# Molecular Regulation of Decision Making in the Interstitial Stem Cell Lineage of *Hydra* Revisited

**Marion Lechable, Matthias Achrainer, Maren Kruus, Willi Salvenmoser and Bert Hobmayer**

**Abstract:** Multipotent interstitial stem cells in the freshwater polyp *Hydra* define one of the best-studied pre-bilaterian adult cell lineages. Most of them represent a population of small, fast-cycling cells that give rise to three somatic differentiation products (neurons, nematocytes, and gland cells) under conditions of continuous asexual growth and reproduction, and they also form the gametes when sexual reproduction is initiated. Few proliferate with a longer cell cycle. Interstitial stem cells in *Hydra* and other marine hydrozoans have been studied intensively using sophisticated cellular and molecular methods over several decades. Here, we discuss the properties of interstitial stem cells in *Hydra* and the known feedback control mechanisms maintaining tissue homeostasis and spatial distribution of interstitial cells along the polyp's major body axis. We summarize the current state of knowledge about molecular regulation of self-renewal and somatic differentiation and put particular emphasis on those molecular factors that have been shown to affect decision making using methods of functional interference.

## 1. Introduction

Interstitial cells (ICs) were discovered in the late 19th century by August Weismann and described as putative migratory germline precursor cells in several colonial marine hydrozoans, laying a basis for his theory of the germline published nearly ten years later (Weismann 1883). Labeling techniques and tissue manipulations revealed the lineage relationships and cellular dynamics of the various types of ICs in hydrozoans, especially in the freshwater polyp *Hydra* (Tardent 1954; Müller 1967; for review also see David et al. 1987; Bode 1996; Plickert et al. 2012). These studies showed that all ICs belong to a single adult stem cell lineage with three somatic cell types—neurons, nematocytes (stinging cells), gland cells—as well as the two types of gametes as differentiation products (Figure 1A).

Classic studies using *Hydra* as a model determined the probabilities for self-renewal and for differentiation into the different somatic interstitial cell types, and they precisely defined cell cycle and differentiation times (Campbell and David 1974; David and Gierer 1974; Schmidt and David 1986). Thus, there is a detailed

quantitative understanding of IC lineage dynamics. More recently, omics approaches, transgenic *Hydra* polyps and genetic up- and down-regulation have provided an advanced understanding of the diversity and plasticity of sub-populations of cells within the IC lineage (Siebert et al. 2008, 2019; Chapman et al. 2010; Hemmrich et al. 2012; Buzgariu et al. 2015). An unexpected, modified model for nerve and gland cell differentiation arose from single-cell transcriptome analysis in *Hydra,* suggesting that these two differentiated cell types arise from a common precursor (Figure 1B; Siebert et al. 2019). Altogether, *Hydra* interstitial stem cells (ISCs), often referred to as "i-cells" in *Hydra* and other hydrozoans, and their differentiation products represent probably the best-studied pre-bilaterian adult stem cell system, and a number of comprehensive reviews have discussed its various features (Bosch 2008; Watanabe et al. 2009; Bosch et al. 2010; Hobmayer et al. 2012; David 2012; Nishimiya-Fujisawa and Kobayashi 2012). Here, after addressing major ISC properties in *Hydra*, we summarize the current state of knowledge about known molecular factors acting in lineage decision making in its asexual reproduction mode, and we focus on those factors shown to be active in functional interference assays. More detailed information about expression, putative function and functional validation of these factors is listed in Table 1. Table 1 also includes some regulators proposed to act in IC decision making on the basis of their cell type-specific gene expression.

**Table 1.** Selected molecular factors acting in the *Hydra* interstitial cell lineage based on available functional interference data and/or cell type-specific gene expression.

| Factor (References) | Cellular Function | *Hydra* Genome Protein Model (Augustus) | Expression Pattern | Experimental Validation |
|---|---|---|---|---|
| | | ISC self-renewal | | |
| HyGSK-3β (Khalturin et al. 2007; Broun et al. 2005) | signal transduction | Sc4wPfr_488.g29970.t1 | ISC | Smi: alsterpaullone |
| Hyβ-Catenin (Gee et al. 2010; Hartl et al. 2019) | signal transduction | Sc4wPfr_975.g7262.t1 | ISC | transgenesis, Smi: alsterpaullone |
| Hy-I-cell1 (Siebert et al. 2019) | unknown | Sc4wPfr_559.g509.t1 | ISC | scRNAseq |
| FoxO (Boehm et al. 2012) | transcription factor | Sc4wPfr_909.g33493.t2 | ISC | RNAi, transgenesis |
| HyMyc1 (Ambrosone et al. 2012; Hartl et al. 2010, 2019) | transcription factor | Sc4wPfr_73.g11571.t1 | ISC | RNAi, smi: 10058-F4 |
| HyMyc2 (Hartl et al. 2014, 2019) | transcription factor | Sc4wPfr_850.1.g5732.t1 | ISC | WISH |

**Table 1.** *Cont.*

| Factor (References) | Cellular Function | *Hydra* Genome Protein Model (Augustus) | Expression Pattern | Experimental Validation |
|---|---|---|---|---|
| Hywi, Hyli (Juliano et al. 2014; Teefy et al. 2020) | RNA binding protein | Sc4wPfr_597.2.g14333.t1, Sc4wPfr_661.g19809.t1 | ISC | transgenesis |
| | | Nerve cell differentiation | | |
| Cnash (Grens et al. 1995) | transcription factor | Sc4wPfr_147.g8607.t1 | ISC, sensory neurons | WISH |
| Myb (Siebert et al. 2019) | transcription factor | Sc4wPfr_423.g13448.t1 | neuronal progenitor cells | scRNAseq |
| HvSoxC (Siebert et al. 2019) | transcription factor | Sc4wPfr_351.g11299.t1 | neuronal progenitor cells | scRNAseq |
| Myc3 (Siebert et al. 2019) | transcription factor | Sc4wPfr_199.g28684.t1 | neuronal progenitor cells | scRNAseq |
| Cnox2 (Miljkovic-Licina et al. 2007) | transcription factor | Sc4wPfr_165.g10051.t1 | apical neurons | RNAi |
| Head activator (Fenger et al. 1994) | signal peptide | - | - | peptide treatment |
| Hym-355 (Takahashi et al. 2000) | neuropeptide | Sc4wPfr_635.g14708.t1 | neurons | peptide treatment, WISH |
| Hym33H (Takahashi et al. 1997) | neuropeptide | Sc4wPfr_59.2.g12471.t1 | neurons | peptide treatment, WISH |
| NDA-1 (Augustin et al. 2017; Siebert et al. 2019) | neuropeptide | Sc4wPfr_824.g11313.t1 | neurons | transgenic overexpression and knock-down |
| prdl-a (Miljkovic-Licina et al. 2007) | transcription factor | Sc4wPfr_1080.g15226.t1 | nerve cells (ectoderm) | WISH |
| prdl-b (Miljkovic-Licina et al. 2007) | transcription factor | Sc4wPfr_372.g27997.t1 | nematocyte, nerve cells | WISH |
| msh (Miljkovic-Licina et al. 2007) | transcription factor | Sc4wPfr_87.g16557.t1 | nerve cells (ectoderm) | WISH |
| COUP-TF (Miljkovic-Licina et al. 2007) | transcription factor | Sc4wPfr_17.g15881.t1 | nerve cells, nematocytes | WISH |
| | | Nematocyte differentiation | | |
| HyZic (Lindgens et al. 2004) | transcription factor | Sc4wPfr_252.1.g15359.t1 Sc4wPfr_237.2.g16165.t1 | early proliferating nematoblast nests (2-8) | BrdU, WISH |
| HvNotch (Käsbauer et al. 2007) | signal transduction receptor | Sc4wPfr_326.g15645.t1 | early nematoblast differentiation | BrdU, smi: DAPT |
| GSK-3β (Khalturin et al. 2007) | phospho-kinase | Sc4wPfr_488.g29970.t1 | early nematoblast differentiation | Smi: alsterpaullone |

**Table 1.** *Cont.*

| Factor (References) | Cellular Function | *Hydra* Genome Protein Model (Augustus) | Expression Pattern | Experimental Validation |
|---|---|---|---|---|
| Cnash (Grens et al. 1995) | transcription factor | Sc4wPfr_147.g8607.t1 | nematoblast differentiation (8 and 16 cells) | WISH |
| HyEED co-expressed with HyEZH2 (Khalturin et al. 2007) | epigenetic regulator | Sc4wPfr_804.g24124.t1 | nematoblast—ISC | transgenesis |
| Myc1 (Ambrosone et al. 2012) | transcription factor | Sc4wPfr_73.g11571.t1 | nematoblast—ISC | RNAi, smi: 10058-F4 |
| Dkk3 (Fedders et al. 2004) | secreted wnt modulator | Sc4wPfr_259_g33632.t1 | differentiating nematocytes | WISH |
| | | Gland cell differentiation | | |
| Myb (Siebert et al. 2019) | transcription factor | Sc4wPfr_839.g4024.t1 | precursor gland cells | scRNAseq |
| Dkk 1/2/4 A-C (Augustin et al. 2006; Guder et al. 2006) | secreted wnt modulator | Sc4wPfr_134_g20117.t1 | endodermal gland cells in gastric region | WISH |
| | | Gametogenesis | | |
| HvNotch (Käsbauer et al. 2007) | signal transduction receptor | Sc4wPfr_326.g15645.t1 | oocyte | smi-DAPT |
| Cnvas1 and Cnvas2 (Mochizuki et al. 2001) | germ-line factor | Sc4wPfr_861.g31120.t1 Sc4wPfr_2009.g19353.t1 | germline—ISC | WISH |
| Cnnos1 and Cnnos2 (Mochizuki et al. 2000) | germ-line factor | Sc4wPfr_366.g23802.t1 Sc4wPfr_169.g29161.t1 | germline—ISC | WISH |
| Hywi (Juliano et al. 2014; Teefy et al. 2020) | RNA-binding protein | Sc4wPfr_597.2.g14333.t1 | germline—ISC | immunocytochemistry |
| Pumilio (Siebert et al. 2019) | RNA-binding protein | Sc4wPfr_112.1.g5130.t1 | body column | scRNAseq |
| HyEED (Genikhovich et al. 2006) | epigenetic regulator | Sc4wPfr_6.g19136.t1 | spermatogonia | WISH |
| HyMyc2 (Hartl et al. 2014) | transcription factor | Sc4wPfr_850.1.g5732.t1 | spermatogenesis, oogenesis | WISH |

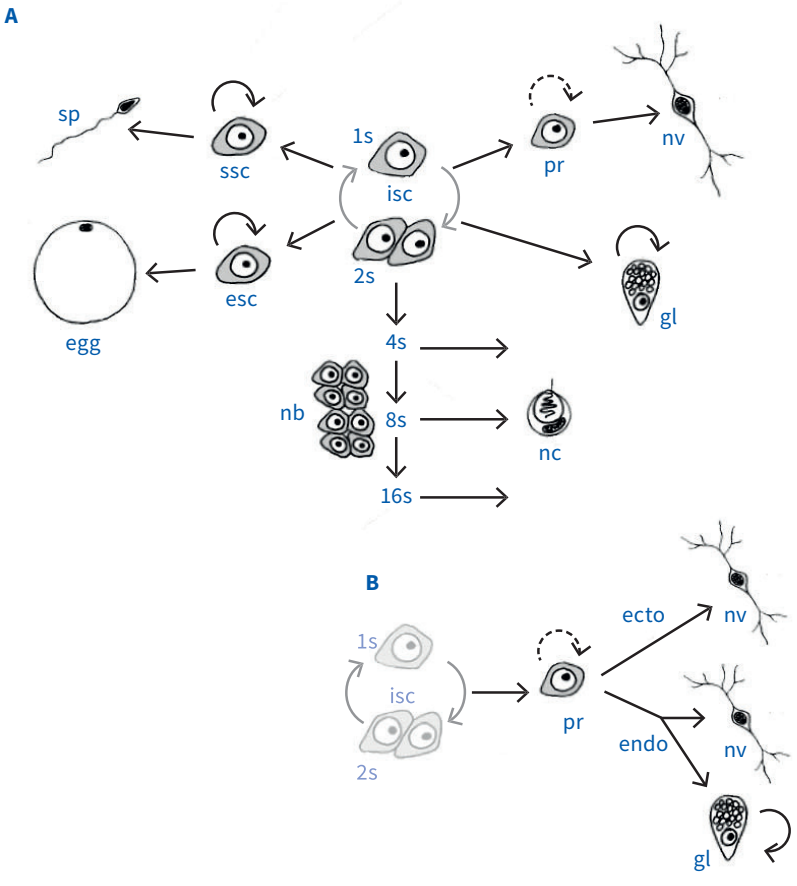


**Figure 1.** Schematics of the *Hydra* interstitial stem cell (ISC) lineage. (**A**) In the classic model, an ISC gives rise to somatic nerve cells (nv), gland cells (gl), and nematocytes (nc). ISCs also form sperm- and egg-restricted stem cells (ssc, esc), which can differentiate mature sperm cells (sp) and eggs during sexual reproduction. Nematocyte differentiation starts with the formation of nematoblast (nb) nests, which differentiate mature nematocytes after going through terminal mitosis. The committed precursor (pr) for nerve cell differentiation has a limited capacity for proliferation. (**B**) Results from single-cell transcriptome analysis suggest a modified model, in which nerve and gland cells derive from a common precursor (pr), whose capacity for proliferation is yet not clear. Source: Graphic by authors.

## 2. Interstitial Stem Cell (ISC) Properties in *Hydra*

### *2.1. ISC Self-Renewal and Stochastic Decision Making*

ISCs represent small, undifferentiated cells appearing as single cells or cell pairs and exhibiting a large nuclear–cytoplasmic ratio, a de-condensed chromatin with conspicuous nucleoli, a poly-ribosome- and mitochondria-rich cytoplasm, and multiple chromatoid bodies (nuage) associated with the nuclear membrane and mitochondria or isolated within the cytoplasm without connections to other organelles (Figure 2; Hobmayer et al. 2012). In vivo stem cell cloning experiments using IC-free host tissue demonstrated the multipotency of *Hydra* ISCs and their capacity to differentiate into somatic cells and gametes (David and Murphy 1977; Bosch and David 1987; Nishimiya-Fujisawa and Sugiyama 1993). ISCs reside in the ectodermal epithelial layer throughout the gastric region. In intact, asexually growing polyps, ISCs continuously grow in contiguous patches and migrate only small distances at most (Bosch and David 1990; Boehm and Bosch 2012). Nearly all ISCs are fast-cycling cells with a cell cycle length of 18–30 h (Campbell and David 1974) and a probability for self-renewal ($P_s$) of around 0.6 (David and Gierer 1974). Notably, after keeping clonal lab strains under conditions of fast and indefinite growth over decades, ISCs do not show any sign of cellular senescence, indicating that they have evolved mechanisms counteracting the known limits to expanded stem cell division such as telomere reduction, mitochondrial dysfunction, DNA damage, etc. (Sun et al. 2020; Tomczyk et al. 2020). There is also a tiny population of slower cycling ISCs showing an expanded cell cycle length of several days, which can be activated to proliferate faster by regeneration signals (Govindasamy et al. 2014). Finally, tracking of DiI vitally labelled ISCs revealed the full capacity for decision making, in which both daughter cells of a stem cell can remain stem cells or become differentiation precursors, or in which asymmetric division yields one stem cell and one differentiation precursor (David 2012). This type of flexible decision making involves communication of ISCs with their environment and rather complex processing of incoming short- and long-range signals.

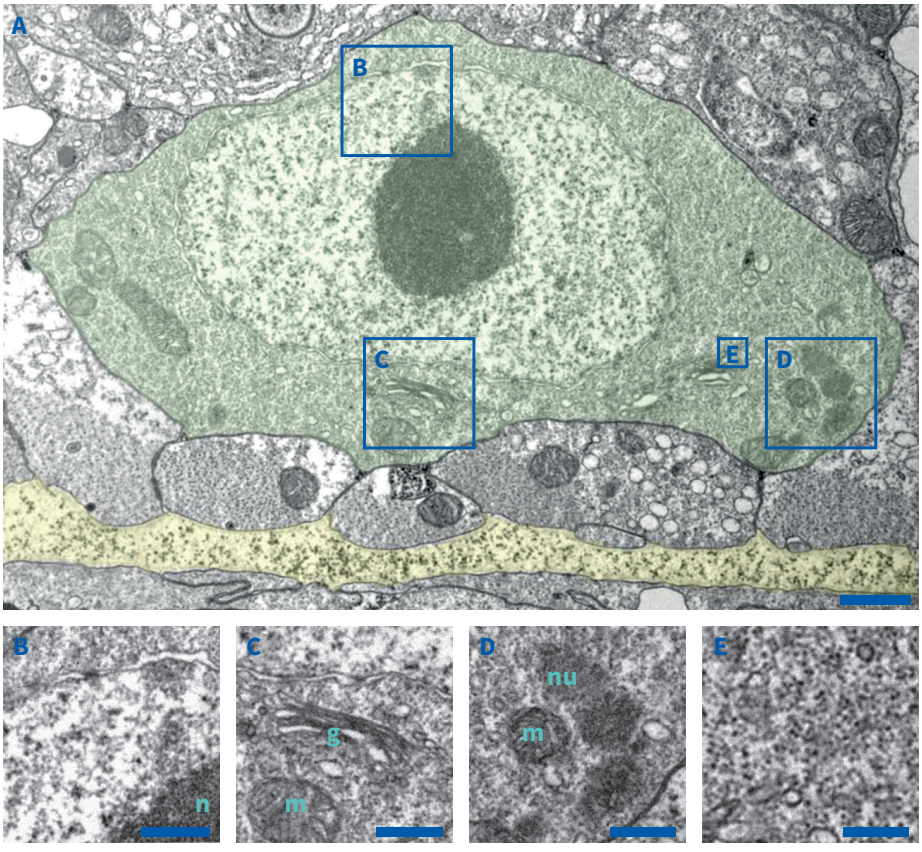


**Figure 2.** Ultrastructure of a *Hydra* interstitial stem cell (ISC). (**A**) ISC (green) positioned apically to the basal epithelial muscle fibers and the underlying mesoglea (yellow). Representative organelles of ISCs are depicted at higher magnification: (**B**) nuclear membrane with a nuclear pore, de-condensed chromatin and part of the nucleolus (n); (**C**) Golgi apparatus (g) and mitochondrium (m); (**D**) chromatoid body/nuage (nu) associated with mitochondria (m); (**E**) abundant ribosomes and chain-like poly-ribosomes in the cytoplasm. Bars: 1000 nm in (**A**), 500 nm in (**B**–**D**), 250 nm in (**E**). Source: Graphic by authors.

### *2.2. A Putative ISC Niche*

*Hydra* ISCs reside in the interstitial spaces between ectodermal epithelial cells usually at the basal level of the epidermal layer close to the muscle fibers. The microenvironment of stem cells (the "niche") is commonly regarded as an important regulatory entity for stem cell decision making and for providing structural, trophic and physiological support. Thus, these interstitial spaces represent distinct niches for ISCs. They may create a communication space for maintaining the

multipotent stem cell state or for becoming a committed precursor cell. However, none of the signals used to communicate has yet been isolated and characterized by now. Light and electron microscopic images reveal the direct contact of ISCs over almost their entire membrane surfaces with the membranes of surrounding epithelial cells (Figure 2). There has been speculation that classic cadherin, as in bilaterian stem cell niches, is involved in ISC–niche interactions (Bosch et al. 2010). The *Hydra* genome indeed encodes one large classic cadherin protein (Chapman et al. 2010), and this gene is transcriptionally activated in ectodermal epithelial cells and ISCs (*Hydra* single-cell transcriptome data available at the Broad Institute Single Cell Portal; Hobmayer lab, unpublished data). Functional analysis is required to validate this view. Furthermore, direct contact between ISCs and the mesoglea, *Hydra*'s extracellular matrix, has been discussed (Bosch et al. 2010), but also here a more detailed analysis using advanced imaging and molecular methods is needed to validate this idea.

### *2.3. Known Feedback Regulation through Signaling from Beyond the Niche*

Two aspects of continuously growing asexual mass cultures of *Hydra* clearly suggest that ISC behavior must be under tight control of complex feedback signaling and global patterning mechanisms. First, ICs exhibit a defined distribution pattern along the polyp's major head–foot body axis. ISCs are restricted to the gastric region, and they do not occur in the differentiated head and foot areas. This was first demonstrated by David and Plotnick (1980) by analyzing the axial origin of self-renewing and clone-forming interstitial cells in host aggregates. Later, it was confirmed using ISC-specific antibody staining and stable transgenic polyps expressing GFP in ISCs (David et al. 1987; Wittlieb et al. 2006). The boundaries to the head and foot areas of differentiation are sharp, raising the question of how such sharp boundaries are maintained under conditions where the entire tissue is constantly growing and cells are permanently changing positions. Positional information provided by the primary axial patterning system was suggested to shift ISC decision making from self-renewal to differentiation at the gastric region-head and gastric region-foot boundaries (Bosch 2008). Wnt/beta-Catenin signaling plays a central role in the *Hydra* head organizer, the polyp's major signaling center for axial patterning and setting up positional information (Hobmayer et al. 2000; Broun et al. 2005). Furthermore, accumulating evidence as discussed below in more detail shows the effects of beta-Catenin on ISC maintenance, as well as on nerve and nematocyte differentiation.

Second, asexual polyp growth strictly follows the rules of homeostasis. All cell types maintain their numbers relative to each other. During permanent tissue growth, they increase in numbers at the same pace, despite the fact that cell cycle lengths and differentiation times of the various cell types differ substantially. Since cell death

plays no role in asexually growing polyps, survival and the production of new cells by proliferation and differentiation are the main players, and they require permanent cell communication throughout the entire body column. By experimentally manipulating the density of selected cell types, feedback mechanisms coming from beyond the ISC niche were uncovered. ISC self-renewal reacts to the ISC density in the surrounding gastric tissue. Low density causes an increase in the probability for self-renewal, and high density a decrease (Bode et al. 1976; David and MacWilliams 1978; Sproull and David 1979; Fujisawa 1992). This feedback mechanism seems to be strain-specific, since ISCs do not respond to host ISC densities in tests using donor and host cells from different *Hydra* strains (David et al. 1991). Transplantation studies introducing ISCs into host tissue with variable nerve cell densities demonstrated that the nerve cell density positively affects ISC proliferation (Heimfeld and Bode 1985; Bosch et al. 1991). Finally, Boehm and Bosch (2012) demonstrated that non-migratory ISCs are stimulated to migrate towards gastric tissue devoid of ISCs. They proposed two alternative models explaining the observed migration patterns. Either attractive signals from empty niches may activate and direct ISC migration over some distance, or gastric tissue holding normal ISC densities may constantly emit signals suppressing ISC migration. None of the proposed signals discussed above has been identified by now. In summary, our understanding of the molecular nature of the described feedback mechanisms is only at its very beginning.

## 3. Molecular Factors Acting in Somatic IC Decision Making in *Hydra*

### *3.1. ISC Maintenance/Self-Renewal Factors*

According to the current paradigm, adult stem cell maintenance and the maintenance of pluri- or multi-potency is a result of the action of a distinct set of molecular factors, mostly stem cell-specific transcription factors. Among these factors, Oct4, Sox2, Kfl4, and c-Myc have become famous for inducing pluripotency in mammalian somatic cells (Takahashi and Yamanaka 2006). Based on this, they have been prime candidates in searches for stemness factors in other animals. However, there is little evidence that they play such a role across the animal kingdom. The *Hydra* genome does not encode homologs of *oct4* and *klf4* genes. Genes of related sub-families are encoded in the *Hydra* genome, but the closest relatives to *oct4* and *klf4* sub-families are not expressed in ISCs. Likewise, paralogs of the *sox* gene family are encoded. However, while several of them are expressed in the interstitial cell lineage, none of them is clearly and specifically activated in ISCs (Siebert et al. 2019). Taken together, these results suggest that different animal lineages have evolved different molecular signatures to maintain adult stem cells. A strong candidate factor for ISC maintenance in *Hydra* and some bilaterians including vertebrates is the transcription factor fork head box O, FoxO (Figure 3; Table 1). Overactivation of FoxO in normal

polyps increased ISC proliferation (Boehm et al. 2012). It also activated expression of *vasa* and *piwi*, two known stem cell genes (see below), in ISCs and in differentiating nematocytes.

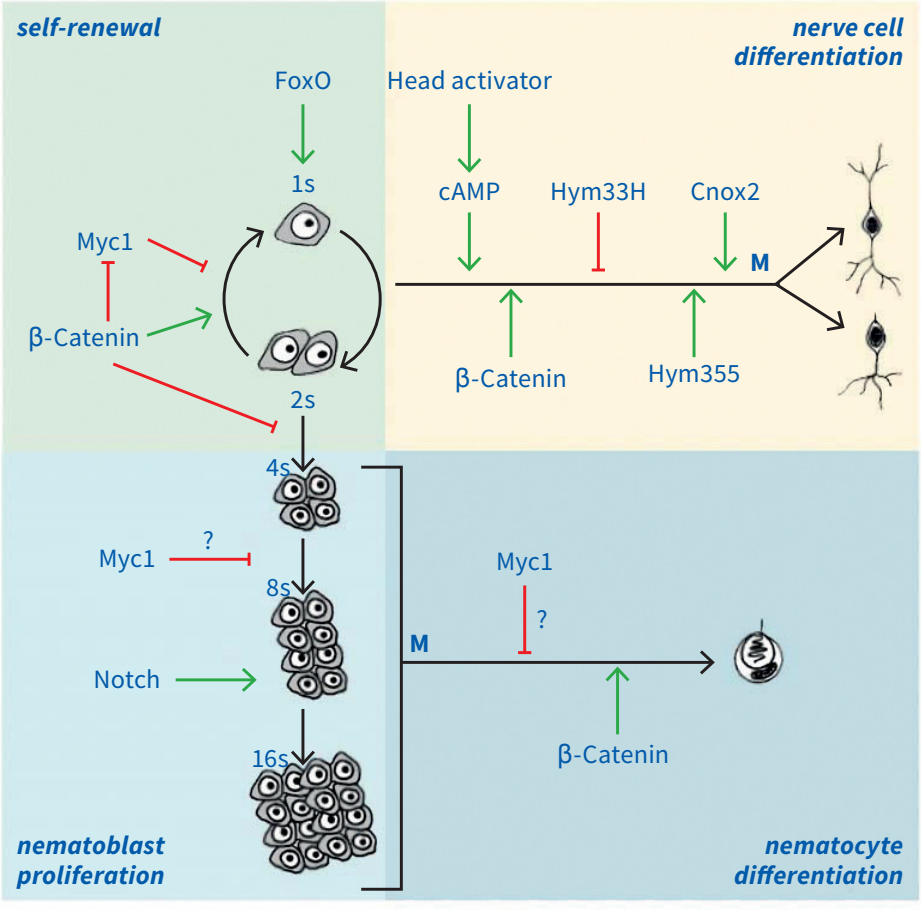


**Figure 3.** Schematic of the known molecular regulators of self-renewal and differentiation in the *Hydra* interstitial cell lineage. Positive and negative regulators are depicted in green and red, respectively. The precise time of action of these factors along a differentiation trajectory is in most cases unknown. Thus, the depicted position does not necessarily represent the precise sequence of events. Source: Graphic by authors.

Among the four *myc* gene homologs identified in *Hydra*, a structural and biochemical characterization showed HyMyc1 and HyMyc2 to share high similarities with c-Myc from vertebrates (Hartl et al. 2010, 2014). RNA interference suggested an initially unexpected role of *hymyc1* to decrease ISC self-renewal (Figure 3; Table 1;

Ambrosone et al. 2012). The *hymyc1* promoter turned out to be a target of repression by Wnt/β-Catenin signaling (Hartl et al. 2019). Furthermore, transgenic animals overexpressing nuclear β-Catenin show an increase in ISC density, which indicates an overall stimulating effect of Wnt/β-Catenin signaling on ISC self-renewal possibly by a double-negative cascade via HyMyc1 (Figure 3; Table 1; Hartl et al. 2019). The precise function of HyMyc2 in ISC maintenance is not clear at the present. The *hymyc2* gene is expressed in all proliferating cells in *Hydra,* including ectodermal and endodermal epithelial cells as well as gamete precursor cells, and its mRNA is a maternal contribution to early embryos (Hartl et al. 2014). Furthermore, the *hymyc2* exon/intron structure and the encoded amino acid sequence are slightly more similar to vertebrate *c-myc* than *hymyc1*. Thus, we proposed that *hymyc2* represents the functional *Hydra* homolog of vertebrate *c-myc* with a corresponding active role in cell cycle regulation and stem cell self-renewal (Hartl et al. 2014). Functional interference experiments to test this view are ongoing in our lab.

There are putative ISC stemness factors proposed primarily based on gene expression data. The so-called germline-specific genes *nanos*, *vasa* and *piwi* are strongly expressed in male- and female-restricted stem cells in *Hydra*, but also in multi-potent ISCs (Table 1; Mochizuki et al. 2000, 2001; Juliano et al. 2014; for review see Nishimiya-Fujisawa and Kobayashi 2012). Activation of these genes in somatic adult stem cells was observed in other sexually and asexually reproducing taxa such as Porifera, various other Cnidaria, Platyhelminthes, and Echinodermata, indicating that there is no clear soma-germline boundary in these species and that these genes contribute to maintaining adult stem cell multipotency (for review see Juliano and Wessel 2010). A recent in-depth *Hydra* single-cell transcriptome analysis identified only a single marker gene expressed specifically in the putative multipotent ISCs (Siebert et al. 2019). This new factor, Hy-icell1, has no homolog in the DNA data bases, and its function in ISCs is unknown at the present. Notably, single-cell transcriptomics failed to identify a specific set of ISC stemness factors (Siebert et al. 2019). Hence, it was argued that ISCs are largely defined by the absence of activity of cell type-specific differentiation genes. How this lack of differentiation activity is maintained is not known, but an understanding of its underlying molecular regulation will be essential to understand the potency and longevity of *Hydra* stem cells.

#### 3.1.1. Nerve Cell Differentiation

*Hydra* exhibits a rather simple nervous system with distinct sub-clusters building three non-overlapping networks (Dupre and Yuste 2017; Siebert et al. 2019). ISC commitment for neuronal differentiation occurs in the late S-phase in the gastric region (Venugopal and David 1981). Committed nerve precursors then either migrate towards the head and foot areas or stay in the gastric region in order to support the

growing neuronal network along the body column and the replacement of neurons lost at the terminal ends. Nerve precursors mostly undergo one terminal mitosis to yield two differentiated neurons; very few undergo one or two more divisions to yield four or eight neurons (Heimfeld and Bode 1985; Hager and David 1997; Technau and Holstein 1996).

Several studies revealed an unexpected action of small peptide signaling in nerve cell differentiation (Figure 3; Table 1). Intact polyps treated with either purified or synthetic Head Activator peptide (pEPPGGSKVILF) showed a significantly increased number of nerve cells throughout the body column (Holstein et al. 1986). This effect can be mimicked by cAMP, indicating that cAMP acts as second messenger in this cascade (Fenger et al. 1994). The gene encoding the Head Activator peptide sequence has yet not been found in the *Hydra* genome. Its origin thus remains elusive. Two other small peptides regulate neurogenesis in *Hydra*. Hym-355, a neuropeptide secreted along the entire body column (FPQSFLPRGa), enhances nerve cell differentiation in the early commitment phase, and treatment with this peptide leads to substantially higher numbers of nerve cells in the polyp (Takahashi et al. 2000). The epitheliopeptide Hym-33H (AALPW) counteracts nerve cell differentiation most likely also acting on early precursors (Takahashi et al. 1997).

Khalturin et al. (2007) showed that the differentiation of Hym-355-positive neurons is stimulated in *Hydra* treated with the β-Catenin-stabilizing small molecule Alsterpaullone. Wnt/β-Catenin signaling is also strongly elevated in transgenic polyps, in which a β-Catenin-GFP fusion protein is driven by the *actin1* promoter. The density of neurons in the body column of these transgenic polyps is more than twice as high as in controls (Hobmayer lab, unpublished data), clearly supporting the view that Wnt/β-Catenin signaling stimulates neurogenesis in *Hydra* (Figure 3; Table 1). Neurogenesis in the head of *Hydra* polyps is suppressed by the knock-down of the transcription factor Cnox-2, as shown by using RNA interference (Figure 3; Table 1). In addition, qPCR-data indicated that cnox-2 is an upstream regulator of the nerve cell marker genes *pradl-a*, *gsc*, *RFamide-B* and *hyCOUP-TF* (Miljkovic-Licina et al. 2007). Single-cell transcriptomics demonstrated that a few other transcription factors are specifically expressed in neuronal progenitor cells (HvSoxC) and in the population of precursors common to nerve and gland cell differentiation (Myb and Myc3; Table 1; Siebert et al. 2019).

#### 3.1.2. Gland Cell Differentiation

Endodermal gland cells are differentiation products of ISCs, but they retain a capacity for proliferation in their differentiated state. Thus, very few gland cells are produced anew, while most of them reproduce by cell division (Schmidt and David 1986). Gland cells actually represent a set of different sub-populations distributed along the body column (Siebert et al. 2019), and they have been shown to change their

phenotype by trans-differentiation when they change their axial position following global tissue movement (Siebert et al. 2008). Gland cell differentiation is not well studied in terms of regulatory molecular factors. As described above, gland cells seem to share a common precursor with differentiating neurons based on specific activation of *myb* and *myc3* genes (Table 1; Siebert et al. 2019).

### 3.1.3. Nematocyte Differentiation

ISCs committed for nematocyte differentiation undergo two to four steps of proliferation, resulting in cell nests of proliferating nematoblasts with a nest size of 4s to 16s. Nematoblast nests then undergo terminal mitosis and thereby form nests of differentiating nematocytes with a nest size of 8s to 32s (David and Challoner 1974). During the differentiation phase, every nest cell builds a fully functional nematocyte capsule. Upon completion of this process, nests break up, and individual and fully mature nematocytes start to migrate. Finally, nematocytes are taken up and mounted at the apical membrane in ectodermal epithelial cells, mostly in ectodermal battery cell complexes in the tentacles.

Wnt/β-catenin signaling may have two modes of action in this pathway (Figure 3; Table 1). First, the total number of nests of proliferating nematoblasts in the body column of a polyp is strongly reduced upon nuclear activation of β-catenin in Alsterpaullone-treated and in β-catenin transgenic polyps (Figure 3; Khalturin et al. 2007); Hobmayer lab, unpublished data). Second, post-mitotic differentiation of nematocytes seems to be strongly enhanced by Wnt/β-catenin signaling based on the observation that differentiating nests expressing the marker gene *nb035* disappear, whereas mature nematocytes expressing the marker gene *nb031* strongly increase in numbers upon Alsterpaullone treatment (Khalturin et al. 2007). In addition, Myc1 seems to be involved. The down-regulation of *myc1* mRNAs by RNA interference resulted in an increase in nests of proliferating nematoblasts and in an increased ratio of mature nematocytes/battery cells in the tentacles (Figure 3; Table 1; Ambrosone et al. 2012). Equivalent results were obtained after treatment of *Hydra* polyps with the c-Myc-specific small-molecule inhibitor 10058-F4 (Ambrosone et al. 2012).

Finally, Notch signaling has been reported to promote nematocyte differentiation in the early post-mitotic phase possibly by acting in nematoblast nests shortly before terminal mitosis (Figure 3; Table 1). This was shown using the small-molecule inhibitor DAPT, which inhibits gamma-secretase and therefore prevents downstream Notch signaling. Treating *Hydra* polyps with DAPT inhibited the expression of the nematocyte marker genes *nb031* and *nb035*, strongly reduced the numbers of nematocyte nests with small vacuoles, and it forced differentiating nematocytes to undergo programmed cell death (Käsbauer et al. 2007; Khalturin et al. 2007).

## 4. Conclusions

Deciphering the molecular regulation of decision making in the *Hydra* IC lineage is at its beginning. A detailed single-cell transcriptomic atlas and advanced methods for stable transgenesis and genetic knock-down join the available molecular tool kit, including a genome annotated at the chromosome level. A large set of sophisticated methods allows the analysis of *Hydra* ISCs and their lineage products at the cellular level. Due to its simple body plan, all this can be carried out in vivo in fully intact polyps. Furthermore, ISC behavior is also studied in related marine hydrozoans such as *Hydractinia* and *Clytia*. The action of the germline factors Nanos, Vasa and Piwi, as well as Myc function, may be conserved. However, there are unexpected differences among the hydrozoan polyp models. Polynem, a POU domain transcription factor more closely related to vertebrate Oct4 than any *Hydra* Pou transcription factor seems to keep cells undifferentiated in *Hydractinia* and is able to induce neoplasia when overactivated (Millane et al. 2011). While AP2 is a core activator for germ cell formation in *Hydractinia* and higher animals (DuBuc et al. 2020), single-cell transcriptome data do not support this role in *Hydra*. *Clytia* Sox proteins, in contrast to those in *Hydra*, seem to affect the balance between self-renewing stem cells and cells undergoing differentiation (Jager et al. 2011). Thus, there is obvious within-class diversity in the action of stem cell and differentiation factors among different hydrozoans, and each lineage may have evolved a stemness regulation adapted to its specific life cycle needs.

What are the imminent questions to be resolved? It is clear that we do not understand most of the key issues well enough. How is stemness and the non-differentiation state of ISCs in *Hydra* and other hydrozoan polyps defined at the molecular level? What are the molecular signals acting in direct niche interactions? How do long-range feedback mechanisms work? Finally, what roles do post-translational modifiers play, and which types of epigenetic mechanisms affect stem cell maintenance and differentiation? Isolating these regulatory factors will clearly contribute to a more general understanding of adult stem cell dynamics and decision making in the common ancestor of Bilateria, and more generally to the evolutionary ancestry of cellular plasticity, regeneration, and ageing.

**Author Contributions:** Conceptualization, B.H., M.L., M.A. and M.K.; formal analysis, M.L., M.A., M.K., W.S. and B.H.; writing—original draft preparation, M.L., M.A., M.K. and B.H.; writing—review and editing, M.L., M.A., M.K., W.S. and B.H.; funding acquisition, B.H.

**Funding:** This research was supported by the EC H2020 Marie Sklodowska-Curie COFUND research grant No 847681 "ARDRE—Ageing, Regeneration and Drug Research" and the EU-COST network 16203 "MARISTEM—Stem Cells of marine/aquatic invertebrates: From basic research to innovative applications".

**Acknowledgments:** The authors thank Lena Seppi, Natalie Kolb for technical support and animal culture, and Kevin Grüner for his help with electron microscopy.

**Conflicts of Interest:** The authors declare no conflict of interests.

## Abbreviations

| | |
|---|---|
| ISC | interstitial stem cell |
| scRNAseq | single-cell RNA Sequencing |
| smi | small molecular inhibitor |
| WISH | whole-mount in situ hybridization |

## References


Ambrosone, Alfredo, Valentina Marchesano, Angela Tino, Bert Hobmayer, and Claudia Tortiglione. 2012. *Hymyc1* downregulation promotes stem cell proliferation in *Hydra vulgaris*. *PLoS ONE* 7: e30660. [CrossRef] [PubMed]

Augustin, René, André Franke, Konstantin Khalturin, Rainer Kiko, Stefan Siebert, Georg Hemmrich, and Thomas C. G. Bosch. 2006. Dickkopf-related genes are components of the positional value gradient in *Hydra*. *Developmental Biology* 296: 62–70. [CrossRef] [PubMed]

Augustin, René, Katja Schröder, Andréa P. Murillo Rincón, Sébastian Fraune, Friederike Anton-Erxleben, Eva-Maria Herbst, Jörg Wittlieb, Martin Schwentner, Joachim Grötzinger, Trudy M. Wassenaar, and et al. 2017. A secreted antibacterial neuropeptide shapes the microbiome of *Hydra*. *Nature Communications* 8: 698. [CrossRef] [PubMed]

Bode, Hans R. 1996. The interstitial cell lineage of hydra: A stem cell system that arose early in evolution. *Journal of Cell Science* 109: 1155–64. [CrossRef] [PubMed]

Bode, Hans R., Kristine M. Flick, and G. Scott Smith. 1976. Regulation of interstitial cell differentiation in *Hydra attenuata*. I. Homeostatic control of interstitial cell population size. *Journal of Cell Science* 20: 29–46. [CrossRef] [PubMed]

Boehm, Anna-Marei, and Thomas C. G. Bosch. 2012. Migration of multipotent interstitial stem cells in *Hydra*. *Zoology* 115: 275–82. [CrossRef]

Boehm, Anna-Marei, Konstantin Khalturin, Friederike Anton-Erxleben, Georg Hemmrich, Ulrich C. Klostermeier, Javier A. Lopez-Quintero, Hans-Heinrich Oberg, Malte Puchert, Philip Rosenstiel, Jörg Wittlieb, and et al. 2012. FoxO is a critical regulator of stem cell maintenance in immortal *Hydra*. *Proceedings of the National Academy of Sciences of the United States of America* 109: 19697–702. [CrossRef]

Bosch, Thomas C. G. 2008. Stem cells in immortal Hydra. In *Stem Cells*. Edited by Bosch TCG. Heidelberg: Springer, pp. 37–58.

Bosch, Thomas C. G., and Charles N. David. 1987. Stem cells of *Hydra magnipapillata* can differentiate into somatic cells and germ line cells. *Developmental Biology* 121: 182–191. [CrossRef]

Bosch, Thomas C. G., and Charles N. David. 1990. Cloned interstitial stem cells grow as contiguous patches in *Hydra*. *Developmental Biology* 138: 513–15. [CrossRef]

Bosch, Thomas C. G., Friederike Anton-Erxleben, Georg Hemmrich, and Konstantin Khalturin. 2010. The *Hydra* polyp: Nothing but an active stem cell community. *Development, Growth & Differentiation* 52: 15–25.

Bosch, Thomas C. G., Rebecca Rollbühler, Birgit Scheider, and Charles N. David. 1991. Role of the cellular environment in interstitial stem cell proliferation in *Hydra*. *Roux's Archives of Developmental Biology* 200: 269–76. [CrossRef] [PubMed]

Broun, Mariya, Lydia Gee, Beate Reinhardt, and Hans R. Bode. 2005. Formation of the head organizer in hydra involves the canonical Wnt pathway. *Development* 132: 2907–16. [CrossRef] [PubMed]

Buzgariu, Wanda, Sarah Al Haddad, Szymon Tomczyk, Yvan Wenger, and Brigitte Galliot. 2015. Multi-functionality and plasticity characterize epithelial cells in *Hydra*. *Tissue Barriers* 3: e1068908. [CrossRef] [PubMed]

Campbell, Richard D., and Charles N. David. 1974. Cell cycle kinetics and development of *Hydra attenuata*. II. Interstitial cells. *Journal of Cell Science* 16: 349–58. [CrossRef] [PubMed]

Chapman, Jarrod A., Ewen F. Kirkness, Oleg Simakov, Steven E. Hampson, Therese Mitros, Thomas Weinmaier, Thomas Rattei, Prakash G. Balasubramanian, Jon Borman, and Dana Busam. 2010. The dynamic genome of *Hydra*. *Nature* 464: 592–96. [CrossRef] [PubMed]

David, Charles N. 2012. Interstitial stem cells in *Hydra*: Multipotency and decision-making. *International Journal of Developmental Biology* 56: 489–97. [CrossRef]

David, Charles N., and Alfred Gierer. 1974. Cell cycle kinetics and development of *Hydra attenuata*. III. Nerve and nematocyte differentiation. *Journal of Cell Science* 16: 359–75. [CrossRef]

David, Charles N., and Diane Challoner. 1974. Distribution of interstitial cells and differentiating nematocytes in nests in *Hydra attenuata*. *American Zoologist* 14: 537–42. [CrossRef]

David, Charles N., and Harry MacWilliams. 1978. Regulation of the self-renewal probability in *Hydra* stem cell clones. *Proceedings of the National Academy of Sciences of the United States of America* 75: 886–90. [CrossRef]

David, Charles N., and Ida Plotnick. 1980. Distribution of interstitial stem cells in hydra. *Developmental Biology* 76: 175–84. [CrossRef]

David, Charles N., and Susan Murphy. 1977. Characterization of interstitial stem cells in hydra by cloning. *Developmental Biology* 58: 372–383. [CrossRef]

David, Charles N., Thomas C. G. Bosch, Engelbert Hobmayer, Thomas W. Holstein, and Tobias Schmidt. 1987. Interstitial stem cells in Hydra. In *Genetic Regulation of Development*. Edited by William F. Loomis. New York: Alan R. Liss, pp. 189–208.

David, Charles N., Toshitaka Fujisawa, and Thomas C. G. Bosch. 1991. Interstitial stem cell proliferation in *Hydra*: Evidence for strain-specific regulatory signals. *Developmental Biology* 148: 501–7. [CrossRef]

DuBuc, Timothy Q., Christine E. Schnitzler, Eleni Chrysostomou, Emma T. McMahon, Febrimarsa, James M. Gahan, Tara Buggie, Sebastian G. Gornik, Shirley Hanley, Sofia N. Barreira, and et al. 2020. Transcription factor AP2 controls cnidarian germ cell induction. *Science* 367: 757–62. [CrossRef] [PubMed]

Dupre, Christophe, and Rafael Yuste. 2017. Non-overlapping neural networks in *Hydra vulgaris*. *Current Biology* 27: 1085–97. [CrossRef] [PubMed]

Fedders, Henning, René Augustin, and Thomas C. G. Bosch. 2004. A Dickkopf-3-related gene is expressed in differentiating nematocytes in the basal metazoan *Hydra*. *Development Genes and Evolution* 214: 72–80. [CrossRef] [PubMed]

Fenger, Ursula, Michael Hofmann, Brigitte Galliot, and H. Chica Schaller. 1994. The role of the cAMP pathway in mediating the effect of head activator on nerve-cell determination and differentiation in hydra. *Mechanisms of Development* 47: 115–25. [CrossRef]

Fujisawa, Toshitaka. 1992. Homeostatic recovery of interstitial cell populations in *Hydra*. *Developmental Biology* 150: 185–92. [CrossRef]

Gee, Lydia, Julia Hartig, Lee Law, Jörg Wittlieb, Konstantin Khalturin, Thomas C. G. Bosch, and Hans R. Bode. 2010. Beta-catenin plays a central role in setting up the head organizer in *Hydra*. *Developmental Biology* 340: 116–24. [CrossRef]

Genikhovich, Griogry, Ulrich Kürn, Georg Hemmrich, and Thomas C. G. Bosch. 2006. Discovery of genes expressed in *Hydra* embryogenesis. *Developmental Biology* 289: 466–81. [CrossRef]

Govindasamy, Niraimathi, Supriya Murthy, and Yashoda Ghanekar. 2014. Slow-cycling stem cells in hydra contribute to head regeneration. *Biology Open* 3: 1236–44. [CrossRef]

Grens, Ann, Elizabeth Mason, J. Lawrence Marsh, and Hans R. Bode. 1995. Evolutionary conservation of a cell fate specification gene: The *Hydra* achaete-scute homolog has proneural activity in *Drosophila*. *Development* 121: 4027–35. [CrossRef] [PubMed]

Guder, Corina, Sonia Pinho, Tanju G. Nacak, Heiko A. Schmidt, Bert Hobmayer, Christof Niehrs, and Thomas W. Holstein. 2006. An ancient Wnt-Dickkopf antagonism in *Hydra*. *Development* 133: 901–11. [CrossRef] [PubMed]

Hager, Gundel, and Charles N. David. 1997. Pattern of differentiated nerve cells in hydra is determined by precursor migration. *Development* 124: 569–76. [CrossRef] [PubMed]

Hartl, Markus, Anna-Maria Mitterstiller, Taras Valovka, Kathrin Breuker, Bert Hobmayer, and Klaus Bister. 2010. Stem cell-specific activation of an ancestral *myc* protooncogene with conserved basic functions in the early metazoan *Hydra*. *Proceedings of the National Academy of Sciences of the United States of America* 107: 4051–56. [CrossRef]

Hartl, Markus, Stella Glasauer, Sabine Gufler, Andrea Raffeiner, Kane Puglisi, Kathrin Breuker, Klaus Bister, and Bert Hobmayer. 2019. Differential regulation of myc homologs by Wnt/β-Catenin signaling in the early metazoan *Hydra*. *The FEBS Journal* 286: 2295–310. [CrossRef]

Hartl, Markus, Stella Glasauer, Taras Valovka, Kathrin Breuker, Bert Hobmayer, and Klaus Bister. 2014. Hydra myc2, a unique pre-bilaterian member of the myc gene family, is activated in cell proliferation and gametogenesis. *Biology Open* 3: 397–407. [CrossRef]

Heimfeld, Shelly, and Hans R. Bode. 1985. Growth regulation of the interstitial cell proliferation in hydra. I. Evidence for global control by nerve cells in the head. *Developmental Biology* 110: 297–307. [CrossRef]

Hemmrich, Georg, Konstantin Khalturin, Anna-Marei Boehm, Malte Puchert, Friederike Anton-Erxleben, Jörg Wittlieb, Ulrich C. Klostermeier, Philip Rosenstiel, Hans-Heinrich Oberg, Tomislav Domazet-Lošo, and et al. 2012. Molecular signatures of the three stem cell lineages in *Hydra* and the emergence of stem cell function at the base of multicellularity. *Molecular Biology and Evolution* 29: 3267–80. [CrossRef]

Hobmayer, Bert, Fabian Rentzsch, Kerstin Kuhn, Christoph M. Happel, Christoph Cramer von Laue, Petra Snyder, Ute Rothbächer, and Thomas W. Holstein. 2000. Wnt signalling molecules act in axis formation in the diploblastic metazoan *Hydra*. *Nature* 407: 186–89. [CrossRef]

Hobmayer, Bert, Marcell Jenewein, Dominik Eder, Stella Glasauer, Sabine Gufler, Markus Hartl, and Willi Salvenmoser. 2012. Stemness in *Hydra*—A current perspective. *International Journal of Developmental Biology* 56: 509–17. [CrossRef]

Holstein, Thomas W., Chica Schaller, and Charles N. David. 1986. Nerve cell differentiation in hydra requires two signals. *Developmental Biology* 115: 9–17. [CrossRef]

Jager, Muriel, Eric Quéinnec, Hervé Le Guyader, and Michael Manuel. 2011. Multiple *sox* genes are expressed in stem cells or in differentiating neuro-sensory cells in the hydrozoan *Clytia hemisphaerica*. *EvoDevo* 2: 1–17. [CrossRef] [PubMed]

Juliano, Celina E., Adrian Reich, Na Liu, Jessica Götzfried, Mei Zhong, Selen Uman, Robert A. Reenan, Gary M. Wessel, Robert E. Steele, and Haifan Lin. 2014. PIWI proteins and PIWI-interacting RNAs function in *Hydra* somatic stem cells. *Proceedings of the National Academy of Sciences of the United States of America* 111: 337–42. [CrossRef] [PubMed]

Juliano, Celina E., and Gary Wessel. 2010. Versatile germline genes. *Science* 329: 640–41. [CrossRef]

Käsbauer, Tina, Par Tow, Olga Alexandrova, Charles N. David, Ekaterina Dall'Armi, Andrea Staudigl, Beate Stiening, and Angelika Böttger. 2007. The Notch signaling pathway in the cnidarian *Hydra*. *Developmental Biology* 303: 376–90. [CrossRef]

Khalturin, Konstantin, Friederike Anton-Erxleben, Sabine Milde, Christine Plötz, Jörg Wittlieb, Georg Hemmrich, and Thomas C. G. Bosch. 2007. Transgenic stem cells in Hydra reveal an early evolutionary origin for key elements controlling self-renewal and differentiation. *Developmental Biology* 309: 32–44. [CrossRef]

Lindgens, Dirk, Thomas W. Holstein, and Ulrich Technau. 2004. Hyzic, the *Hydra* homolog of the zic/odd-paired gene, is involved in the early specification of the sensory nematocytes. *Development* 131: 191–201. [CrossRef]

Miljkovic-Licina, Marijana, Simona Chera, Luiza Ghila, and Brigitte Galliot. 2007. Head regeneration in wild-type hydra requires de novo neurogenesis. *Development* 134: 1191–201. [CrossRef]

Millane, R. Cathriona, Justyna Kanska, David J. Duffy, Cathal Seoighe, Stephen Cunningham, Günter Plickert, and Uri Frank. 2011. Induced stem cell neoplasia in a cnidarian by ectopic expression of a POU domain transcription factor. *Development* 138: 2429–39. [CrossRef]

Mochizuki, Kazufumi, Chiemi Nishimiya-Fujisawa, and Toshitaka Fujisawa. 2001. Universal occurrence of the *vasa-related* genes among metazoans and their germline expression in *Hydra*. *Development Genes and Evolution* 211: 299–308. [CrossRef]

Mochizuki, Kazufumi, Hiroko Sano, Satoru Kobayashi, Chiemi Nishimiya-Fujisawa, and Toshitaka Fujisawa. 2000. Expression and evolutionary conservation of *nanos-related* genes in *Hydra*. *Development Genes and Evolution* 210: 591–602. [CrossRef] [PubMed]

Müller, Werner A. 1967. Differenzierungspotenzen und Geschlechtsstabilität der I-Zellen von *Hydractinia echinata*. *Wilhelm Roux'Archiv für Entwicklungsmechanik der Organismen* 159: 412–32. [CrossRef] [PubMed]

Nishimiya-Fujisawa, Chiemi, and Satoru Kobayashi. 2012. Germline stem cells and sex determination in *Hydra*. *International Journal of Developmental Biology* 56: 499–508. [CrossRef] [PubMed]

Nishimiya-Fujisawa, Chiemi, and Tsutomu Sugiyama. 1993. Genetic analysis of developmental mechanisms in *Hydra*. XX. Cloning of interstitial stem cells restricted to the sperm differentiation pathway in *Hydra magnipapillata*. *Developmental Biology* 157: 1–9. [CrossRef]

Plickert, Günter, Uri Frank, and Werner A. Müller. 2012. *Hydractinia*, a pioneering model for stem cell biology and reprogramming somatic cells to pluripotency. *International Journal of Developmental Biology* 56: 519–34. [CrossRef]

Schmidt, Tobias, and Charles N. David. 1986. Gland cells in *Hydra*: Cell cycle kinetics and development. *Journal of Cell Science* 85: 197–215. [CrossRef]

Siebert, Stefan, Jeffrey A. Farrell, Jack F. Cazet, Yashodara Abeykoon, Abby S. Primack, Christine E. Schnitzler, and Celina E. Juliano. 2019. Stem cell differentiation trajectories in *Hydra* resolved at single-cell resolution. *Science* 365: eaav9314. [CrossRef]

Siebert, Stefan, Friederike Anton-Erxleben, and Thomas C. G. Bosch. 2008. Cell type complexity in the basal metazoan *Hydra* is maintained by both stem cell based mechanisms and transdifferentiation. *Developmental Biology* 313: 13–24. [CrossRef]

Sproull, Frederick, and Charles N. David. 1979. Stem cell growth and differentiation in *Hydra attenuata*. I. Regulation of the self-renewal probability in multiclone aggregates. *Journal of Cell Science* 38: 155–69. [CrossRef]

Sun, Shixiang, Ryan R. White, Kathleen E. Fischer, Zhengdong Zhang, Steven N. Austad, and Jan Vijg. 2020. Inducible aging in *Hydra oligactis* implicates sexual reproduction, loss of stem cells, and genome maintenance as major pathways. *GeroScience* 42: 1119–32. [CrossRef]

Takahashi, Kazutoshi, and Shinya Yamanaka. 2006. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–76. [CrossRef] [PubMed]

Takahashi, Toshio, Osamu Koizumi, Yuki Ariura, Anna Romanovitch, Thomas C. Bosch, Yoshitaka Kobayakawa, Shirou Mohri, Hans R. Bode, Seungshic Yum, Masayuki Hatta, and et al. 2000. A novel neuropeptide, Hym-355, positively regulates neuron differentiation in *Hydra*. *Development* 127: 997–1005. [CrossRef] [PubMed]

Takahashi, Toshio, Yojiro Muneoka, Jan Lohmann, Maria S. Lopez de Haro, Gaby Solleder, Thomas C. G. Bosch, Charles N. David, Hans R. Bode, Osamu Koizumi, Hiroshi Shimizu, and et al. 1997. Systematic isolation of peptide signal molecules regulating development in hydra: LWamide and PW families. *Proceedings of the National Academy of Sciences of the United States of America* 94: 1241–46. [CrossRef] [PubMed]

Tardent, Pierre. 1954. Axiale Verteilungs-Gradienten der interstitiellen Zellen bei *Hydra* und*Tubularia* und ihre Bedeutung für die Regeneration. *Wilhelm Roux'Archiv für Entwicklungsmechanik der Organismen* 146: 593–643. [CrossRef] [PubMed]

Technau, Ulrich, and Thomas W. Holstein. 1996. Phenotypic maturation of neurons and continuous precursor migration in the formation of the peduncle nerve net in *Hydra*. *Developmental Biology* 177: 599–615. [CrossRef] [PubMed]

Teefy, Bryan B., Stefan Siebert, Jack F. Cazet, Haifan Lin, and Celina E. Juliano. 2020. PIWI-piRNA pathway-mediated transposable element repression in *Hydra* somatic stem cells. *RNA* 26: 550–63. [CrossRef] [PubMed]

Tomczyk, Szymon, Nenad Suknovic, Quentin Schenkelaars, Yvan Wenger, Kazadi Ekundayo, Wanda Buzgariu, Christoph Bauer, Kathleen Fischer, Steven Austad, and Brigitte Galliot. 2020. Deficient autophagy in epithelial stem cells drives aging in the freshwater cnidarian *Hydra*. *Development* 147: dev177840. [CrossRef]

Venugopal, Gopalan, and Charles N. David. 1981. Nerve commitment in *Hydra*: II. Localization of commitment in S Phase. *Developmental Biology* 83: 361–65. [CrossRef]

Watanabe, Hiroshi, Van Thanh Hoang, Robert Mättner, and Thomas W. Holstein. 2009. Immortality and the base of multicellular life: Lessons from cnidarian stem cells. *Sem Cell Developmental Biology* 20: 1114–25. [CrossRef]

Weismann, August. 1883. *Die Entstehung der Sexualzellen bei den Hydromedusen*. Jena: Gustav Fischer Verlag.

Wittlieb, Jörg, Konstantin Khalturin, Jan U. Lohmann, Friederike Anton-Erxleben, and Thomas C. G. Bosch. 2006. Transgenic *Hydra* allow in vivo tracking of individual stem cells during morphogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 103: 6208–11. [CrossRef]