Somatic Expression of Stemness Genes in Aquatic Invertebrates

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Abstract: Adult stem cells (ASCs) of aquatic invertebrates are involved in important biological processes such as regeneration and asexual reproduction. Unlike in vertebrates, they share pluripotency and even totipotency, and do not reside in permanent niches. Aquatic invertebrates represent the widest phylogenetic animal radiation on earth, but until now, limited research data have been available on their ASCs. Although less studied than their vertebrate counterparts, aquatic invertebrate ASCs express orthologues of many vertebrate genes usually associated with stemness. With this review, we aim at providing a database for current and future studies on ASC properties through a comprehensive literature analysis of intra- and inter-phylum comparisons of gene expressions and their functions in aquatic invertebrate ASCs. We concentrate on major gene families where sufficient data are available; gaps in our results will be filled by future studies on ASCs of aquatic invertebrates.

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1. Introduction

Aquatic invertebrates present the widest metazoan radiation, and by virtue of their intraphylum diversity, they form large assemblages of multicellular animals and represent many model species in a wide range of biological disciplines (Ballarin et al. 2018). They include sponges (phylum Porifera), diploblastic jellyfish, anemones and corals (phylum Cnidaria), and triploblastic animals, the latter further divided into protostomes and deuterostomes. Protostomes include Spiralia (e.g., phyla Platyhelminthes, Annelida, Mollusca) and Ecdysozoa (e.g., phyla Nematoda and Arthropoda), whereas deuterostomes have Echinodermata and the subphyla Cephalochordata and Tunicata of the phylum Chordata as the prominent representatives (Figure 1). However, only limited research data are available on adult stem cells (ASCs) in general and ASC characteristics in particular in aquatic invertebrates (Rinkevich and Matranga 2009; Ballarin et al. 2021; Rinkevich et al. 2022). This is in clear contrast to the fact that ASCs in aquatic invertebrates are key players in many biological processes, such as regeneration, asexual reproduction/budding, torpor phenomena and more (Rinkevich et al. 2022).

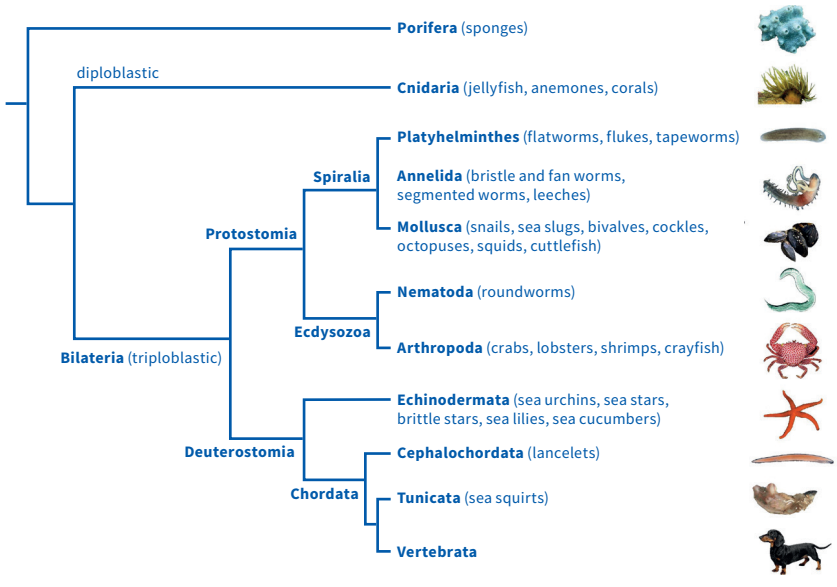

Figure 1. Phylogenetic relationship among the main invertebrate phyla. Graphic by authors.

The vertebrates possess ASCs homed to defined niches that are known to hold multipotency at best, representing lineage-specific self-renewing cells with tissue/organ-specific activities that generate limited numbers of daughter cell types (self-renewing progenitors and differentiated cells; Wagers and Weissman 2004; Clevers and Watt 2018). A careful examination of the animal phylogenetic tree (Rinkevich et al. 2022) reveals that ASCs have been studied only in a few metazoan phyla outside of the vertebrates, mostly taxa with high capabilities for asexual reproduction and regeneration (including whole-body regeneration) such as sponges, cnidarians, platyhelminthes, tunicates and echinoderms. These ASCs reveal dramatic disparities from vertebrate ASCs. Many of them are pluripotent and even totipotent, they do not follow the germline-sequestering model of the vertebrates, many exhibit morphologies of highly differentiated cells, they may generate the entire repertoire of cell types in adult animals, and some of them do not reside in stem cell niches (Rinkevich et al. 2022; Martinez et al. 2022).

It is thus of obvious importance to evaluate the properties of aquatic invertebrate ASCs using inter- and intra-phylum comparative and mechanistic analyses focusing on biological processes or specific properties. Indeed, aquatic invertebrate ASCs express orthologues of many vertebrate "stemness" genes (associated or disparate from the biological phenomena studied in the vertebrates), even though the molecular

machinery by which these organisms hold sustainable ASC stocks along their lifespan is still unsolved (Conte et al. 2009; Rinkevich et al. 2022).

In the following sections, we provide a comprehensive literature analysis of intra- and inter-phylum comparisons of gene expressions and their functions in aquatic invertebrate ASCs. We concentrated on major gene families where sufficient data are available, and we aim at providing a database for current and future studies on ASC properties and at deducing general aspects of ASC gene-regulatory programs across the metazoan tree of life. The most important current models for the study of aquatic invertebrate ASCs are represented by sponges, cnidarians, flatworms, annelids, echinoderms and colonial ascidians, but limited data are available also for ctenophores (comb jellies), xenacelomorphs (acoel flatworms), hemichordates (acorn worms) and cephalochordates (lancelets). It must be noted that since most of the cited studies were not performed using the most cutting-edge methodological approaches and techniques (including using well-annotated genome assemblies and in-depth, single-cell-transcriptome data), there may be gaps in our results, for which future studies are required to resolve the presence or absence of stem cell gene expression.

2. RNA-Binding Proteins (RBPs)

One main commonality that characterizes the pluripotent ASCs of aquatic invertebrates is the abundance of expressed RBPs. They are represented by several families of proteins situated in the cytoplasm and/or the nucleus, and account for the major differences between transcribed mRNA and protein levels eventually synthesized by the cells. RBPs have a role in every aspect of mRNA post-transcriptional regulation (mRNAs biogenesis, stability, function, transport, structure and interactions with other RNAs and proteins). Specific RBPs and mRNA combinations in stem cells (ribonucleoprotein, RNPs) lead to mRNA alternative splicing, 3' UTR cleavage and polyadenylation, mRNA sequence alteration at control regions, and altogether impact the specific function or stability of mRNAs in stems cells (Shigunov and Dallagiovanna 2015). Germ stem cells and early differentiating cells of a germline lineage contain condensed protein–mRNA complexes called nuage/pole plasm/germ plasm/germ granule/chromatoid bodies, similar in content to complexes found in some pluri/multipotent somatic cells (Juliano et al. 2010). The RBP families listed below are all part of the nuage-like structures and function in post-transcriptional regulation and in curtailing the activity of transposable elements (TEs) in order to assure genome integrity. These proteins are believed to be part of the "germline multi-potency program" (GMP), but are also expressed in adult somatic stem cells.

2.1. Argonaute (Ago)/Piwi Family

The ago/piwi family is composed of three subfamilies, two of which—ago and piwi—have vital functions in all multicellular organisms. Ago/piwi proteins form

complexes with small noncoding RNAs. These complexes silence transposons and specific genes at various stages of RNA metabolism, perform chromatin modifications or inhibit mRNA translation. Piwi proteins associate with piwi-interacting RNAs (piRNAs) and are restricted to the germ lineage and pluripotent stem cells. Piwi proteins not only pair with piRNA, but also participate in piRNA biogenesis through the so-called "ping-pong" amplification that occurs in the nuage-like regions (Czech et al. 2018). The ago proteins are involved in the formation of the RNA-induced silencing complex (RISC), which specifically targets mRNA or DNA sequences in the genome and silences them. They bind to micro RNAs (miRNAs) and silence complementary transcripts by either destroying them or preventing their correct translation (Höck and Meister 2008; Meister 2013).

Expression of *ago* genes in ASCs has been reported in choanocytes, pinacocytes and type 1 vacuolar cells of the homoscleromorph sponge *Oscarella lobularis* (Fierro-Constaín et al. 2017). *ago* genes are also present in placozoan and cnidarian genomes (Grimson et al. 2008), although their expression was not studied in detail. An *ago2* gene is transcribed by neoblasts of the planarian *Dugesia japonica* (Rouhana et al. 2010), as well as by some somatic cells. Another ago protein is present in neoblasts of the fluke *Schistosoma mansoni* (Collins et al. 2013). No data on the presence of ago proteins in ASCs of coelomate metazoans are present in the literature.

As far as piwi proteins are concerned, they are expressed in ASCs of almost all the metazoans characterized by high regenerative power. Two *piwi* genes have been described in sponges, active in choanocytes and archeocytes of the demosponge *Ephydatia fluviatilis* (Funayama et al. 2010; Alié et al. 2015) and in choanocytes, pinacocytes and mesohyl type 1 vacuolar cells of the homoschleromorph sponge *O. lobularis* (Fierro-Constaín et al. 2017). No *piwi* genes are present in Placozoans (Grimson et al. 2008), whereas a *piwi* gene is present in the comb jelly *Pleurobrachia pileus*, expressed in progenitors of colloblasts, muscle cell, cells of the forming combs and of the aboral sense organ (Alié et al. 2011). In Cnidaria, two *piwi* orthologues (*hywy* and *hyli*) are actively transcribed by i-cells and epidermal cells of *Hydra vulgaris* and *Hydra magnipapillata*: in the former species, their mRNAs are also located in nematoblasts, the precursors of stinging cells (Juliano et al. 2014). A single *piwi* gene is transcribed in the i-cells of the hydroid *Hydractinia echinata* (Plickert et al. 2012) and the siphonophoran *Nanomia bijuga* (Siebert et al. 2015), in transdifferentiating epitheliomuscular cells of *Podocoryne carnea* medusae (Seipel et al. 2004), and in nematoblasts of the *Clytia hemisferica* medusae (Denker et al. 2008). Among bilateria, *piwi* expression has been demonstrated in the neoblasts of the acoelomorph worm *Isodiametra pulchra* (Egger et al. 2009) and of the planarians *Macrostomum lignano* (two piwi proteins; Pfister et al. 2007; Zhou et al. 2015), *Schmidtea mediterranea* (three active piwi genes; Reddien et al. 2005a, 2005b; Palakodeti et al. 2008; Rouhana et al. 2014) and *D. japonica* (six piwi genes; Rossi et al. 2006, 2007; Rouhana et al. 2010;

Shibata et al. 2016). Two piwi proteins are located in cells of the posterior growth zone and of the regeneration blastema of the polychaete annelids *Platynereis dumerilii* (Rebscher et al. 2007; Gazave et al. 2013; Planques et al. 2019), *Alitta virens* (Kozin and Kostyuchenko 2015) and *Capitella teleta* (Giani et al. 2011), whereas a *piwi* gene is transcribed by proliferating, undifferentiated cells of the growth zone and fission zone of the oligochaete worm *Pristina leidyi* undergoing asexual reproduction by paratomy (Özpolat and Bely 2015). Two expressed *piwi* genes have also been reported in the gastropod mollusk *Lymnaea stagnalis* and the bivalve *Crassostrea gigas*; in addition to the reproductive tract, their mRNAs are located in cells of the gills and lung, musculature, brain and labial palps (Jehn et al. 2018). In the sea slug *Aplysia californica*, a piwi protein is present in the central nervous system (CNS), where it is involved in the epigenetic control of memory-related synaptic plasticity (Rajasethupathy et al. 2012). Among nonchordate deuterostomes, *piwi* expression has been demonstrated in a series of somatic tissues, including coelomocytes, esophagus and tube feet epithelium, the epithelium of the spines, and the musculature of the sea urchins *Lytechinus variegatus*, *Strongylocentrotus purpuratus* and *Mesocentrotus franciscanus* (Reinardy et al. 2015; Bodnar and Coffman 2016). Anti-piwi-positive cells were also observed in the coelomic cells, the coelomic epithelium and the connective tissue of the sea cucumber *Eupentacta fraudatrix* (Dolmatov et al. 2021): these cells reach a maximum number within 4 h after evisceration and contribute to the regeneration of the intestine (Dolmatov 2021). In addition, *piwi* transcripts were also found in the adult nerve cord of the holothurian *Holothuria glaberrima* (Mashanov et al. 2015a). As for invertebrate chordates, data are limited to ascidians. In the solitary species *Ciona intestinalis*, two *piwi* orthologues are actively transcribed in cells inside the vessels of the branchial basket, where the lymph nodes, representing hematopoietic organs, are located. They are also expressed in the endostyle, gut epithelium, cells of the basal stalk, cell clusters of the siphon walls, and cells of the atrial epithelium. They assure the growth and the continuous turnover of cells of the body (Jeffery 2014). In colonial botryllid ascidians, one *piwi* gene is present in *Botryllus schlosseri*, *Botryllus primigenus*, *Botrylloides leachii* and *Botrylloides violaceus*. The protein is located in phagocytes near the endostyle, in tunic cells and in cells of the stomach of zooids of *B. schlosseri* along the ontogeny (Rosner et al. 2009; Rinkevich et al. 2010). The gene is also expressed by the epithelial monolayers developed from extirpated palleal buds and isolated floating buds in vitro (Rabinowitz and Rinkevich 2011). In *B. schlosseri* and *B. leachii*, the *piwi* gene is also transcribed by activated dormant cells lining the vasculature epithelium during whole-body regeneration (Rinkevich et al. 2010). At the onset of hibernation, in *B. leachii*, Hyams et al. (2017) observed high expression levels of *piwi* within the cell islands, the stem cell niches at both sides of the endostyle; in the advanced hibernation state, *piwi* was expressed in the multinucleated cells, the probable reservoir cells for the generation of new zooids at the end of the torpor. In *B.*

primigenus, *piwi* is expressed in coelomic cells (Kawamura and Sunanaga 2011). In *B. violaceus*, *piwi*-positive cells are found in the endostyle and hemocytes of adult zooids and in hemocytes and a few cells of the peribranchial epithelium of the developing bud (Brown et al. 2009).

2.2. Tudor Domain-Containing Proteins

The tudor proteins allow for the reading of protein methylations and include methylarginine- and methyllysine-binding proteins. Piwi proteins contain symmetrically dimethylated arginine (sDMA) in their N-termini and form tudor–piwi interactions that are required for the proper function of the piwi-piRNA pathway. Tudor proteins also participate in the proper assembly of the nuage and control of gametogenesis (Pek et al. 2012).

tudor genes, which are part of the piwi machinery, show high expression in archeocytes of the sponge *E. fluviatilis* (Alié et al. 2015). *oltudor1* is expressed in choanocytes, pinacocytes and type 1 vacuolar cells of the sponge *O. lobularis* (Fierro-Constaín et al. 2017). *tdrd9* of the cnidarian *H. magnipapillata* is associated with both *piwi* orthologues, *hywi* and *hyli*, at nuage perinuclear granules of i-cells, and contribute to piRNA biogenesis (Lim et al. 2014); *tdrd5* of *H. vulgaris* is also expressed in i-cells (Alié et al. 2015). In the flatworm *Schmidtea polychroa*, the Tudor domain-containing protein, Spoltud-1, was identified as a chromatoid body component of neoblasts, essential for proliferation and differentiation; it is also expressed in cells of the CNS (Solana et al. 2009); *smtdrd5* of the planarian *S. mediterranea* is expressed in neoblasts (Alié et al. 2015). In the annelid *Platynereis dumerilii* there are three *tudor* genes expressed in a very similar way in cells situated in the segment addition zone and in germ cells (Gazave et al. 2013).

2.3. DEAD and "DEAH-Box"-Containing Helicases

The "DEAD-box" helicases form a family of proteins present in all eukaryotic cells, and are characterized by the existence of a domain of 400 amino acids that can be further divided into 12 characteristic motifs, one of which—the Asp-Glu-Ala-Asp (DEAD) motif—confers the name to the family (Cordin et al. 2006; Rosner and Rinkevich 2007). The conserved domain serves as a binding site for ATP and RNA to facilitate helicase activities. The motifs participate in various interactions, endowing the proteins with multifunctionality in many aspects of RNA metabolism, from transcription to decay. Despite the high conservation between "DEAD-box" proteins, they participate in different processes, some of which having very specific roles. "DEAD-box" proteins often function within large multiprotein complexes such as the exon junction complex, and are involved in processes such as the export of mRNA and translation initiation (Gilman et al. 2017; Perčulija and Ouyang 2019). This is a large family of proteins and an individual genome may contain dozens of members

of genes encoding for proteins of this family. *vasa* (*ddx4*) and *pl10* (*ddx3*) are among the most prominent and well-studied members of this family.

The vasa proteins were considered for many years as specific markers of the germline lineage; vasa proteins are among the most important components of the nuage localized at the nuclear envelope, transporting piRNA transcripts to the cytoplasmic piRNA machinery. Additionally, they function as translational initiating factors involved in the translation of several stem cell-specific mRNAs (Poon et al. 2006; Liu et al. 2009; Xiol et al. 2014).

Pl10 (ddx3) has been extensively studied in many organisms and has been shown to participate in transcription and translation regulation, mRNA maturation and mRNA export. Additionally, it may be associated with stress responses and stress granules, innate immune response, and regulation of apoptosis (Chang and Liu 2010). *ddx3* is the closest paralogue of *vasa*, both of them being highly expressed in germ cells and indispensable for their integrity. However, vasa is more restricted to germ lineages and pluripotent stem cells.

Ddx6, also known as me31b (in *Drosophila*) and dhh1 (in yeast), is one of the GMP proteins found in P-bodies and in stress granules that function in both gene translation inhibition and deadenylation-dependent mRNA degradation, by forming complexes with other proteins (Chang and Liu 2010).

In the sponge *E. fluviatilis*, all the three "DEAD box" genes, *efvasa*, *efpl10*, and *efddx6,* are continuously expressed in archeocytes (Alié et al. 2015). In the calcarean sponge *Sycon ciliatum,* there are two *vasa* and two *pl10* orthologues. In mature animals, only one *vasa* (*scivasab*) and one *pl10* (*scipl10b*) genes are expressed in choanocytes (Leininger et al. 2014). In the homoscleromorph sponge *O. lobularis*, *olvasa* and *olpl10* are both expressed in choanocytes, pinacocytes and mesohyl type 1 vacuolar cells (Fierro-Constaín et al. 2017). The hydrozoan *H. magnipapillata* has two *vasa* orthologues (*cnvas1* and *cnvas2*) that are actively transcribed in i-cells and epidermal cells (Mochizuki et al. 2001). The *pl10* of this animal (*cnpl10*) is expressed in i-cells and epidermal cells as well as in nematoblasts (Mochizuki et al. 2001). In the medusa of the hydrozoan *P. carnea*, *vasa* in transcribed in nematoblasts of the tentacle bulbs and manubrium (Plickert et al. 2012). In the siphonophoran *N. bijuga*, *vasa* and *pl10* are expressed in the i-cells of both the epidermis and the gastrodermis (Siebert et al. 2015). In the ctenophoran *P. pileus*, one *vasa* and one *pl10* gene have been reported, and they are transcribed in progenitors of colloblasts and muscle cells, cells of the forming combs and cells of the aboral sense organ (Alié et al. 2011). In the flatworm *M. lignano*, the *vasa* homologue, *macvasa*, is expressed in neoblasts: *macvasa* knockdown does not affect ASCs population but dramatically reduces the quantity of piRNAs, suggesting that *macvasa* functions in piRNA biogenesis (Pfister et al. 2008). In the planarian *D. japonica*, *djvas1* is transcribed in neoblasts and is required for regeneration and differentiation, but not for neoblast maintenance. *D. japonica djvlga*

is expressed in neoblasts and the CNS, while *djvlgb* is detected in a limited fraction of neoblasts (Shibata et al. 1999; Rouhana et al. 2010; Wagner et al. 2012). In the same species, the *djcbc1* gene, the orthologue of *ddx6*, similarly to *djvlga*, is expressed in neoblasts and cells of the CNS (Rouhana et al. 2010; Juliano et al. 2014) and is abundant in chromatoid bodies, different from those in which the *piwi* orthologue *djpiwic* is detected (Kashima et al. 2016). In the planarian *S. mediterranea*, smedvasa1 and smedvasa2 proteins are upregulated in neoblasts; *smedvasa1* is essential for proliferation and for promoting differentiation (Shibata et al. 1999; Wagner et al. 2012). The *vasa* orthologues of *D. japonica* (phylum Platyhelminthes) are involved in regeneration but not in cell proliferation (Shibata et al. 1999; Rossi et al. 2007; Rouhana et al. 2010; Wagner et al. 2012). In the polychaete worms *P. dumerilii* and *A. virens*, *vasa* and *pl10* are actively transcribed in the proliferating, undifferentiated cells of the growth (via posterior elongation) zone in metamorphosing larvae and in growing adults, as well as in the blastema of regenerating animals (Rebscher et al. 2007; Gazave et al. 2013; Planques et al. 2019). A similar location was reported for the *vasa* mRNA of *C. teleta* (Dill and Seaver 2008). In the oligochaete worm *P. leidy*, in addition to the posterior growth zone of metamorphosing larvae and adults, *vasa* is expressed in the fission zone of animals undergoing asexual reproduction by fragmentation (Özpolat and Bely 2015). Analogously, in the oligochaete annelid *Enchytraeus japonensis*, *ejvlg2* mRNA is located in undifferentiated cells of mesodermal origin in the posterior surface of the septa during asexual reproduction by autotomy (Sugio et al. 2012).

Arthropods are animals with low regenerative power and no asexual reproduction. Usually in this taxon, genes of the nuage proteins are expressed only in the germ line. However, in the crab *Eriocheir sinensis*, *esddx6* has low expression in some somatic tissues, such as heart, stomach, muscle, hemocytes, and cells of the thorax and intestine (Li et al. 2015). Moreover, in the rhizocephalan cirripede *Polyascus polygenea*, *ppvlg* and *ppdrh1* are expressed in cells of the stolons and buds of *interna* that will give rise to both the germline and the soma (Shukalyuk et al. 2007).

As for deuterostomes, one *vasa* gene was reported in the echinoids *L. variegatus*, *S. purpuratus* and *M. franciscanus*—it is expressed in muscles, epithelium of the gut, tube feet and spines, CNS and coelomocytes (Bodnar and Coffman 2016), and its protein product is located in the epithelium of tube feet, spines and esophagus, as well as in neurons and a fraction of coelomocytes (Reinardy et al. 2015).

In the colonial ascidian *B. schlosseri*, *bsvasa* is expressed in germ cells (Rosner and Rinkevich 2011) and two additional cell populations: (i) cells, resembling the PGCs, that aggregate exterior to the developing gonads or in hemolymphatic vessels; (ii) phagocytes in the cell island adjacent to the endostyle. Anti-vasa antibodies also stain the epithelium of the stomach and the intestine. Bspl10 protein is present in

PGCs, many germ-cell types and somatic cells of the epithelium of the digestive tract. In addition, *pl10* is upregulated in differentiating bud and budlet tissues, and in a fraction of hemocytes, and has low expression in mature tissue. Knockdown of *pl10*, in addition to reducing the number of germ cells, causes malformation in the developing buds and the alteration of the morphology of adult zooid tissues; however, high expression of *pl10* can be detected even in malformed tissues (Rosner et al. 2006, 2009). In the same species, *bsddx1* is expressed in hemocytes of the cell islands along ontogeny and astogeny (Rosner et al. 2013). In the ascidian *B. violaceus*, 3–6% of circulating hemocytes and some tunic cells closely associated with the vasculature stain positively with the *vasa* antisense probe (Brown and Swalla 2007).

2.4. Nanos Family Proteins

Nanos is a zinc finger protein with two C2HC zinc finger motifs and represents an additional component of the nuage-like structures. Nanos acts as a translational repressor of specific mRNAs by forming a complex with pum2 proteins. The complex associates with the 3′-UTR of mRNA targets and inhibits their translation (De Keuckelaere et al. 2018). Nanos is mainly a regulator of the germ lineage in embryos and adults. In adult tissues, *nanos* is mainly expressed in the spermatogonia and other early differentiating germ cells, and in some invertebrates (sponges, cnidarians, flatworms, annelids, echinoderms, lancelet and ascidians) it is expressed in ASCs.

The *nanos* mRNA in *O. lobularis* (phylum Porifera) is located in choanocytes, pinacocytes and mesohyl type 1 vacuolar cells (Fierro-Constaín et al. 2017). The cnidarian *H. magnipapillata* contains two *nanos* genes: *cnnos1*, expressed in the multipotent i-cells and cells of the germline, and *cnnos2*, expressed in the gastrodermal cells and a subset of germ cells (Mochizuki et al. 2000). In the hydroid *H. echinata*, in addition to the i-cells, the *nanos-2* gene is actively transcribed in nematoblasts and maturing nematocytes (Kanska and Frank 2013). Two *nanos* genes, *nanos1* and *nanos2*, are transcribed in the i-cells located in the siphonosomal buds and young zooids of the siphonophoran *N. bijuga* (Siebert et al. 2015). In the planarian *D. japonica*, *djnos* is expressed in a subset of germline-committed neoblasts, and in the early differentiating oogonia and spermatogonia (Sato et al. 2006). Furthermore, in the planarian *S. mediterranea*, *smednos* is expressed in eye precursor cells during regeneration (Handberg-Thorsager and Saló 2007), whereas *smnanos2* mRNA of the fluke *S. mansoni* is located in neoblasts (Collins et al. 2013). In the annelid *P. dumerilii*, *pdunos* is expressed in the posterior zone of segmental growth and cells of the CNS, and is upregulated during regeneration (Rebscher et al. 2007; Gazave et al. 2013; Planques et al. 2019). A similar location was reported for the *capinanos* mRNA of the worm *C. teleta* (Dill and Seaver 2008). The *bpnos* of the colonial tunicate *Botryllus primigenus* is strongly expressed in immature and mature male germ cells, and to a lesser extent, in the multipotent epithelia of the buds and in a fraction of blood cells. Knockdown of *bpnos* strongly interferes with

male germ-cell differentiation, but does not affect the formation of female germ cells (Sunanaga et al. 2008).

2.5. PUF Family Proteins

The PUF family proteins associate with nanos and regulate the translation of specific genes by binding to a specific Pumilio Response Element situated at their 3′ UTR (De Keuckelaere et al. 2018).

In the sponge *O. lobularis*, *pumilio* is expressed in choanocytes, pinacocytes and mesohyl type 1 vacuolar cells (Fierro-Constaín et al. 2017). The *djpum* orthologue of the flatworm *D. japonica* is predominantly expressed in neoblasts, and RNAi-mediated gene silencing of it causes loss of nematoblasts and reduced regeneration (Salvetti et al. 2005; Rouhana et al. 2010). Similar expression was reported for *smedpumilio* of the planarian *S. mediterranea* (Solana et al. 2009). *pumilio*, and its related genes *pufa* and *pufb*, are expressed both in soma (posterior growth zone) and germ stem cells in *P. dumerilii* (phylum Annelida); however, the *pumilio* genes are not upregulated during posterior elongation, and they are also expressed in the gut of untreated animals (Gazave et al. 2013).

2.6. Mago-Nashi (or Mago)

Originally identified in *Drosophila*, Mago-nashi has emerged as essential for germ plasm assembly. It is characterized by a specific domain localized at the 5′ end of the molecule, and it is an integral part of a protein complex that forms the exon junction complex (Kataoka et al. 2001). In *Drosophila*, Mago-nashi acts during germ stem cell differentiation and is required for the polarization of the oocyte and the formation of perpendicular axes (Micklem et al. 1997; Parma et al. 2007).

The *mago-nashi* orthologue of the demosponge *E. fluviatilis*, *efmago-nashi*, is transcribed to a higher extent in the totipotent archeocytes than in other cells, however, its functions in these cells has not yet been defined (Alié et al. 2015). In the freshwater sponge *Lubomirskia baicalensis*, a *mago-nashi* orthologue is expressed at the top of the branches that characterize the deep-water morphs (Wiens et al. 2006).

3. RNA Recognition Motif (RRM) Containing Proteins

RRM is a 90 amino acid domain consisting of three aromatic side chains located between two conserved motives: RNP1 (octamer) and RNP2 (hexamer). RRMs usually binds a variable number of nucleotides, ranging from two to eight, within a single-strand RNA (ssRNA), but it can interact with single-strand DNA (ssDNA) as well. The number of RRMs varies among different subfamilies of proteins. For example, Bruno has three RRMs and Bruli has two domains. Both function in pre-mRNA alternative splicing, mRNA translation and stability (Maris et al. 2005).

3.1. Mbnl, Bruli and Bruno

Alternative splicing occurs with the involvement of highly regulated RBPs that bind pre-mRNA at specific sequences and regions and modulate the inclusion or exclusion of exons. In stem cells and their descendants, this regulation is an interplay between two kinds of RBPs with opposing functions in controlling splicing: Mbml (muscleblind-like splicing regulator), Bruli and Bruno. The former is a CCCH zinc finger protein that represses gene isoforms active in stem cells and is upregulated in differentiating cells, while Bruli and Bruno assist in the expression of gene isoforms that are active and upregulated in stem cells.

The sponge *O. lobularis* contains two copies of *bruno* genes: *bruno* and *brunob*. Both are continuously expressed in choanocytes, pinacocytes and mesohyl type 1 vacuolar cells. Similar expression was reported for the *boule* gene (Fierro-Constaín et al. 2017), acting as regulator of the translation of specific mRNAs and required for meiotic entry and germline differentiation at the transition between G2 and M phases of meiosis I (Shah et al. 2010). The sponge *E. fluviatilis* also shows high expressions of *bruno* in archeocytes and low expression in other cells, while *mbnl* has exactly the opposite expression: low in archeocytes and high in other somatic cells (Alié et al. 2015). In the ctenophoran *P. pileus*, *ppibruno* is expressed in progenitors of various somatic cell lineages (Alié et al. 2011). In the planarian *S. mediterranea*, neoblasts contain *smedbruli* mRNA; depletion of *bruli* results in neoblast loss and lack of regeneration. *smedbruli* is also expressed in cells of the CNS; loss of *mbnl* function results in slower regeneration (Guo et al. 2006; Solana et al. 2016). In the same species, *smedmbnl1* and three *smedmbnl-like* genes are present: *smedmbnl1* mRNA is present in differentiated cells of the body parenchyma, whereas *smedmbnl-like1* and *smedmbnl-like2* are transcribed in differentiated cells of the epidermis and gut tissues; no data on *smedmbnl-like3* expression are present in the literature (Solana et al. 2016). *djbruli* of the planarian *D. japonica* is actively transcribed in neoblasts and cells of the CNS (Rouhana et al. 2010). *pdubruno* of *P. dumerilii* is expressed in proliferating, undifferentiated cells of the of the posterior growth zone (Gazave et al. 2013).

3.2. Musashi

The Musashi proteins contain two RRMs and are expressed in stem and in neural lineage cells, including neural stem cells. Musashi proteins are involved in stem cell self-renewal. They function through binding of the 3'UTR of target mRNAs that prevent their translation, and by inhibiting 80 S ribosome assembly (Park et al. 2014).

In the sponge *E. fluviatilis*, *eflmsia*, the *musashi* orthologue is transcribed in archeocytes and the protein product is localized in their nucleus. Based on *eflmsia* expression at M-phase, archeocytes can be divided into a group undergoing self-renewal and expressing high quantities of the *eflmsia*, and another one expressing 30–60% of the quantity of mRNA of the previous group and protein and committed to

differentiation (Okamoto et al. 2012). In the starlet sea anemone *Nematostella vectensis*, *nvmsi* is expressed in precursor cells of the CNS (Marlow et al. 2009). In the flatworm *D. japonica*, *musashi-like genes -a, -b, -c* are expressed in differentiated neural cells, and therefore are not markers of stem cells, whereas *djdmlg* (DAZAP-like/musashi-like gene) is expressed in stem cells and additional types of soma cells (Higuchi et al. 2008). In the polychaete worm *P. dumerilii*, the *musashi* gene is upregulated in the posterior zone of segment formation and in cells of the nervous system, but it is not transcribed in germ cells nor upregulated during regeneration (Gazave et al. 2013). In the holothurian *H. glaberrima*, *msi1/2* mRNA is located in the outermost part of the adult radial nerve cord (Mashanov et al. 2015a). In the colonial ascidian *B. schlosseri*, *dazap1* is transcribed in buds and in differentiating tissues of both, germ line and soma (Gasparini et al. 2011). Two additional genes were described in ascidians, *hrmsi* from *Halocynthia roretzi* and *cimsi* from *C. intestinalis*; however, their expressions were only tested in embryos (Kawashima et al. 2000).

4. Signal Transduction Factors

4.1. Wnt

Wnts represent a family of secreted, lipid-modified signaling glycoproteins that are 350–400 amino acids in length. The lipid modification of Wnts is required to bind its carrier protein Wntless (WLS) and to be transported to the plasma membrane for secretion and binding to the receptor Frizzled. Three Wnt signaling pathways have been characterized: (i) the canonical Wnt pathway leading to regulation of gene transcription by nuclear localization of β-Catenin; (ii) the noncanonical planar cell polarity pathway that regulates the cytoskeleton and thereby modulates cell shape and migration; (iii) the noncanonical Wnt/calcium pathway that regulates intracellular calcium. All three pathways are activated by the binding of an Wnt-protein ligand to a Frizzled family receptor, which passes the biological signal to the Dishevelled protein inside the cell (Zhan et al. 2017). In the hydrozoan *H. magnipapillata*, *wnt* mRNA is located in the epidermis and gastrodermis of the hypostome, the oral end of the buds and the apical tip of regenerating animals (Hobmayer et al. 2000; Lengfeld et al. 2009). In embryos of the anthozoan *N. vectensis*, it is present around the blastopore and later in the oral end of growing polyps (Kusserow et al. 2005). Both the oral hypostome of hydrozoan polyps and the blastoporal region in anthozoan embryos represent the main inductive organizers for patterning the entire cnidarian oral–aboral body axis. Moreover, the canonical Wnt/β-Catenin pathway represents a core element of these inductive signaling centers. There is also accumulating evidence that Wnt/β-Catenin signaling is involved in self renewal in cnidarian and vertebrate ASCs. In fact, recent data show that global activation of Wnt/β-Catenin signaling along the major body axis in *H. vulgaris* enhances self-renewal in i-cells, strongly activates neurogenesis

and inhibits the differentiation of nematocytes (stinging cells) (Khalturin et al. 2007; Hartl et al. 2019). Enhanced i-cell maintenance in *Hydra* is most likely mediated by β-Catenin regulating the activity of myc transcription factors (see below; see also the accompanying chapter in this book by Lechable et al. 2022). In the related colonial hydrozoan *H. echinata*, *wnt3a* is expressed in i-cells of the epidermis and gastrodermis, as well as in nematoblasts along the body column of the polyps (Müller et al. 2007). Furthermore, action of Wnt/β-Catenin signaling in global patterning and ASC decision making is reported in several bilaterians, particularly in flatworms, cephalochordates and tunicates. *wntA*, *wnt4*, *wnt6*, *wnt16*, *frizzled1/2/7*, *frizzled4* and *frizzled5/8* are overexpressed in somatic tissues of the holothurian *E. fraudatrix* during the regeneration of the internal organs after the induction of evisceration (Girich et al. 2017). A *wnt* orthologue is transcribed in cells of the tail-regenerating blastema of the lancelet *Branchiostoma lanceolatum* (Somorjai 2017), whereas two *wnt* orthologues, *wnt2b* and *wnt5a*, have been identified in the ascidian *B. schlosseri*: they are actively transcribed in all the tissues of the early buds (Di Maio et al. 2015). In the regular blastogenetic cycle of *B. schlosseri*, Wnt is an important signal transduction pathway, and the administration of both Wnt agonist and antagonist imposed significant alterations in the prosecution of the cycle and bud development (Rosner et al. 2014). In whole-body regeneration of the colonial ascidian *B. leachii*, differential gene expression analysis of the transcriptome revealed upregulation of genes involved in developmental signaling pathways including *wnt* (Blanchoud et al. 2018). In the congeneric species *Botrylloides diegensis*, *frizzled5/8*, *β-catenin* and *disheveled* increase their transcription during whole-body regeneration (Kassmer et al. 2020).

4.2. TGF-β/BMP

The transforming growth factor-beta (TGF-β)/bone morphogenetic protein (BMP) signaling pathway plays a fundamental role ni regulating cell development and growth through the activation of receptor serine/threonine kinases (Guo and Wang 2009). Smad (small mother against decapentaplegic) is the main signal transducer for receptors of the transforming growth factor beta (TGF-β) superfamily, which are critically important in cell proliferation and differentiation (Blobe et al. 2000).

Choanocytes of the calcarean sponge *S. ciliatum* weakly express two *smad* orthologues: *smad1/5* and *smad4* (Leininger et al. 2014). The same cell type in the demosponge *Chondrosia reniformis* expresses *tgf6* mRNA (Pozzolini et al. 2019). The gene orthologue *smed-smad-6/7* is expressed by neoblasts of *S. mediterranea* (Van Wolfswinkel et al. 2014), whereas *bssmad1/5/8* is expressed by a fraction of circulating phagocytes of the colonial ascidian *B. schlosseri* (Rosner et al. 2013). In the cephalochordate *B. lanceolatum*, *chordin* and *bmp2/4* increase their transcription in the regenerating tail (Somorjai et al. 2012; Somorjai 2017; Liang et al. 2019; Ferrario et al.

2020). TGF-β, together with Wnt/β-catenin and MAPK/ERK, emerged as important signal transduction pathways in organizing the colony of the ascidian *B. schlosseri* (Rosner et al. 2014).

5. Other Transcription Factors

Many transcription factors have been described as being expressed in ASCs of aquatic invertebrates, and most of them derive from studies in acoel flatworms and planarians, some from studies in sponges, cnidarians, cephalochordates and tunicates. *scibra1*, *scibra2*, *scigata*, *smad1/5* and *smad4* are expressed by choanocytes of the calcarean sponge *S. ciliatum* (Leininger et al. 2014). Among cnidarians, *hymyc1* and *hymyc2* are transcribed in proliferating i-cells and other proliferating somatic cells and gamete precursors of *H. vulgaris* (Hartl et al. 2010, 2014; Hobmayer et al. 2012). Interstitial (i-) cells of *H. vulgaris* also express *foxo* (Boehm et al. 2012). Functional interference suggests that the action of *myc* genes and *foxo* is directed to *Hydra* i-cell maintenance (Boehm et al. 2012; Hartl et al. 2019). In the stolon of the colonial hydroid *H. echinata*, i-cells express the POU protein Pln (Millane et al. 2011). In the acoel flatworm *I. pulchra*, *ipptx*, *ipsix1/2*, *ipfox a1*, *ipfox a2*, *ipfox c*, *ipgata4/5/6*, *iptwist1*, *iptwist2* are transcribed in the neoblasts and the musculature (Chiodin et al. 2013). In the planarian *S. mediterranea*, *smedprox-1*, *smedpbx1*, *smednkx2.2*, *smedsoxb1*, *smedsoxP1*, *smedsoxP3*, *smedgata4/5/6*, *smedtcf15*, *smedjunl1*, *smedzfmym1*, *smedzf2071*, *smedfhl1*, *smedzfp1*, *smedprog1*, *smedprog2* and *smedegr1* are transcribed by neoblasts, whereas *smedpax3/7* is expressed in differentiating sensory neurons (Wagner et al. 2012). The annelid *P. dumerilii* expresses *pdumyc*, *pduhes2*, *pduhes4*, *pduhes5*, *pduhes6*, *pduhes8*, *pduid*, *pduap2*, *pdugcm*, *pducdx*, *pduevx*, *pduhox3* in cells of its posterior growth zone and in the regenerating blastema (Gazave et al. 2013). In the sea cucumber *H. glaberrima*, *myc* is transcribed by progenitor cells in the adult radial nerve cord and by scattered cells of the neural parenchyma. The same cells also host the transcripts of a series of stem cell-associated genes, such as *soxb1*, *foxj1*, *hes, klf1/2/4* and *oct1/2/11*. Most of the cells are located in the outer layer of the adult radial nerve cord and correspond to the radial glial cells that also express a series of proneural genes (Mashanov et al. 2015a, 2015b). In the holothurian *E. fraudatrix*, *efsox9/10* and *efsox17* are actively transcribed by cells of the epithelia of the coelom and of the gut anlage during the regeneration of the internal organs consequent to evisceration (Dolmatov et al. 2021). A *pax3/7* orthologue is transcribed in the tail-regenerating blastema of *B. lanceolatum* (Somorjai et al. 2012; Somorjai 2017). In colonial ascidians, *myc* is expressed by cells of the peribranchial epithelium and fibroblast-like cells involved in organogenesis of the developing buds of *P. misakiensis* (Fujiwara et al. 2011), and by cells of the peribranchial epithelium of growing palleal and vascular buds as well as by some circulating hemocytes of *B. primigenus* (Kawamura et al. 2008). In the colonial species *B. schlosseri*, *bspitx* is transcribed by cells of the peribranchial

epithelium of buds at early developmental stages, the inner wall of the oral siphon and the oral tentacles, the forming cerebral ganglion and the developing gut of young zooids (Tiozzo et al. 2005; Tiozzo and de Tomaso 2009); Bsoct4 protein is present in the epithelial cells of the branchial sac along ontogeny and astogeny (Rosner et al. 2009). In the same species, *bspou*3 is expressed by few cells of the proximal side of the bud atrial epithelium (Ricci et al. 2016). In *B. schlosseri*, *bsgata4/5/6* is transcribed by cells of the posterior side of the new bud (Ricci et al. 2016).

6. Chromatin Modification/Cell Cycle/Differentiation

6.1. Proliferation Markers

Proliferating Cell Nuclear Antigen (PCNA) is a protein found in the nucleus and serves as an auxiliary protein of DNA polymerase delta. High expression levels of this molecule correlate with high rates of division.

In the demosponges *Hymeniacidon perleve* and *E. fluviatilis*, *pcna* is actively transcribed in archeocytes (Sun et al. 2007; Alié et al. 2015). mRNAs for HvPCNA of *H. vulgaris*, and DjPCNA of the planarian *D. japonica* are located in the i-cells and neoblasts, respectively (Orii et al. 2005; Alié et al. 2015). In addition, *smedpcna* of *S. mediterranea* is actively transcribed in neoblasts (Eisenhoffer et al. 2008; Onal et al. 2012; Alié et al. 2015). *pcna* of the annelid *P. dumerilii* is actively transcribed in the posterior growth zone (Gazave et al. 2013; Planques et al. 2019). In the enteropneust hemichordate *P. flava*, *pcna* is expressed in cells of the regeneration blastema (Rychel and Swalla 2008). In early regenerating fragments of the tunicate *B. leachii*, *pcna* predominantly stains piwi-positive cells attached to the vascular epithelium, directly involved in the formation of new buds, whereas later on, it also labels piwi-positive cells within the lumen of the colonial vasculature (Rinkevich et al. 2010). In the colonial ascidian *B. leachii*, at the onset of hibernation, leading to the resorption of the colonial zooids, a high expression level of *pcna* was recorded within the cell islands, the stem cell niches on both sides of the endostyle, whereas in a deep hibernation state, *pcna* was expressed in the multinucleated cells, probably a reservoir of cell types for fast regeneration of zooids from the circulation during arousal from torpor (Hyams et al. 2017). In the colonial ascidian *P. misakiensis*, *pcna* is transcribed in cells of the epithelia of the developing buds (Kawamura et al. 2012), whereas in the tunicate *B. violaceus*, *pcna* is expressed by clusters of hemocytes during whole-body regeneration (Brown et al. 2009).

Mini chromosome Maintenance Complex Component 2 (MCM2) forms a complex with additional proteins that function in the initiation of eukaryotic genome replication. The gene is transcribed by archeocytes of the demosponge *E. fluviatilis* (Alié et al. 2015), i-cells of the cnidarian *H. vulgaris* (Alié et al. 2015), and neoblasts of

the planarians *D. japonica* and *S. mediterranea* (Salvetti et al. 2000; Rossi et al. 2007; Onal et al. 2012).

Cyclin B1 (CCNB1) is necessary for proper control of the G2/M transition phase of the cell cycle. The protein encoded by this gene, together with phospho-histone H3, is considered a good marker for cell proliferation. Its mRNA is present in archeocytes of the sponge *E. fluviatilis* (Alié et al. 2015), i-cells of the hydrozoan *H. vulgaris* (Alié et al. 2015) and neoblasts of the flatworm *S. mediterranea* (Reddien et al. 2005b; Eisenhoffer et al. 2008); in addition, two cyclin genes are expressed in proliferating, undifferentiated cells of the growth zone and regeneration blastema of the annelid *P. dumerilii* (Planques et al. 2019). A cyclin gene is over-transcribed during whole body regeneration in the colonial ascidian *B. diegensis* (Kassmer et al. 2020).

6.2. Genes for Chromatin Modifications

Many genes involved in the epigenetic modification of chromatin are actively transcribed in ASCs of aquatic invertebrates. Three transcriptional silencers are expressed by the planarian *S. mediterranea* neoblasts (Eisenhoffer et al. 2008; Resch et al. 2012; Trost et al. 2018); the same cells express various genes involved in methylation/demethylation and acetylation/deacetylation as well as histone modifications in the flatworms *S. mediterranea* (Reddien et al. 2005a, 2005b; Eisenhoffer et al. 2008; Onal et al. 2012; Cao et al. 2019) and *D. japonica* (Rossi et al. 2007; Bonuccelli et al. 2010; Cao et al. 2019).

6.3. Telomere Protection

Telomeres are guanine-rich DNA repeats ((TTAGGG)n) located at the termini of chromosomes, that stabilize and protect chromosome ends through a protein complex called shelterin, which also serves to recruit telomerase to the telomeres. In most mature cells, telomeres progressively shorten through each cell division and trigger DNA damage responses that, eventually, mediate cell cycle arrest or apoptosis. Homeostasis can be achieved via telomere lengthening by a telomerase, a process that occurs in germline and somatic stem cells. Telomerase is a cellular reverse transcriptase that synthesizes telomeric DNA directly onto chromosome ends. Although the gene encoding the telomerase has been detected in many metazoans, equivalent telomerase activity has not been demonstrated in many organisms. Other molecules associated with telomerase activity are Pot1 and RTEL1. Protection of telomeres 1 (Pot1), a component of shelterin, contributes to the suppression of unnecessary DNA damage response at the telomeres and their maintenance. RTEL1 is an ATP-dependent DNA helicase implicated in telomere-length regulation, DNA repair and the maintenance of genomic stability. It also regulates meiotic recombination and crossover homeostasis (Udroiu et al. 2017).

The archeocytes of the demosponge *H. perleve*, when cultured in vitro, show the presence of mRNAs for telomerase and telomerase reverse transcriptase (Sun et al. 2007). Similarly, the archeocytes of the sponge *E. fluviatilis* transcribe *efrtel1*, and the i-cells of the solitary cnidarian *H. vulgaris* contain mRNA for HvRTEL1 (Alié et al. 2015). The neoblasts of the flatworm *S. mediterranea* express *smrtel1* and the telomerase reverse transcriptase orthologue *smedtert*. Four different alternative splice isoforms are encoded by the latter gene, only one of which is coding for a full active enzyme. Following fission or regeneration, there is an increased expression of the genes for all four *smedtert* isoforms in ASCs of the asexual individuals, with an increase in the relative proportion of the full-length isoform. The combined result of about an eight-fold increase in telomerase mRNA may contribute to indefinite somatic telomerase activity in proliferating stem cells during regeneration or reproduction by fission (Tan et al. 2012). In the same species, *smedob1*, a *pot1* orthologue, is ubiquitously expressed, however its knockdown impairs homeostasis and regeneration (Yin et al. 2016). The same phenomenon occurs in the planarian *D. japonica* (Yin et al. 2016). A telomerase gene is also actively transcribed by undifferentiated cells of the posterior surface of the septa, and during asexual reproduction by autotomy in the oligochaete annelid *E. japonensis* (Sugio et al. 2012). *tert* is also transcribed in muscles, the esophagus, CNS and coelomocytes of the sea urchins *L. variegatus*, *S. purpuratus* and *M. franciscanus* (Bodnar and Coffman 2016). The multipotent bud epithelia of the colonial ascidian *B. schlosseri* express *bspot1* for telomere protection and an orthologous of telomerase (Laird and Weissman 2004; Ricci et al. 2016).

7. Discussion and Conclusions

ASC evolution is associated with the origin of multicellular animals. Beyond the obvious role in tissue homeostasis, ASCs in aquatic invertebrates often are key participants in sustaining important biological processes for indeterminate growth, regeneration, asexual reproduction (agametic cloning), torpor phenomena and more (Sköld et al. 2009; Vogt 2012; Rinkevich et al. 2022). The technologies for ASC isolation and growth under in vitro conditions (Odintsova 2009; Rinkevich 2011; Zahiri and Zahiri 2016), for the study of their expression repertoires, for proteomic metabolomics and bioinformatic approaches to the biology of ASC, are advancing rapidly (Ballarin et al. 2018). The application of the above techniques will offer the possibility to obtain, in the near future, unprecedented insights into the biology of ASCs from aquatic invertebrates.

A wide range of disparate characteristics have been found when comparing vertebrate and aquatic invertebrate ASCs, including features such as morphology, differentiation states and somatic/germ lineage characteristics. As these and numerous other important traits in aquatic invertebrates differ significantly from those recorded in the vertebrates' ASCs (Isaeva et al. 2009; Rinkevich et al. 2022), it is of great

importance to find common shared characteristics, many of which are associated with stemness gene expressions. Stem cells in various marine taxa, including members of Porifera, Cnidaria, Ctenophora, Annelida, Acoela, Platyhelminthes, Echinodermata, Cephalochordata and Tunicata, express stemness genes and other key genes. As they exist throughout the lifespan of these organisms and are not rare (may contribute >25–30% of the total animals' cells), with high potency (pluripotency and totipotency), a comparative analysis of their gene expression may add relevant information to our understanding of their nature (e.g., Lai and Aboobaker 2018; Alié et al. 2015; Fierro-Constaín et al. 2017). Indeed, in order to clarify the biological phenomena evolved, there is a need to understand the nature, the biology and gene expressions of ASCs from aquatic invertebrates (Rinkevich et al. 2022). Such an analysis is of further importance since ASCs from aquatic invertebrates participate in biological phenomena not found in the vertebrates, such as whole-body regeneration, asexual budding and dormancy (Vogt 2012; Rinkevich et al. 2022).

The data collected in this report are summarized in Table 1; a detailed version can be found in Appendix A. From these data, we propose a few general conclusions. First, it is difficult to identify clear ASC gene-expression signatures across and even within phyla. This is in agreement with initial in-depth single-cell-transcriptome data sets established in cnidarians and flatworms, where it was not possible to characterize core programs of ASC-specific gene activation within the studied species (Fincher et al. 2018; Plass et al. 2018; Siebert et al. 2019). Based on this, it is difficult to define conserved molecular mechanisms for maintenance of long-term ASC stocks and to define conserved elements for communication between ASCs and their short- and long-range environment (Martinez et al. 2022). Much larger data sets at this level of resolution in many more species studied in the future may improve this dilemma.

Second, our analysis shows clear coexpression of somatic- and germ line-specific genes in ASCs across and within phyla, confirming earlier reports (Juliano et al. 2010; Alié et al. 2015; Fierro-Constaín et al. 2017). This indicates that an ancestral conserved multi- or toti-potency program may coordinate ASC dynamics during tissue homeostasis, (indefinite) asexual growth and sexual reproduction. Third, ASC transcriptional regulation, when compared to vertebrate ASC programs, is limited when we consider the activation of genes encoding for somatic transcription factors, but rich if referring to RNA regulatory genes known from pan-metazoan embryonic stem cells (Rinkevich et al. 2022).

Table 1. Main stem cell genes expressed by ASCs of aquatic invertebrates.

Protein Family	Porifera	Ctenophora	Cnidaria	Acoelomorpha	Platyhelminthes	Annelida	Mollusca	Arthropoda Rhizocephala	Echinodermata	Hemichordata	Cephalochordata	Tunicata
RNA-binding proteins												
ago/piwi family	✓	✓	✓	✓	✓	✓	✓		✓			✓
tudor domain-containing proteins	✓		✓		✓	✓						
DEAD and "DEAD-box"-containing helicases	✓	✓	✓	✓	✓	✓		✓	✓			✓
nanos family proteins	✓		✓		✓	✓						✓
PUF family proteins	✓				✓	✓						✓
mago-nashi	✓											
RRM-containing proteins												
mbnl, bruli and *bruno*	✓	✓			✓	✓						
musashi	✓		✓		✓	✓						✓
Signal transduction factors												
wnt			✓								✓	✓
tgf-β/bmp	✓				✓						✓	✓
Chromatin modification/cell cycle/differentiation												
pcna	✓	✓			✓	✓				✓		✓
mcm2	✓				✓							
cyclin b1	✓	✓			✓	✓						✓

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Appendix A

On the basis of the codified proteins, genes were grouped as follows: RNA-binding proteins, RNA recognition motif (RRM)-containing proteins, signal transduction proteins, transcription factors, chromatin modification proteins, proteins involved in autophagy, cell-cycle proteins, control of differentiation proteins, niche interaction proteins and genes for miRNAs. Each category of genes (titles in boxes with green background) can contain various subgroups (titles in boxes with blue background). Within each subgroup, genes are listed according to the phylogenetic position of the organisms.

Table A1. Genes expressed in invertebrate ASCs and progenitor cells during potency state changes.

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
RNA-binding proteins						
Argonaute family silencing genes						
Ephydatia fluviatilis (Porifera, Demospongiae)	*efpiwia* *efpiwib*	choanocytes archeocytes	choanocyte chambers mesohyl	throughout ontogeny	ISH, scRNAsec	Funayama et al. (2010); Alié et al. (2015)
Oscarella lobularis (Porifera, Homoscleromorpha)	*olpiwia*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny during wound healing during wound healing	ISH	Fierro-Constaín et al. (2017)
	olpiwib *olago*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny	ISH	Fierro-Constaín et al. (2017)
Podocoryne carnea (Cnidaria, Hydrozoa)	*cniwi*	epitheliomuscular cells	epiderm	throughout ontogeny	ISH	Seipel et al. (2004); Plickert et al. (2012)
Hydra vulgaris (Cnidaria, Hydrozoa)	*hywi* *hyli*	i-cells nematoblasts epidermal cells gastrodermal cells	body column	throughout ontogeny	ISH, IB	Juliano et al. (2014)
Hydra magnipapillata (Cnidaria, Hydrozoa)	*hywi* *hyli*	i-cells epidermal cells	body column	throughout ontogeny	ISH, IB	Juliano et al. (2014)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Nanomia bijuga (Cnidaria, Hydrozoa, Siphonophora)	*piwi*	i-cells (epiderm and gastroderm)	siphonosomal horn buds young zooids	throughout ontogeny	ISH	Siebert et al. (2015)
Clytia hemisphaerica (Cnidaria, Hydrozoa)	*piwi*	nematoblasts	tentacle bulb	throughout ontogeny	ISH	Denker et al. (2008); Plickert et al. (2012)
Hydractinia echinata (Cnidaria, Hydrozoa)	*piwi*	i-cells	stolon and polyp	throughout ontogeny	ISH	Rebscher et al. (2008)
Pleurobrachia pileus (Ctenophora)	*ppipiwi1*	progenitors of colloblasts muscle cell progenitors cells of the forming combs cells of the aboral sense organ	tentacle roots tentacle roots comb rows aboral sense organ	throughout ontogeny	ISH	Alié et al. (2011)
Isodiametra pulchra (Acoelomorpha)	*ipiwi*	neoblasts	whole animals	throughout ontogeny	ISH	Egger et al. (2009)
Macrostomum lignano (Platyhelminthes, Rhabditophora)	*macpiwi1*	neoblasts	whole animals	throughout ontogeny	ISH	Pfister et al. (2007)
	macpiwi2	neoblasts γ-radiation resistant cells	whole animals regeneration blastema	throughout ontogeny during regeneration	ISH	Pfister et al. (2007); Zhou et al. (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedwi1*	neoblasts	whole animals	throughout ontogeny	ISH, FACS	Reddien et al. (2005a, 2005b)
	smedwi2	neoblasts	whole animals	throughout ontogeny	ISH, FACS,	Reddien et al. (2005a, 2005b); Palakodeti et al. (2008)
	smedwi3	neoblasts	whole animals	throughout ontogeny	ISH, FACS	Palakodeti et al. (2008)
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djpiwi1*	neoblasts of the dorsal parenchyma	whole animals	throughout ontogeny	ISH, RNAseq	Rossi et al. (2006, 2007)
	djpiwi2 *djpiwi3*	neoblasts	whole animals	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007)
	djpiwi-a *(djpiwi4)*	neoblasts and CNS (cytoplasm)	whole animals	throughout ontogeny	ISH	Rouhana et al. (2010); Shibata et al. (2016)
	djpiwi-b	neoblasts and their descendants (nucleus)	whole animals	throughout ontogeny	ICC, ISH	Shibata et al. (2016)
	djpiwi-c	neoblasts (cytoplasm)	whole animals	throughout ontogeny	ICC, ISH	Shibata et al. (2016)
	djago2	neoblasts, brain, intestine	whole animals	throughout ontogeny	ISH	Rouhana et al. (2010)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Schistosoma mansoni (Platyhelminthes, Neodermata, Trematoda)	*smago2-1*	neoblasts	whole animals	throughout ontogeny	ISH, iRNA	Collins et al. (2013)
Platynereis dumerilii (Annelida, Polychaeta)	*piwia* *piwib*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration metamorphosing larva	ICC, ISH	Rebscher et al. (2007); Gazave et al. (2013); Planques et al. (2019)
Alitta virens (Annelida, Polychaeta)	*piwil1*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Kozin and Kostyuchenko (2015)
	avipiwil2	proliferating, undifferentiated cells of the blastema	blastema	regeneration	ISH	Kozin and Kostyuchenko (2015)
Capitella teleta (Annelida, Polychaeta)	*piwi1* *piwi2*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Giani et al. (2011)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Pristina leidyi (Annelida, Oligochaeta)	*piwi1*	proliferating, undifferentiated cells of the growth zone and fission zone blastema	posterior growth zone fission zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) asexual reproduction regeneration metamorphosing larva	ISH	Özpolat and Bely (2015)
Lymnaea stagnalis (Mollusca, Gastropoda)	*piwil1* *piwil2*	not reported	muscle lung brain	throughout ontogeny	qPCR	Jehn et al. (2018)
Aplysia californica (Mollusca. Gastropoda)	*piwi*	not reported	central nervous system, heart	throughout ontogeny	NB, ICC	Rajasethupathy et al. (2012)
Crassostrea gigas (Mollusca, Bivalvia)	*piwil1* *piwil2*	not reported	labial palps gills adductor muscle mantle	throughout ontogeny	qPCR	Jehn et al. (2018)
Lytechinus variegatus (Echinodermata, Echinoidea)	*piwi*	muscle, epithelia of the esophagus, spines, tube feet, spines, coelomocytes, radial nerve	tube feet spines coelomocytes central nervous system gut	throughout ontogeny	ICC, qRT PCR	Reinardy et al. (2015); Bodnar and Coffman (2016)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Strongylocentrotus purpuratus (Echinodermata, Echinoidea)	*piwi*	muscles, epithelia of the esophagus, spines, tube feet, spines, coelomocytes radial nerve	tube feet spines coelomocytes central nervous system gut	throughout ontogeny	qRT PCR	Bodnar and Coffman (2016)
Mesocentrotus franciscanus (Echinodermata, Echinoidea)	*piwi*	muscle, epithelia of the esophagus, spines, tube feet, spines, radial nerve	tube feet spines central nervous system gut	throughout ontogeny	qRT PCR	Bodnar and Coffman (2016)
Eupentacta fraudatrix (Echinodermata, Holothoroidea)	*piwi*	coelomic cells, coelomic epithelium, connective tissue	coelomic cells, coelomic epithelium, connective tissue	throughout ontogeny	IHC, ISH	Dolmatov et al. (2021)
Holothuria glaberrima (Echinodermata, Holothoroidea)	*piwi*	nerve cord	neuroepithelium of the radial nerve cord cells of the neural parenchyma	adults	ISH	Mashanov et al. (2015a)
Botryllus schlosseri (Chordata, Tunicata)	*piwi*	phagocytes near the endostyle (Ab) tunic cells (Ab) stomach cells (Ab)	hemolymph, tunic	throughout ontogeny and astogeny	ISH, IHC; iRNA	Rosner et al. (2009)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Botrylloides leachii (Chordata, Tunicata)	*piwi*	activated dormant cells lining the vasculature epithelium cell islands	colonial vasculature	during WBR	IHC; iRNA	Rinkevich et al. (2010)
Botrylloides violaceus (Chordata, Tunicata)	*piwi*	endostyle, hemocytes hemocytes, few epithelial cells	filtering zooids regenerating buds	throughout ontogeny buds from stage 3–6	IHC with commercial Abs	Brown et al. (2009)
Botrylloides diegensis (Chordata, Tunicata)	*piwi2*	hemoblasts	colonial vasculature	throughout ontogeny	ISH	Kassmer et al. (2020)
Ciona intestinalis (Chordata, Tunicata)	*piwi-like (1,2)*	stem cells	gut epithelium vessels of the branchial sac basal stalk cell clusters in the siphon walls lymph nodes in pharynx, endostyle, atrial epithelium	continuous turnover of cells forming body growth zone	IHC with commercial Abs	Jeffery (2014)
Styela plicata (Chordata, Tunicata)	*piwi*	hemoblasts	intestine submucosa	adults	IHC with commercial Abs	Jiménez-Merino et al. (2019)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
DEAD and "DEAH-box"-containing helicases						
Ephydatia fluviatilis (Porifera, Demospongiae)	*efvasa* *efpl10* *ef-ddx6*	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Sycon ciliatum (Porifera, Calcarea)	*scivasab* *scipl10b*	choanocytes	choanocyte chambers	throughout ontogeny	ISH	Leininger et al. (2014)
Oscarella lobularis (Porifera, Homoscleromorpha)	*olvasa* *olpl10*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny during wound healing throughout ontogeny	ISH	Fierro-Constaín et al. (2017)
Hydra magnipapillata (Cnidaria, Hydrozoa)	*cnvas1* *cnvas2*	i-cells (+) epidermal cells	whole animals body column	throughout ontogeny	ISH	Mochizuki et al. (2001)
	cnpl10	i-cells nematoblasts epidermal cells	whole animals whole animals body column	throughout ontogeny	ISH	Mochizuki et al. (2001)
Hydractinia echinata (Cnidaria, Hydrozoa)	*vasa*	i-cells	epidermis of stolon and polyp	throughout ontogeny	ISH	Rebscher et al. (2008); Plickert et al. (2012)
Podocoryne carnea (Cnidaria, Hydrozoa)	*vasa*	nematoblasts	tentacle bulbs and manubrium	medusa	ISH	Plickert et al. (2012)
Nanomia bijuga (Cnidaria, Hydrozoa, Siphonophora)	*vasa1* *pl10*	i-cells (epiderm and gastroderm)	siphonosomal horn buds young zooids	throughout ontogeny	ISH	Siebert et al. (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Pleurobrachia pileus (Ctenophora)	*vasa* *pl10*	progenitors of colloblasts muscle cell progenitors cells of the forming combs cells of the aboral sense organ	tentacle roots tentacle roots comb rows aboral sense organ	whole, adult animals	ISH	Alié et al. (2011)
Macrostomum lignano (Platyhelminthes, Rhabditophora)	*macvasa*	neoblasts (Ab + ISH))	body parenchyma	whole animals	ICC, ISH	Pfister et al. (2008)
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djvas1*	neoblasts	body parenchyma	whole animals	ISH	Rouhana et al. (2010); Wagner et al. (2012)
	djvlga	neoblasts and CNS	body parenchyma	regenerating animals	ISH, iRNA	Shibata et al. (1999); Rouhana et al. (2010); Wagner et al. (2012)
	djvlgb	neoblasts	body parenchyma	whole animals	ISH, RNAseq	Shibata et al. (1999); Rossi et al. (2007)
	djtud1	neoblasts	body parenchyma	ontogeny	ISH	Rouhana et al. (2010)
	djdhx-8c	neoblasts and CNS	body parenchyma and brain	whole animals	ISH	Rouhana et al. (2010)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	djcbc1 (ddx6)	neoblasts and CNS	body parenchyma and brain	throughout ontogeny	ISH	Rouhana et al. (2010); Juliano et al. (2014)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedvasa1*	neoblasts	body parenchyma	adult animals	ICC, ISH, iRNA	Pfister et al. (2008); Wagner et al. (2012)
	smedvasa2	neoblasts	body parenchyma	adult animals	ISH, iRNA, NB	Shibata et al. (1999); Wagner et al. (2012)
	smedtud1a *smedtud1b*	neoblasts	body parenchyma	throughout ontogeny	ISH	Solana et al. (2009)
	smedtdrd1l2	neoblasts	body parenchyma	throughout ontogeny	ISH, scqPCR, cell transplantation	Van Wolfswinkel et al. (2014)
Platynereis dumerilii (Annelida, Polychaeta)	*vasa* *pl10*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ICC, ISH	Rebscher et al. (2007); Gazave et al. (2013); Planques et al. (2019)
Alitta virens (Annelida, Polychaeta)	*vasa* *pl10*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Kozin and Kostyuchenko (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Capitella teleta (Annelida, Polychaeta)	*vasa*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration metamorphosing larva	ISH	Dill and Seaver (2008)
Pristina leidyi (Annelida, Oligochaeta)	*vasa*	proliferating, undifferentiated cells of the growth zone and fission zone blastema	posterior growth zone fission zone	metamorphosing larva throughout ontogeny (posterior elongation) asexual reproduction regeneration metamorphosing larva	ISH	Özpolat and Bely (2015)
Enchytraeus japonensis (Anellida, Oligochaeta)	*ejvlg2*	neoblasts and N-cells (only for mesoderm)	posterior surface of septa (N-cells dorsal to neoblasts)	during asexual reproduction by autotomy throughout	ISH	Sugio et al. (2012)
Polyascus polygenea (Cirripedia, Rhizocephala)	*ppvlg*	stem cells	stolons and buds of the asexual organism	asexual reproduction	ISH	Shukalyuk et al. (2007)
	ppdrh1	stem cells	stolons and buds of *interna*	*interna* asexual reproduction	ISH	Shukalyuk et al. (2007)
Eriocheris sinensis (Crustacea, Decapoda)	*esddx6*	cells of various tissues	heart, stomach muscle, hemocytes, thorax, intestine	throughout ontogeny	IHC with commercial Abs	Li et al. (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Lytechinus variegatus (Echinodermata, Echinoidea)	*vasa*	muscle, epithelia of the esophagus, spines, tube feet, spines, coelomocytes, radial nerve	tube feet spines coelomocytes central nervous system gut	throughout ontogeny	ICC, qPCR	Reinardy et al. (2015); Bodnar and Coffman (2016)
Strongylocentrotus purpuratus (Echinodermata, Echinoidea)	*vasa*	muscles, epithelia of the esophagus, spines, tube feet, spines, coelomocytes radial nerve	tube feet spines coelomocytes central nervous system gut	throughout ontogeny	qRT PCR	Bodnar and Coffman (2016)
Mesocentrotus franciscanus (Echinodermata, Echinoidea)	*vasa*	muscle, epithelia of the esophagus, spines, tube feet, spines, radial nerve	tube feet spines central nervous system gut	throughout ontogeny	qRT PCR	Bodnar and Coffman (2016)
Botryllus schlosseri (Chordata, Tunicata)	*bsvasa*	epithelial cells phagocytes in cell islands (Ab) stomach cells (Ab)	bud tissues hemolymph tissues of filtering zooids	throughout astogeny throughout ontology and astogeny	ISH, IHC	Rosner et al. (2009)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	bspl10	epithelial cells some blood cells, phagocytes in cell islands (Ab) stomach cells (Ab)	bud tissues hemolymph tissues of filtering zooids	throughout astogeny throughout ontology and astogeny	ISH, IHC, ICC	Rosner et al. (2006, 2009)
	bsddx1	cell islands	hemolymph	throughout ontogeny and astogeny	IHC, ISH	Rosner et al. (2013)
Botrylloides violaceus (Chordata, Tunicata)	*bvvasa*	hemocytes in vasculature (3–6%) cells outside the vessels in proximity of the tunic	hemolymph	throughout ontogeny and astogeny	ISH	Brown and Swalla (2007)
Botrylloides diegensis (Chordata, Tunicata)	*vasa*	hemoblasts	colonial vasculature	throughout ontogeny	ISH	Kassmer et al. (2020)
			tudor domain-containing proteins			
Ephydatia fluviatilis (Porifera, Demospongiae)	*eftudor9* *eftdrkh* *eftdrd1* *eftdrd5*	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Oscarella lobularis (Porifera, Homoscleromorpha)	*oltudor1*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny	ISH	Fierro-Constaín et al. (2017)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Hydra magnipapillata (Cnidaria, Hydrozoa)	*tdrd9*	i-cells	body column	throughout ontogeny	ICC	Lim et al. (2014)
Hydra vulgaris (Cnidaria, Hydrozoa)	*tdrd5*	i-cells	body column	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smtdrd5*	neoblasts	whole animals	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Schmidtea polychroa (Platyhelminthes, Rhabditophora)	*spoltud-1*	neoblasts central nervous system	whole animals	throughout ontogeny	ISH	Solana et al. (2009)
Platynereis dumerilii (Annelida, Polychaeta)	*tdrd1* *tdrd2* *tdrd3*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
			PUF family proteins			
Oscarella lobularis (Porifera, Homoscleromorpha)	*pumilio*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny throughout ontogeny throughout ontogeny	ISH	Fierro-Constaín et al. (2017)
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djpum*	neoblasts	whole animals	throughout ontogeny	ISH	Salvetti et al. (2005) Rouhana et al. (2010)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smed-pumilio*	neoblasts	whole animals	throughout ontogeny	ISH	Solana et al. (2009)
Platynereis dumerilii (Annelida, Polychaeta)	*pumilio* *pufa* *pufb*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
			nanos family proteins			
Oscarella lobularis (Porifera, Homoscleromorpha)	*nanos*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny throughout ontogeny throughout ontogeny	ISH	Fierro-Constaín et al. (2017)
Hydra magnipapillata (Cnidaria, Hydrozoa)	*cnnos1*	i-cells	whole animals	throughout ontogeny	ISH	Mochizuki et al. (2000)
	cnnos2	endodermal epithelial cells	hypostome	throughout ontogeny	ISH, ICC	Mochizuki et al. (2000); Kanska and Frank (2013)
Hydractinia echinata (Cnidaria, Hydrozoa)	*nanos2*	nematoblasts, maturing nematocytes i-cells	whole animals	throughout ontogeny	ISH, ICC	Kanska and Frank (2013)
Nanomia bijuga (Cnidaria, Hydrozoa, Siphonophora)	*nanos1* *nanos2*	i-cells (epiderm and gastroderm)	siphonosomal horn buds young zooids	throughout ontogeny	ISH	Siebert et al. (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djnos*	neoblasts	asexual and sexual individuals mesenchyme	throughout ontogeny	ISH	Sato et al. (2006)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smednos*	eye precursor cells	body parenchyma	during regeneration	ISH	Handberg-Thorsager and Saló (2007)
Schistosoma mansoni (Platyhelminthes, Neodermata, Trematoda)	*smnanos2*	neoblasts	body parenchyma	throughout ontogeny	ISH, iRNA	Collins et al. (2013)
Platynereis dumerilii (Annelida, Polychaeta)	*pdunos*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ICC, ISH	Rebscher et al. (2007); Gazave et al. (2013); Planques et al. (2019)
Capitella teleta (Annelida, Polychaeta)	*capinanos*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration metamorphosing larva	ISH	Dill and Seaver (2008)
Botryllus primigenus (Chordata, Tunicata)	*bpnos*	weak staining of pharyngeal epithelia of the developing budlets	pharyngeal epithelia	blastogenesis (budlets stages 1–6)	ISH, IHC	Sunanaga et al. (2008)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Mago nashi family proteins			
Ephydatia fluviatilis (Porifera, Demospongiae)	*efmago-nashi*	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Lubomirskia baicalensis (Porifera, Demospongiae)	*mago-nashi*	?	top of the branches	throughout ontogeny	ISH	Wiens et al. (2006)
			RNA recognition motif (RRM)-containing proteins			
Ephydatia fluviatilis (Porifera, Demospongiae)	*bruno*	archeocytes	mesohyl	throughout ontogeny	ISH, ICC	Alié et al. (2015)
	msia	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Okamoto et al. (2012)
Chondrosia reniformis	*msi1*	archeocytes	mesohyl	throughout ontogeny	ICC	Pozzolini et al. (2019)
Oscarella lobularis (Porifera, Homoscleromorpha)	*boule* *bruno* *brunob*	choanocytes pinacocytes vacuolar cells type 1	choanocyte chambers pinacoderm mesohyl	throughout ontogeny during wound healing throughout ontogeny	ISH	Fierro-Constaín et al. (2017)
Nematostella vectensis (Cnidaria, Anthozoa)	*nvmsi*	neuronal progenitors	tentacle epidermis	young polyp	ISH	Marlow et al. (2009)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Pleurobrachia pileus (Ctenophora)	*ppibruno*	progenitors of colloblasts muscle cell progenitors cells of the forming combs cells of the aboral sense organ	tentacle roots tentacle roots comb rows aboral sense organ	whole animals	ISH	Alié et al. (2011)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedbruli*	neoblasts, CNS	body parenchyma and brain	throughout ontogeny	ISH	Guo et al. (2006); Solana et al. (2016)
	smedkhd1 *smedcip29*	neoblasts	body parenchyma	throughout ontogeny	ISH	Mochizuki et al. (2001)
	smedsmb	neoblasts	body parenchyma	throughout ontogeny	ISH, iRNA, FACS	Fernandéz-Taboada et al. (2010)
	smedmbnl1	neoblasts differentiated cells	body parenchyma	throughout ontogeny	ISH	Okamoto et al. (2012)
	smedmbnl-like1 *smedmbnl-like2* *smedmbnl-like3*	epidermis gut tissues	whole animals	throughout ontogeny	ISH	Solana et al. (2016)
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djtial1* *djtial2* *djtial3* *djtial4* *djtial5*	neoblasts	body parenchyma	throughout ontogeny	ISH	Rouhana et al. (2010)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	djbruli *djpabpc2* *djedc4* *djcnot6* *djgemin5* *djdicer1* *djlsm14* *djsgm7* *djrbm18* *djfmrp1*	neoblasts and CNS	body parenchyma and brain	throughout ontogeny	ISH	Rouhana et al. (2010)
	djxrn1 *djg3bp* *djcnot7* *djdcp1l* *djupf1*	neoblasts, brain, intestine	whole animals	throughout ontogeny	ISH	Rouhana et al. (2010)
	djdmlg	neoblasts neural precursors and additional cell types	body parenchyma	throughout ontogeny	ISH, FACS, scPCR	Higuchi et al. (2008)
Platynereis dumerilii (Annelida, Polychaeta)	*pdubruno* *pdusmb*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	pdumusashi	proliferating, undifferentiated cells of the growth zone ventral nerve cord brain	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
Holothuria glaberrima (Echinodermata, Holothuroidea)	*msi1/2*	radial nerve cord	radial nerve cord	adult	ISH	Mashanov et al. (2015a, 2015b)
Botryllus schlosseri (Chordata, Tunicata)	*bsdazap1*	all tissues	buds	during blastogenesis	ISH	Gasparini et al. (2011)
Signal transduction pathways						
Helix–Loop–Helix-domain-containing proteins						
Isodiametra pulchra (Acoelomorpha)	*iptwist1* *iptwist2*	neoblasts musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)
Sycon ciliatum (Porifera, Calcarea)	*scibra1*	choanocytes	choanocyte chambers	throughout ontogeny	ISH	Leininger et al. (2014)
Hydra magnipapillata (Cnidaria, Hydrozoa)	*wnt*	epidermis and gastrodermis	hypostome apical end of buds apical end of regenerating animals	throughout ontogeny budding during regeneration	ISH	Lengfeld et al. (2009)
Hydractinia echinata (Cnidaria, Hydrozoa)	*wnt3*	i-cells, nematoblasts	epidermis and gastrodermis	polyp	ISH	Müller et al. (2007)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Nematostella vectensis (Cnidaria, Anthozoa)	*wnt*	epidermis and gastrodermis	apical end of the animal	throughout ontogeny	ISH	Kusserow et al. (2005)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedjunl1* *smedtcf15*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
Eupentacta fraudatrix (Echinodermata, Holothuroidea)	*wntA* *wnt4* *wnt6* *wnt16,*	somatic tissues	somatic tissues	during the regeneration of the internal organs	qRT PCR	Girich et al. (2017)
Eupentacta fraudatrix (Echinodermata, Holothuroidea)	*frizzled1/2/7,* *frizzled4* *frizzled5/8*	somatic tissues	somatic tissues	during the regeneration of the internal organs	qRT PCR	Girich et al. (2017)
Branchiostoma lanceolatum (Chordata, Cephalochordata)	*wnt5*	cells of the blastema	regenerating tail	throughout ontogeny, regeneration	ISH	Somorjai (2017)
Botryllus schlosseri (Chordata, Tunicata)	*wnt2b*	all the tissues	secondary buds	stages 1–3	ISH	Di Maio et al. (2015)
	wnt5a	mesenchymal cells	developing gonads	primary buds	ISH	Di Maio et al. (2015)
	wnt9a	all the tissues	secondary buds	emerging secondary buds	ISH	Di Maio et al. (2015)
Botrylloides diegensis (Chordata, Tunicata)	*frizzled5/8* *β-catenin* *dishevelled*	cycling hemoblasts	colonial vasculature	during WBR	ISH	Kassmer et al. (2020)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			TGF-β/BMP			
Sycon ciliatum (Porifera, Calcarea)	*smad1/5*	choanocytes (weak)	choanocyte chambers	throughout ontogeny	ISH	Leininger et al. (2014)
	smad4	choanocytes (weak) mesohyl cells (weak)	choanocyte chambers mesohyl	throughout ontogeny throughout ontogeny	ISH	Leininger et al. (2014)
Chondrosia reniformis (Porifera, Demospongiae)	*tgf6*	choanocytes	choanocyte chambers	during regeneration	ISH	Pozzolini et al. (2019)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedsmad6/7*	neoblasts	body parenchyma	throughout ontogeny	ISH, scqPCR, cell transplantation	Van Wolfswinkel et al. (2014)
Branchiostoma lanceolatum (Chordata, Cephalochordata)	*chordin*	cells of the regenerating notochord	regenerating tail	throughout ontogeny, regeneration	ISH	Somorjai et al. (2012); Di Maio et al. (2015); Somorjai (2017)
Branchiostoma japonicum (Chordata, Cephalochordata)	*bmp2/4*	cells around wound edge	regenerating tail	throughout ontogeny, regeneration	ISH, IHC, RNASeq	Ferrario et al. (2020); Liang et al. (2019); Kaneto and Wada (2011)
Botryllus schlosseri (Chordata, Tunicata)	*bssmad1/5/8*	phagocytes	hemocytes	throughout ontogeny	IHC, ISH	Rosner et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Notch			
Platynereis dumerilii (Annelida, Polychaeta)	*pdudelta* *pdunotch*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva	ISH	Gazave et al. (2017)
	pduhes4 *pduhes5* *pduhes6* *pduhes8*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2014)
Botrylloides diegensis (Chordata, Tunicata)	*notch1* *notch2* *hes1*	cycling hemoblasts	colonial vasculature	during WBR	ISH	Kassmer et al. (2020)
			Hedgehog			
Sycon ciliatum (Porifera, Calcarea)	*scigli*	choanocytes	choanocyte chambers	throughout ontogeny	IHC; iRNA	Rinkevich et al. (2010)
			Kinases			
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smednlk1* *smedfgfr1* *smedfgfr4*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
Schistosoma mansoni (Platyhelminthes, Neodermata, Trematoda)	*smfgfra*	neoblasts	body parenchyma	throughout ontogeny	ISH, iRNA	Collins et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Polyandrocarpa misakiensis (Chordata, Tunicata)	*pmrack1*	atrial epithelium undifferentiated mesenchymal cells associated with epidermis pharynx epithelium	developing buds whole zooids	during dedifferentiation throughout ontogeny	ISH, IHC	Tatzuke et al. (2012)
Pair rule and segment polarity genes						
Platynereis dumerilii (Annelida, Polychaeta)	*pduhunchback*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
	runt	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Gazave et al. (2013); Planques et al. (2019)
Branchiostoma japonicum (Chordata, Cephalochordata)	*runx*	distal cells of the regenerating oral cirrus	regenerating cirrus	throughout ontogeny, regeneration	ISH	Kaneto and Wada (2011)
Other transcription factors						
Homeobox-containing proteins						
Hydractinia echinata (Cnidaria, Hydrozoa)	*pln (pou protein)*	i-cells	stolons	throughout ontogeny	ISH	Millane et al. (2011)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Isodiametra pulchra (Acoelomorpha)	*ippitx* *ipsix1/2*	neoblasts musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedprox1*	neoblasts	body parenchyma	throughout ontogeny	ICC, ISH	Pfister et al. (2008)
	smedpbx1 *smednkx2.2*	neoblasts	body parenchyma	throughout ontogeny	ISH	Lim et al. (2014)
	smedpax3/7	differentiating sensory neurons			ISH	Lim et al. (2014)
Platynereis dumerilii (Annelida, Polychaeta)	*pducdx* *pduhox3*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
	pduevx	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Planques et al. (2019)
Branchiostoma lanceolatum (Chordata, Cephalochordata)	*pax3/7*	cells of the blastema and regenerating nerve cord	tail regenerate	throughout ontogeny, regeneration	ISH, IHC	Somorjai et al. (2012); Somorjai (2017)
	msx	cells of the blastema	regenerating tail	throughout ontogeny, regeneration	ISH	Somorjai et al. (2012); Somorjai (2017)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Botryllus schlosseri (Chordata, Tunicata)	*bspitx*	cells of the peribranchial epithelium inner wall of the oral siphon and tentacles; forming cerebral ganglion, developing gut	budlets (stage 1–3); left peribranchial chamber (stage 4–6) zooids at stage 8–9	during budding and blastogenesis	ISH, qPCR	Tiozzo et al. (2005); Tiozzo and de Tomaso (2009)
	bsoct4	epithelial cells (Ab)	branchial sac	throughout ontogeny and astogeny	ISH, IHC	Rosner et al. (2009)
	bspou3	few cells of the proximal side of the bud	atrial epithelium	bud at stage 3	ISH	Ricci et al. (2016)
Botrylloides diegensis (Chordata, Tunicata)	*pou3*	hemoblasts	colonial vasculature	throughout ontogeny	ISH	Kassmer et al. (2020)
			Sox family proteins			
Clytia hemisphaerica (Cnidaria, Hydrozoa)	*chesox1, chesox3, chesox10 chesox12*	i-cells	tentacle bulb	medusa	ISH	Jager et al. (2011)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedsoxb1*	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Onal et al. (2012)
	smedsoxp1	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	smedsoxp3	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
Holothuria glaberrima (Echinodfermata, Holothuroidea)	*soxb1*	progenitor cells in the radial nerve cord and by scattered cells of the neural parenchyma	radial nerve cord	adult	ISH	Mashanov et al. (2015a, 2015b)
E. fraudatrix (Echinodfermata, Holothuroidea)	*efsox9/10* *efsox17*	cells of the epithelia of the coelom and of the gut anlage	coelomic epithelium gut	during regeneration of internal organs after evisceratio	ISH	Dolmatov et al. (2021)
Branchiostoma lanceolatum (Chordata, Cephalochordata)	*soxb2*	cells of the regenerating nerve cord	tail regenerate	throughout ontogeny, regeneration	ISH	Somorjai et al. (2012); Somorjai (2017)
Branchiostoma japonicum (Chordata, Cephalochordata)	*soxe*	distal cells of the regenerating oral cirrus	cirrus regenerate	throughout ontogeny, regeneration	ISH	Kaneto and Wada (2011); Ferrario et al. (2020)
			Fox family proteins			
Hydra vulgaris (Cnidaria, Hydrozoa)	*foxo*	i-cells	polyp	polyp	ISH	Boehm et al. (2012)
Isodiametra pulchra (Acoelomorpha)	*ipfoxa1* *ipfoxa2* *ipfoxc*	neoblasts musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Holothuria glaberrima (Echinodermata, Holothuroidea)	*foxj1*	progenitor cells in the radial nerve cord and by scattered cells of the neural parenchyma	radial nerve cord	adult	ISH	Mashanov et al. (2015a, 2015b)
			Zinc finger proteins			
Sycon ciliatum (Porifera, Calcarea)	*scigata*	choanocytes	choanocyte chambers	throughout ontogeny	ISH	Leininger et al. (2014)
Hydra (Cnidaria, Hydrozoa)	*hymyc1*	proliferating i-cells nematoblast gland cells	whole animals	polyp	ISH	Hobmayer et al. (2012); Hartl et al. (2014)
	hymyc2	proliferating i-cells (+++) epidermal cells (+) gastrodermal cells (+)	whole animals	polyp	ISH	Hobmayer et al. (2012); Hartl et al. (2014)
Hydractinia echinata (Cnidaria, Hydrozoa)	*myc2*	i-cells	stolon	polyp	ISH	Plickert et al. (2012)
Isodiametra pulchra (Acoelomorpha)	*ipgata456*	neoblasts musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedgata4/5/6*	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Flores et al. (2016)
	smedzfmym1 *smedzf2071* *smedfhl1* *smedzfp1* *smedegr1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
Platynereis dumerilii (Annelida, Polychaeta)	*pdumyc*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
Holothuria glaberrima (Echinodermata, Holothuroidea)	*myc*	coelomic epithelium, intestinal cells, neuroepithelial and glial cells	regenerating intestine and radial nerve cord	during regeneration	ISH	Mashanov et al. (2015b)
Botryllus schlosseri (Chordata, Tunicata)	*bsgata4/5/6*	atrial epithelium of the bud	posterior side of the budlet	bud at stage 3	ISH	Ricci et al. (2016)
Botryllus primigenus (Chordata, Tunicata)	*myc*	cells of the branchial epithelia circulating hemocytes	growing palleal and vascular buds hemocoel of developing zooids	Strong signal during blastogenesis, in budlets (stages 1–6) and weak signal in the early primary buds.	ISH	Kawamura et al. (2008); Kawamura and Sunanaga (2011)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Polyandrocarpa misakiensis (Chordata, Tunicata)	*myc*	cells of the atrial epithelium and fibroblast-like cells involved in organogenesis	developing bud	more than one day before the dedifferentiation	ISH	Fujiwara et al. (2011)
Helix–Loop–Helix-domain-containing proteins						
Isodiametra pulchra (Acoelomorpha)	*iptwist1* *iptwist2*	neoblasts musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)
Initiation factors						
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djeif2a* *djeif3a* *djeif4e* *djeif4g* *djeif5a* *djeif4a3*	neoblasts	body parenchyma	throughout ontogeny	ISH	Rouhana et al. (2010)
T-box proteins						
Sycon ciliatum (Porifera, Calcarea)	*scibra2*	choanocytes	choanocyte chambers	throughout ontogeny	ISH	Leininger et al. (2014)
Other transcription factors						
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djekf2a*	neoblasts	body parenchyma	throughout ontogeny	ISH	Rouhana et al. (2010)
	djelk3 *djprohibitin2* *djctbp1*	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Platynereis dumerilii (Annelida, Polychaeta)	*pduid* *pdugcm*	proliferating, undifferentiated cells of the growth zone	posterior growth zone	metamorphosing larva throughout ontogeny (posterior elongation)	ISH	Gazave et al. (2013)
	pduap2	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Gazave et al. (2013); Planques et al. (2019)
Holothuria glaberrima (Echinodfermata, Holothuroidea)	*hes* *klf1/2/4* *oct1/2/11*	progenitor cells in the radial nerve cord and by scattered cells of the neural parenchyma	radial nerve cord	adult	ISH	Mashanov et al. (2015a, 2015b)
Chromatin modification/cell cycle/differentiation						
Transcriptional silencers						
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedbcl11a*	neoblasts	body parenchyma	throughout ontogeny	iRNA, FACS ISH	Resch et al. (2012); Trost et al. (2018)
	smedsirt	neoblasts	body parenchyma	throughout ontogeny	ISH	Ziman et al. (2020)
	smedcbx1	neoblasts	body parenchyma	throughout ontogeny	ISH, FACS	Eisenhoffer et al. (2008)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Proteins involved in methylation-demethylation						
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djhrjda djhrjda*	neoblasts differentiated cells	whole animals	throughout ontogeny	ISH	Cao et al. (2019)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedsedt8*	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
	smhrjda smhrjdb	neoblasts differentiated cells	whole animals	throughout ontogeny	ISH	Cao et al. (2019)
	smedsetd81	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012); Torre et al. (2017)
	smednsd1 smedmrg1 smedrbbp41	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
	smedbrg1l	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
Proteins involved in acetylation–deacetylation						
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djtaf1β*	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007)
	djbap48	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007); Bonuccelli et al. (2010)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedash2l smedprmt5*	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
	smedhdac1	neoblasts	body parenchyma	throughout ontogeny	ISH, FACS	Eisenhoffer et al. (2008)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Histones			
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djh2az* *djrbp4* *djcip-29* *djhp1*	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedxrn1* *smedsmarcc2* *smedssrp1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
Branchiostoma lanceolatum (Chordata, Cephalochordata)	*ph3*	Cells of the blastema and regenerating nerve cord and notochord (many pax3/7+)	Tail regenerate	Throughout ontogeny, regeneration	IHC	Somorjai et al. (2012)
Branchiostoma japonicum (Chordata, Cephalochordata)	*ph3*	Isolated cells in the regenerating oral cirrus	Oral cirrus regenerate	regeneration	IHC	Kaneto and Wada (2011)
Botryllus schlosseri (Chordata, Tunicata)	*ph3*	Budlet and primary buds Zooidal stomach	adults, buds	throughout ontogeny and astogeny	IHC with commercial antibodies	Rosner et al. (2014)
Botrylloides diegensis (Chordata, Tunicata)	*ph3*	hemoblasts	colonial vasculature	throughout ontogeny	ISH	Kassmer et al. (2020)
Styela plicata (Chordata, Tunicata)	*ph3*	hemoblasts	intestine submucosa	adults	putative ASC	Jiménez-Merino et al. (2019)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Polycomb group proteins			
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedezh*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
	smedezh2	neoblasts	body parenchyma	throughout ontogeny	ISH, IHC	Onal et al. (2012)
	smedsz12-1	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)
	smedeed-1	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012); Onal et al. (2012)
	smedbmi1 *smedrnf2* *smedsuz12*	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
			Control of transcription			
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedhcf1* *smedleo1* *smedctr9*	neoblasts	body parenchyma	throughout ontogeny	ISH	Onal et al. (2012)
	smedthoc4	neoblasts	body parenchyma	throughout ontogeny	ISH	Eisenhoffer et al. (2008); Bonuccelli et al. (2010)
	smedrrm2-1	neoblasts	body parenchyma	throughout ontogeny	ISH	Eisenhoffer et al. (2008)
	smedchd4	neoblasts	body parenchyma	throughout ontogeny	ISH, IHC	Scimone et al. (2010)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Proliferation markers			
Hymeniacidon perleve (Porifera, Demospongiae)	*pcna*	archeocytes	mesohyl	cultured cells	ICC with commercial Ab	Sun et al. (2007)
Ephydatia fluviatilis (Porifera, Demospongiae)	*pcna* *efmcm2* *efccnb1*	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Hydra vulgaris (Cnidaria, Hydrozoa)	*hvpcna* *hvmcm2* *hvccnb1*	i-cells	body column	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djmcm2*	neoblasts polymorphic large cells	whole animals macerates of tissues excised just below the wound	proliferating cells after X-ray irradiation proliferating cells of intact and regenerating planaria 30–60 min after wound infliction	ISH, RNAseq	Salvetti et al. (2000); Rossi et al. (2007)
	djpcna	neoblasts	body parenchyma	proliferating cells after X-ray irradiation	IHC	Orii et al. (2005)
	djkif3a *djkif3b* *djkif19b*	neoblasts and CNS	body parenchyma and brain	throughout ontogeny	ISH	Rouhana et al. (2010)
.5*Schmidtea mediterranea* (Platyhelminthes, Rhabditophora)	*smedrb*	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Onal et al. (2012)
	smedpcna *smedmcm2*	neoblasts	body parenchyma	throughout ontogeny	ISH, scRNAsec	Onal et al. (2012); Alié et al. (2015)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
	smedcyclinb	neoblasts	body parenchyma	throughout ontogeny	ISH	Eisenhoffer et al. (2008); Alié et al. (2015)
	smedp53	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Onal et al. (2012)
	smedpp32a *smedprohibitin-1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Eisenhoffer et al. (2008)
Macrostomum lignano (Platyhelminthes, Rhabditophora)	*duf2366/tim29*	neoblasts	body parenchyma		ISH, iRNA, RNAseq	Mouton et al. (2021)
Platynereis dumerilii (Annelida, Polychaeta)	*pdupcna*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Gazave et al. (2013); Planques et al. (2019)
	pducycb1 *pducycb3*	proliferating, undifferentiated cells of the growth zone and blastema	posterior growth zone blastema	metamorphosing larva throughout ontogeny (posterior elongation) regeneration	ISH	Planques et al. (2019)
Ptychodera flava (Hemichordata, Emteropneusta)	*pcna*	cells of blastemal (Ab)	regeneration blastema	throughout ontogeny	IHC with commercial Abs	Rychel and Swalla (2008)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Polyandrocarpa misakiensis (Chordata, Tunicata)	*pmpcna*	cells of the atrial epithelium cells associated with epithelia	developing buds	during dedifferentiation	IHC	Kawamura et al. (2012)
Botrylloides violaceus (Chordata, Tunicata)	*pcna*	hemocytes	regenerating buds, during WBR	buds from stage 4	IHC with commercial Abs	Brown et al. (2009)
Botrylloides diegenesis (Chordata, Tunicata)	*cyclin b*	hemoblasts	colonial vasculature	during WBR	ISH	Kassmer et al. (2020)
			Cytostactic proteins			
Polyandrocarpa misakiensis (Chordata, Tunicata)	*tc14-1 (lectin)*	atrial epithelial cells hemoblasts	bud	primordial bud stage growing buds	IHC	Kawamura et al. (1991)
	tc14-3 (lectin)	hemocytes, atrial epithelium	adults, buds	throughout ontogeny and astogeny in hemocytes, only in growing buds for epithelium	IHC	Matsumoto et al. (2001)
			Regulators of mitochondrial dynamics			
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djsam68*	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2006)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedarmc1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Telomere protection			
Hymeniacidon perleve (Porifera, Demospongiae)	*telomerase*	archeocytes	mesohyl	cultured cells	PCR	Sun et al. (2007)
	tert	archeocytes	mesohyl	cultured cells	activity	Sun et al. (2007)
Ephydatia fluviatilis (Porifera, Demospongiae)	*efrtel1*	archeocytes	mesohyl	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Hydra vulgaris (Cnidaria, Hydrozoa)	*hvrtel1*	i-cells	body column	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smrtel1*	neoblasts	body parenchyma	throughout ontogeny	ISH, scRNAsec	Alié et al. (2015)
	smedtert	neoblasts	body parenchyma	throughout ontogeny	ISH	Tan et al. (2012)
	smedob1	all cells	Whole body	throughout ontogeny	ISH	Yin et al. (2016)
Enchytraeus japonensis (Anellida, Oligochaeta)	*telomerase*	neoblasts and N-cells (only for mesoderm)	posterior surface of the septa	during asexual reproduction by autotomy	ISH	Sugio et al. (2012)
Litechinus variegatus (Echinodermata, Echinoidea)	*tert*	muscles, esophagus, CNS, coelomocytes	muscles, esophagus, CNS, coelomocytes	adult	qRT PCR	Bodnar and Coffman (2016)
Strongylocentrotus purpuratus (Echinodermata, Echinoidea)	*tert*	muscles, esophagus, CNS, coelomocytes	muscles, esophagus, CNS, coelomocytes	adult	qRT PCR	Bodnar and Coffman (2016)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Mesocentrotus franciscanus (Echinodermata, Echinoidea)	*tert*	muscles, esophagus, CNS, coelomocytes	muscles, esophagus, CNS, coelomocytes	adult	qRT PCR	Bodnar and Coffman (2016)
Botryllus schlosseri (Chordata, Tunicata)	*pot1*	multipotent epithelia	budlets	throughout blastogenesis	ISH	Tiozzo and de Tomaso (2009)
	telomerase	earliest asexual bud	budlets	throughout blastogenesis	ISH	Laird and Weissman (2004)
Other nucleic acid binding-proteins						
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedpairbp1* *smedhmg1* *smedhmg2*	neoblasts	body parenchyma	throughout ontogeny	ISH	Eisenhoffer et al. (2008)
Markers of postmitotic cells						
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedprog1* *smedprog2* *smedporcna* *smedmhc1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Wagner et al. (2012); Van Wolfswinkel et al. (2014)
Other genes						
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedhnf4*	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Onal et al. (2012)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
Proteins involved in autophagy						
Polyandrocarpa misakiensis (Chordata, Tunicata)	*pmatg7*	atrial epithelium	developing buds	during dedifferentiation	ISH	Kawamura et al. (2018)
Control of differentiation						
Dugesia japonica (Platyhelminthes, Rhabditophora)	*djjy1*	neoblasts, neurons	body parenchyma and brain	throughout ontogeny	ISH	Rouhana et al. (2010)
	dhsp60 djahnak	neoblasts	body parenchyma	throughout ontogeny	ISH, RNAseq	Rossi et al. (2007)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedmhc1*	neoblasts	body parenchyma	throughout ontogeny	ISH	Van Wolfswinkel et al. (2014)
	cbp-2	ubiquitous	body tissues	throughout ontogeny	ISH, iRNA	Fraguas et al. (2021)
Botrylloides leachii (Chordata, Tunicata)	*blraldh*	circulating phagocytes	hemolymph	throughout ontogeny and astogeny	ISH	Rinkevich et al. (2007)
	if-b	atrial epithelium of buds	budlets	buds at stage 1–3	ISH	Ricci et al. (2016)
	bsraldh	inner epithelium of the bud	posterior side of the budlet	bud at stage 3	ISH	Ricci et al. (2016)
myocyte enhancer factor-2 (Mef2) genes						
Isodiametra pulchra (Acoelomorpha)	*ipmef2*	neoblasts, musculature	whole animals	throughout ontogeny	ISH	Chiodin et al. (2013)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			Niche interaction			
Ephydatia fluviatilis (Porifera)	*efannexin*	choanocytes	choanocyte chambers	throughout ontogeny	ISH	Funayama et al. (2010)
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*smedinx13*	neoblasts	body parenchyma	throughout ontogeny	IHC, ISH	Van Wolfswinkel et al. (2014)
Botryllus schlosseri (Chordata, Tunicata)	*bscadherin*	aggregates of hemoblasts aggregates of phagocytes near the endostyle bud epithelia	ampullae cell islands buds	throughout ontogeny and astogeny buds at stage 1–5	ISH, IHC	Rosner et al. (2007)
	bscd133	budding ampullar epithelium some hemocytes	circulation and vasculature	during vasculature regeneration	ISH, FACS	Braden et al. (2014)
Botrylloides diegensis	*integrin α6*	hemoblasts	colonial vasculature	throughout ontogeny	ISH	Kassmer et al. (2020)
			Others			
Polyandrocarpa misakiensis (Chordata, Tunicata)	*pmpumpa*	atrial epithelium	developing buds	during dedifferentiation	ISH	Kawamura et al. (2018)

Table A1. *Cont.*

Species	Gene	Cell Types	Tissue/Organ	Interval of Expression	Identification Methods	References
			miRNA			
Schmidtea mediterranea (Platyhelminthes, Rhabditophora)	*let7a* *mir71b* *mir756* *mir13* *mir752*	neoblasts	body parenchyma	throughout ontogeny	differential expression after irradiation	Lu et al. (2009); Friedländer et al. (2009)
	let7b *mir2160*	neoblasts	body parenchyma	throughout ontogeny	differential expression after irradiation	Lu et al. (2009)
	mir36b *mir2a* *mir2d*	neoblasts	body parenchyma	throughout ontogeny	differential expression after irradiation	Friedländer et al. (2009)

FACS: fluorescence-activated cell sorter; IB: immunoblot; ICC: immunocytochemistry; IHC: immunohistochemistry; iRNA: RNA interference; ISH: in situ hybridization; NB: Northern blot; qPCR: quantitative RT PCR; RNA seq: RNA sequencing; scRNAseq: single-cell RNA sequencing; scqPCR: single-cell qRT PCR.

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