH is expressed in both differentiating cnidocytes (Grens et al. 1995; Lindgens et al. 2004) as well as in neurons (Hayakawa et al. 2004) adds strong support to the view that (i) cnidocytes are mechanosensory and/or chemosensory cells (Hausmann and Holstein 1985; Brinkmann et al. 1996) and (ii) that genetic cascades of neural development are highly conserved during animal evolution (Sasai 2001).

The first identification of a Dickkopf gene outside the vertebrates (Fedders et al. 2004) with a deduced amino acid sequence closely related to that of chicken *Dkk-3*, *HyDkk-3*, in *Hydra* adds further support to the view that cnidocytes represent neuronal sensory cells. In vertebrates, Dkk-3 in contrast to Dkk-1 and Dkk-4 is expressed in brain and some other tissue, indicating that it might be involved in neuron differentiation or function (Krupnik et al. 1999). In *Hydra*, *HyDkk*-3 is expressed in all four types of cnidocytes at a late stage of differentiation (Fedders et al. 2004). This differentiation step is characterized by changes in morphology and cell behavior that allow extended cell migration of cnidocytes from the gastric region towards the tentacles. Thus, due to the neuronal function of Dkk3 in vertebrates, not yet understood parallels appear to exist between *Dkk*-3 function in *Hydra* and vertebrates.

7
Pathways that may Suppress Activation of the Stem Cell Differentiation Program in *Hydra*

Epigenetic genome modifications are important for specifying pluripotency and lineage commitment (Azuara et al. 2006). In higher organisms, PcG proteins form multiple Polycomb Repressive Complexes (PRCs) which are epigenetic chromatin modifiers involved in maintenance of embryonic and adult stem cells (Ng et al. 2000; Wang et al. 2002; Plath et al. 2003; Valk-Lingbeek et al. 2004). The PRC2 complex, comprising embryonic ectoderm development (EED), enhancer of zest (EZH2), and additional components, initiates gene silencing and catalyzes histone H3 methylation on lysine 27 (H3K27) at target loci (Kirmizis et al. 2004; Kuzmichev et al. 2004). When screening the *Hydra* transcriptome for genes expressed during embryogenesis (Genikhovich et al. 2006), one of the genes found to be expressed strongly during both early embryogenesis and in adult polyps was the embryonic ectoderm development (EED) homolog HyEED. While in early embryos it is ubiquitously expressed, at later stages of embryogenesis HyEED expression becomes restricted to a subset of cells in the endoderm and ectoderm of the embryos, which morphologically resemble interstitial cells (Genikhovich et al. 2006). In adult polyps HyEED is expressed in all interstitial cells, cnidoblasts and in spermatogonia. Terminally differentiated interstitial cell derivatives such as cnidocytes , gland cells or neurons, do not express HyEED (Genikhovich et al. 2006). Semi-thin sections of male polyps hybridized in situ with the *HyEED* probe revealed that *HyEED* transcripts are localized in the proximal zone of the testis, which is known (Tardent 1974; Kuznetsov et al. 2001) to contain spermatogonia and spermatocytes. No *HyEED* transcripts were found in the distal part of testis containing spermatids and mature sperm. Since chromatin remodeling in male germ cells is required for completion of spermatogenesis (Grimes 2004), HyEED appears to play a role in this process. Interestingly, in male polyps *HyEED* is co-expressed with the *Hydra* homologue of *EZH2* suggesting that, similar to mammals and *Drosophila*, the PRC2 complex exists in *Hydra*. Since sperm precursors in the testis expressing *HyEED* show high levels of histone methylation (Genikhovich et al. 2006) and co-express putative *HyEZH2*, *HyEED* – similar to EED proteins in *Drosophila*, *C. elegans* and mouse (Rideout et al. 2001; Leatherman and Jongens 2003; Cao et al. 2002; Plath et al. 2003; Okamoto et al. 2004; Kirmizis et al. 2004) – may be involved in remodeling and silencing sperm chromatin and thereby play an important role in spermatogenesis. In support of the view that HyEED is actively suppressing final differentiation steps in *Hydra* interstitial stem cells, Konstantin Khalturin in my lab recently has produced transgenic *Hydra* which constitutively express HyEED under the control of the

Hydra actin promoter in their interstitial cells (Khalturin and Bosch, in preparation). Preliminary data indicate that interstitial cells expressing a HyEED/GFP fusion protein – in contrast to control cells expressing the GFP protein alone – do not differentiate into cnidocytes or nerve cells but remain located exclusively in the gastric region. HyEED/GFP fusion protein expressing interstitial cells can never be found in the head or foot region where in control polyps most of the terminally differentiated interstitial cells end up as neurons or cnidocytes. Thus, HyEED is not only the earliest embryonic marker of the interstitial cell precursors known to-date, but appears to be causally involved in suppressing terminal differentiation of interstitial cells.

8 Conclusions and Perspectives

Are studies in *Hydra* telling us anything relevant with respect to stem cells in man? Control of asymmetry and cell fate is as critical to human stem cells as it is to *Hydra* stem cells. Recent studies suggest that Cnidaria – in contrast to the well known model organisms *Drosophila* and *C. elegans* – have retained all key regulatory genes required for cell decision making and share most of their genes with human (Kortschak et al. 2003; Technau et al. 2005). I have outlined above that certain key elements of the mechanisms that specify self-renewal and prevent differentiation of stem cells may be conserved through evolution from *Hydra* to man. These elements include paracrine signalling pathways, negative feed back loops that limit the response to mitogenic signals, and pathways that suppress activation of the differentiation program in stem cells. In *Hydra* both the "pan-metazoan" features such as a tissue-level organization, stem cells with an enormous developmental potential, and a net of nerve cells as well as the unexpected molecular equivalence to human cells are complemented by unique biological and experimental opportunities. The model system *Hydra* offers fully worked out cell lineages and a nearly unlimited potential for tissue manipulation combined with a completely transparent tissue consisting mostly of stem cells which continuously undergo self-renewing mitotic divisions. Furthermore, transgenic *Hydra* are paving the way for many applications including in vivo imaging to analyze stem cell behaviour and niche function in an animal that diverged from the main line of metazoan evolution about 560 million years. Thus, since fundamental processes that are relevant for understanding asymmetric division and self-renewal are expected to be conserved in the animal kingdom, the basal metazoan *Hydra* is showing its worth when it comes to unlocking the mystery of "stemness" and deciphering the components controlling pluripotency and lineage commitment.

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