4 The Early Evolution of Cellular Reprogramming in Animals

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4.1 INTRODUCTION

One of the hallmarks of animal development is progressive determination of cell fate, whereby cellular states become increasingly specialized as development proceeds, for example, cell-layer specification during gastrulation followed by cell-type differentiation during organogenesis. Developmental potential of a cell is channeled by the action of cytoplasmic determinants such as maternal factors that are asymmetrically distributed during cell division and/or by inductive interactions with other cells. Pools of stem cells, defined as undifferentiated cells with the capacity to self-renew and generate more specialized cells, often segregate from differentiating cells and tissues during animal development.

Segregated stem cells can be important not only for tissue homeostasis and sexual maturation of animals but also for replenishing damaged or lost cells during regeneration and for generating new postembryonic cell types during life cycle transition. In sea urchins, for instance, most adult tissues as well as germ line cells derive from coelomic sac cells of the larva referred to as "set-aside" cells (Davidson et al. 1995; Peterson et al. 1997), which are pluripotent stem cells segregated during

embryogenesis and that remain mitotically quiescent until metamorphosis (Pehrson and Cohen 1986). We use the term *pluripotent stem cells* to refer to stem cells capable of generating somatic cells and germ cells.

Other examples of segregated stem cells include the migratory pluripotent stem cells of planarians referred to as neoblasts (reviewed by Reddien and Alvarado 2004) and those of hydrozoan cnidarians known as *interstitial stem cells* (i-cells) (reviewed by Gahan et al. 2016). Similarly, a pluripotent stem cell type—archeocytes—occurs in some sponges (poriferans) and is thought to be a major source of differentiated cells during development/metamorphosis, regeneration, reproduction, and tissue homeostasis (reviewed by Funayama 2008). Interestingly, a conserved set of germ line determinants (*piwi, vasa*, *bruno*, and *pl-10*) is expressed in these pluripotent stem cell populations—set-aside cells of a sea urchin (Juliano et al. 2006); i-cells in hydrozoans (Leclere et al. 2012; Rebscher et al. 2008; Seipel et al. 2004); and archeocytes of a sponge (Funayama et al. 2010). These comparative gene expression data led to the proposal that stem cells that are segregated early in development and have both somatic and germ potential—referred to as "primordial stem cells"—are a fundamentally conserved cell type of animals (Solana 2013). Yet, i-cells and archeocytes appear to be lineage-specific cell types within Cnidaria (Gold and Jacobs 2013) and Porifera (Ereskovsky 2010), respectively, casting doubt on whether early animal ancestors indeed generated pluripotent stem cells.

In addition to questions of common ancestry of pluripotent stem cells across basally branching metazoan groups, stem cells are not the only source of cells during regeneration and metamorphosis. Although it is often assumed that development generates irreversible, terminally differentiated cell types such as neurons, "terminally" differentiated cells frequently change their cellular states via reprogramming—referred to as transdifferentiation—during development and regeneration in animals (reviewed by Sanchez Alvarado and Yamanaka 2014 and by Okada 1991). For instance, eye lens cells of adult newts can regenerate from epithelial cells of the dorsal iris (Eguchi and Shingai 1971). In *Caenorhabditis elegans,* a rectal epithelial cell Y transdifferentiates into a motor neuron during larval development (Borisenko et al. 2015). Likewise, in zebrafish larvae, transdifferentiation of dorsal root ganglia sensory neurons into sympathetic neurons has been reported (Wright et al. 2010). In vitro, the striated muscle cells of hydrozoan jellyfish can transform into a variety of somatic cell types such as smooth muscle cells and neurons (Schmid et al. 1988). Given that some differentiated somatic cells are capable of altering cellular states via reprogramming, it seems reasonable to consider the contribution of differentiated somatic cells, along with stem cells, as potential sources of postembryonic cells.

What, then, is the ancestral cellular mechanism of generating postembryonic cell types in the context of development and regeneration in animals? Is it differentiation of resident pluripotent stem cells, reprogramming of differentiated somatic cells, or both? Resolving this problem requires an understanding of the processes of postembryonic cell differentiation and regeneration in early-branching lineages of animals (Figure 4.1). To this end, we review (i) developmental origins of cells that are produced at life cycle transition or during regeneration in the early-branching animal groups Cnidaria, Ctenophora, and Porifera and (ii) evidence for cell differentiation in the closest relative of animals, the choanoflagellates. We begin by examining
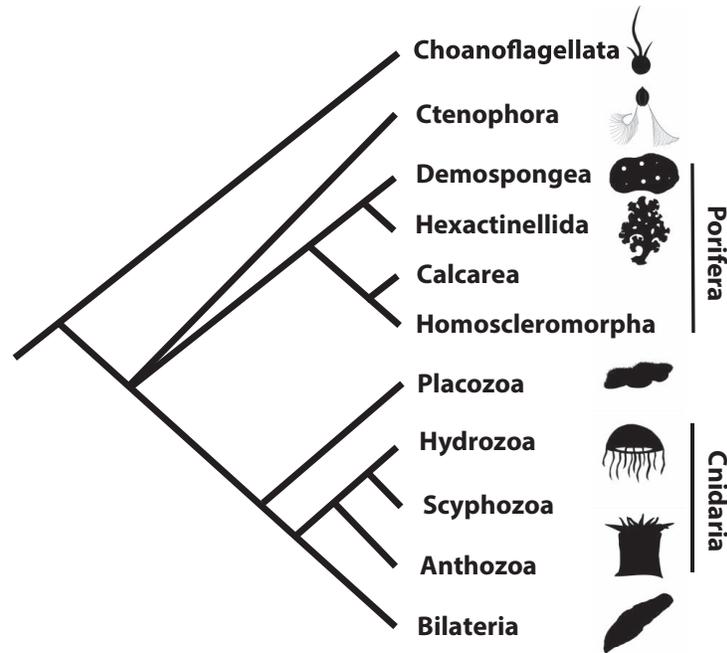
FIGURE 4.1 A consensus animal phylogeny based on current phylogenetic/phylogenomic evidence, rooted by the sister group of animals, Choanoflagellata. Note polytomy at the base of the animal tree, representing uncertainties about the branching order of Porifera and Ctenophora. Silhouette images are from phylopic.org and are under the Public Domain. Based on data from numerous sources, notably Dunn et al. (2008), Moroz et al. (2014), Ryan et al. (2013), Simion et al. (2017), Carr et al. (2008), Collins et al. (2006), Medina et al. (2001), Putnam et al. (2007), Zapata et al. (2015), Erpenbeck and Worheide (2007), and Gazave et al. (2012).

Cnidaria, a group of animals that include jellyfish, sea anemones, and corals, all with complex life cycles. We then explore Porifera (sponges), which have a biphasic life cycle, and Ctenophora (comb jellies), which undergo direct development. Finally, we briefly consider Choanoflagellata.

4.2 CNIDARIA

Cnidarians constitute a diverse group of animals that thrive in marine and freshwater environments. They include sea anemones, corals, and a variety of jellyfishes such as sea wasps and Lion's mane. Cnidaria is sister group to Bilateria and consists of Anthozoa (sea anemones and corals) and Medusozoa (jellyfishes; Staurozoa, Hydrozoa, Scyphozoa, and Cubozoa) (Collins et al. 2006; Medina et al. 2001; Putnam et al. 2007; Zapata et al. 2015). Cnidarians are generally conceived of as diploblastic, having the outer ectodermal layer and the inner endodermal layer separated by an extracellular matrix, the mesoglea, although it should be noted that cnidarian epithelia can be complex and are not always simple single-cell layers.

A cnidarian life cycle typically begins with a free-swimming planula larva that metamorphoses into a sessile polyp, which sexually matures in anthozoans

and some medusozoans such as the hydrozoan *Hydra*. In most medusozoans, the polyp undergoes another round of metamorphosis to form free-swimming medusae either through lateral budding (in Hydrozoa) or transverse fission/strobilation (in Scyphozoa and Cubozoa). Each life cycle stage is characterized not only by distinct body plans but also by sets of stage-specific cell types (e.g., motor nerve net neurons of scyphozoan medusa or hair cells of anthozoan polyps).

The transition between life cycle phases appears to involve either cellular reprogramming or differentiation of segregated stem cells, depending on the taxa. In the scyphozoan *Aurelia,* metamorphosis of a planula larva into a polyp entails renewal of the endoderm, a process that has been referred to as secondary gastrulation (Yuan et al. 2008). During this process, the planula endoderm appears to undergo apoptosis (Yuan et al. 2008), and ectodermal epithelial cells of the planula larva transform into endodermal epithelial cells of a polyp (Gold et al. 2016). These data indicate that metamorphosis of a planula into a polyp in Scyphozoa involves transdifferentiation.

On the other hand, at the planula-polyp transition in hydrozoans, ectoderm undergoes apoptosis, as seen in the colonial marine hydroid *Hydractinia echinata* (Seipp et al. 2001), and endodermally derived migratory stem cells—referred to as interstitial stem cells (i-cells)—move into the ectoderm as demonstrated in the feather hydroid *Pennaria tiarella* by Summers and Haynes (1969) and in *H. echinata by* Weis and Buss (1987). Such cells presumably contribute to the formation of the polyp ectoderm, as i-cells are known to give rise to a variety of cell types, including neurons, stinging cells (cnidocytes), gland cells, and gametes in *Hydra* and *Hydractinia* (reviewed by (Bode 1996 and by Gahan et al. 2016). We note, however, that i-cells are a hydrozoan-specific cell type and that homologous cell types have not been demonstrated in nonhydrozoan cnidarians (reviewed by Gold and Jacobs 2013). Thus, while in hydrozoans it is likely that i-cells generate new cells at the planula to polyp transition, there is currently no evidence that segregated stem cells contribute to morphogenesis during life cycle transition in nonhydrozoan cnidarians.

The planula to polyp transition in the anthozoan starlet sea anemone *Nematostella vectensis* is not nearly as drastic; there is no evidence that it involves reorganization of cell layers. Nonetheless, cell shape changes occur in the body column and tentacle primordia during polyp formation (Fritz et al. 2013; Nakanishi et al. 2012). Moreover, a new polyp-tentacle-specific somatic cell type, the hair cell, develops (Nakanishi et al. 2012). The developmental origin of tentacular hair cells has not been resolved.

Limited data indicate that transformation of polyps into medusae may involve both differentiation of segregated stem cells and transformation of differentiated somatic cells. In the hydrozoan *Podocoryne carnea,* an electron microscopic study suggests that somatic cells of a polyp, namely ectodermal epithelial cells and endodermal digestive cells, transdifferentiate into exumbrellar epithelial cells and manubrial digestive cells of a medusa, while i-cells generate other cell types such as muscle cells, cnidocytes, gland cells, and gametes (Boelsterli 1977).

Helm et al. (2015) examined the development of medusa muscles in two closely related scyphozoan cnidarians, *Chrysaora quinquecirrha*, with a complete life cycle, and *Pelagia noctiluca,* which lost the polyp stage. They found that polyp muscles

did not directly transform into medusa muscles in *Chrysaora* and did not transiently appear during the development of medusa muscles in direct-developing *Pelagia*. Thus, "remodeling" by which preexisting larval structures are modified to generate adult structures was ruled out in favor of "compartmentalization" by which adult structures develop de novo from segregated stem cells. However, the identity of these stem cells remains unknown. In addition, the possibility of transdifferentiation of polyp somatic cells into medusa muscle cells has not been examined.

Cnidarians generally have high regenerative potential; regeneration appears to result from either cellular reprogramming or differentiation of segregated stem cells. In *Hydra,* three stem cell lineages—tissue-specific epithelial stem cells of ectoderm and endoderm, and pluripotent interstitial stem cells—contribute to regeneration of a head or foot, without requiring cell proliferation (Cummings and Bode 1984; Hicklin and Wolpert 1973). This process of regeneration that involves repatterning of the existing tissues without growth is referred to as *morphallaxis.* i-cells can generate neurons, secretory cells, cnidocytes, and gametes but do not generate ectodermal and endodermal epithelial cells in *Hydra* (reviewed by Bode 1996). In another hydrozoan *Hydractinia,* the developmental potential of i-cells is less restricted; i-cells generate all somatic and germ cell types, including ectodermal and endodermal epithelial cells (Muller et al. 2004). During head regeneration in *Hydractinia* polyps, i-cells migrate to the decapitated site and proliferate to form a blastema from which a head is regenerated (Bradshaw et al. 2015). Thus, i-cells appear to be the major source of tissues during head regeneration in *Hydractinia.* In the anthozoan *N. vectensis*, oral structures of the polyp regenerate upon amputation, which, as in *Hydractinia,* requires cell proliferation (Passamaneck and Martindale 2012); however, cellular sources of regenerated tissues are not known.

Transdifferentiation-mediated regeneration also has been observed in Cnidaria. In the scyphozoan *Aurelia,* ectodermal fragments of a polyp—devoid of i-cells—can reconstitute the entire polyp (Steinberg 1963). Light microscopic evidence indicates that during this process of regeneration, ectodermal epithelial cells proliferate and generate endodermal cells via a dedifferentiated intermediate stage of amoeboid cells with little mitotic activity. Therefore, in *Aurelia,* ectodermal epithelial cells can transform into endodermal cells, not only during metamorphosis as discussed above but also during regeneration.

Another example of transdifferentiation-mediated regeneration comes from green hydra (*Hydra viridis*), in which the gastrodermal digestive cells contain symbiotic algae, and i-cells and cnidocytes occur exclusively in the epidermis and not in the gastrodermis (Haynes and Burnett 1963). In this hydra species, the isolated gastrodermis (lacking i-cells), but not epidermis (having i-cells), can regenerate a complete polyp, and histological observations indicated that gastrodermal cells (gland cells and/or digestive cells) directly gave rise to epidermal epitheliomuscular cells during regeneration (Haynes and Burnett 1963). Subsequent electron microscopy studies of regeneration from the isolated gastrodermis in *H. viridis* provided evidence that endodermal digestive cells directly transdifferentiated into epidermal epitheliomuscular cells (Davis et al. 1966), while gland cells transformed into cnidoblasts (cnidocyte precursor cells; Davis et al. 1966) and germ cells (Burnett et al. 1966) via an i-cell intermediate.

Similarly, a whole medusa can regenerate from i-cell-free umbrellar fragments in the hydrozoan *Clytia hemisphaerica* (formerly *Campanularia johnstoni;* Schmid and Tardent 1971). Moreover, in another hydrozoan jellyfish *Podocoryne carnea*, striated muscle cells can be induced in vitro to transform into a variety of somatic cell types, including ciliated smooth muscle cells and anti-FMRFamide-immunoreactive neurons, to form manubria or tentacles, but not the whole medusa (Schmid and Alder 1984; Schmid et al. 1988). Transdifferentiation is triggered by digesting the mesoglea adhered to mechanically isolated striated muscle cells using extracellular matrix–specific enzymes such as collagenase; without destabilization of extracellular matrix, isolated striated muscle cells remain differentiated (Schmid 1978). As expected for reprogramming, transcription and translation are required for transdifferentiation of striated muscle cells (Weber et al. 1987). Taken together, these observations suggest that differentiated somatic cells can contribute to regeneration via reprogramming in medusozoan cnidarians.

Interestingly, transformation of an advanced life cycle phase such as polyps and medusae back into earlier life cycle phases, referred to as *reverse development*, has been reported in some cnidarians (reviewed by (Piraino et al. 2004). For example, in the hydrozoan *Turritopsis,* medusae can transform into the colonial polyps connected by tube-like stolons regardless of the status of sexual maturity when exposed to environmental stress such as a sudden increase or decrease in water temperature (Piraino et al. 1996). Similarly, in the scyphozoan *Aurelia*, a strobilating polyp can generate a stack of polyps, instead of ephyrae, in response to heat stress (Kakinuma 1975), and juvenile and sexually mature medusae, as well as their tissue fragments, have been reported to transform back into a polyp (He et al. 2015). Moreover, newly settled polyps of the scleractinian cauliflower or lace coral *Pocillopora damicornis* can revert to a planula-like free-swimming form under unfavorable environmental conditions (Richmond 1985).

A few lines of evidence suggest that reverse development in *Turritopsis* primarily involves transdifferentiation (Piraino et al. 1996). First, i-cells do not appear sufficient for reverse development; isolated manubria containing a large number of replicating i-cells cannot generate polyps. Second, tissues from the exumbrellar epidermis and the gastrovascular system, which are poor in i-cells, are required for reverse development. Third, the exumbrellar epidermis lacks i-cells but is the only tissue capable of generating the secretory epidermis of stolons. Hence, differentiated ectodermal cells of the exumbrellar epidermis of the medusa must transform into those of the stolons during reverse development. The possibility that i-cells contribute to reverse development cannot be ruled out, however. The cellular bases of reverse development in *Aurelia* and *Pocillopora* remain unexplored.

In summary, cnidarians appear capable of generating postembryonic cells by either differentiation of segregated stem cells or transformation of differentiated somatic cells. Hydrozoans use a pluripotent stem cell type, the i-cells, to generate new cells at the transition from the planula to polyp, and at the transition from the polyp to medusa, and to replenish cells during head regeneration in polyps. There is also evidence that transdifferentiation of somatic cells contributes to regeneration and reverse development in hydrozoans. In nonhydrozoan cnidarians, there is currently no evidence for the presence of segregated pluripotent stem cells, but a

cell lineage tracing study provides evidence for transdifferentiation in a scyphozoan cnidarian, where ectodermal cells of planulae transform into endodermal cells of polyps at metamorphosis.

4.3 PORIFERA

Sponges (Porifera) are marine and freshwater benthic animals, characterized by internal epithelial structures known as the choanocyte chambers that consist of ciliated epithelial cells (choanocytes) to filter-feed and generate a water current through the body (Bergquist 1978).

Sponges represent one of the earliest-evolving metazoan lineages composed of four diverse clades: Demospongiae, Calcareae, Homoscleromorphae, and Hexactinellidae with the phylogenetic interrelationship ([Demospongiae, Hexactinellidae], [Calcareae, Homoscleromorphae]; (Erpenbeck and Worheide 2007; Gazave et al. 2012). Fossil and biomarker evidence indicates that sponges have thrived on earth since at least 635 million years ago (Maloof et al. 2010; Love et al. 2009; Gold et al. 2016).

The sponge internal epithelial layer is typically made up of choanocytes and endopinacocytes, while the outer epithelial layer is composed of exopinacocytes that are exposed to the outer environment, and basopinacocytes that are in contact with the underlying substrate. Sandwiched between the internal and external layers is the mesohyl enriched with mesenchymal cells and collagen fibers. Following fertilization, embryogenesis typically generates elongated, radially symmetrical ciliated swimming larvae that settle onto a substrate by attaching their anterior region. The settled larvae metamorphose and grow into a sexually mature adult. Choanocyte chambers typically develop during metamorphosis, but they have been observed in larvae in some taxa, for example in the trichimella larvae of Hexactinellidae.

Two mechanisms for generating choanocytes—cellular reprogramming and differentiation from segregated stem cells—have been proposed. In some demosponges with parenchymella larvae, morphological evidence indicates that choanocyte chambers form directly from internally localized mesenchymal stem cells referred to as *archeocytes* (Brien and Meewis 1938, 1973; Meewis 1939; Bergquist and Green 1977), akin to hydrozoan i-cells. Here, all the larval epithelial cells are phagocytized by archeocytes during metamorphosis, as demonstrated in *Ephydatia fluviatilis* (Brien and Meewis 1938; Meewis 1939), *Microciona prolifera* (Meewis 1939; Misevic and Burger 1982; Misevic et al. 1990), or shed to the external media as in *Halichondria moorei, Ulosa* sp., and *Microciona rubens* (Bergquist and Green 1977). Thus, larval epithelial cells seem to play no role in the development of choanocytes. More recently, a cell lineage tracing study demonstrated that the larval archeocytes can generate choanocytes in the demosponge *Amphimedon queenslandica* (Nakanishi et al. 2014). However, development of choanocytes from larval archeocytes is unlikely to be an ancestral mechanism in sponges; larval archeocytes are not observed outside Demospongiae (e.g. Calcinea (Amano and Hori 2001); Hexactinellida (Boury-Esnault et al. 1999)).

In contrast, ciliated epithelial cells of larvae transform into choanocytes during metamorphosis across sponges. In Demospongiae, electron microscopy and cell

lineage tracing studies support transformation of larval ciliated epithelial cells into choanocytes during metamorphosis in diversely represented taxa with different larval types. They include

1. parenchymella larvae (*A. queenslandica,* Nakanishi et al. 2014; Leys and Degnan 2002; Sogabe et al. 2016), the freshwater sponge *Spongilla lacustris* (Evans 1899), *Hamigera hamigera*, (Boury-Esnault 1976), *Mycale contarenii* (Borojevic and Lévi 1965), and the purple encrusting sponge *Haliclona permollis* (Amano and Hori 1996);
2. coeloblastula larvae lacking internal cells as in the melted chocolate sponge *Chondrilla australiensis* (Usher and Ereskovskyz 2005), and
3. dispherula larvae with a transient internal epithelial layer (Ereskovsky et al. 2007).

Likewise, among calcareous sponges, the amphiblastula larvae in Calcaronea and the coeloblastula/calciblastula larvae in Calcinea contain few internalized cells. Morphological data in these groups indicate that larval flagellated cells dedifferentiate into amoeboid cells via a loss of cilia and then differentiate into choanocytes (Minchin 1896; Amano and Hori 2001, 1993), similar to the pattern observed in the demosponge *A. queenslandica* (Nakanishi et al. 2014; Sogabe et al. 2016). Moreover, in the cinctoblastula larvae in Homoscleromorpha, internalized cells are rare or absent (Boury-Esnault et al. 2003; de Caralt et al. 2007), and electron microscopic evidence suggests that the juvenile internal epithelial layer is generated by tissue movement via invagination and involution of either anterior or posterior epithelium of the larva and transdifferentiation of the larval ciliated cells during metamorphosis (Ereskovsky et al. 2007). It is not known whether the outer layer epithelial cells of Hexactinellida trichimella larvae can generate choanocytes, if the larval choanocyte chambers simply grow or whether a combination of both occurs. Taken together, it is most parsimonious to assume that the last common ancestor of sponges developed choanocytes by transdifferentiation of larval ciliated epithelial cells during metamorphosis.

Similar to cnidarians, sponges have strong regenerative potential (reviewed by Simpson 1984). Morphological evidence indicates that cellular sources of regenerated tissues in demosponges are typically archeocytes (reviewed by Simpson 1984; Funayama 2008), but it remains unclear whether archeocytes contribute to regeneration in hexactinellids (Leys et al. 2007). Archeocytes are absent in homoscleromorphs and calcareans, despite their ability to regenerate (Ereskovsky et al. 2015; Korotkova 1963, 1970; Tuzet and Paris 1963). Thus, archeocytes are not a requirement for regeneration across sponges. We also note that, importantly, the lack of archeocytes in homoscleromorphs and calcareans makes it ambiguous whether the last common ancestor of sponges developed archeocytes, despite this being often assumed to be the case (e.g., Funayama 2008; Solana 2013).

Choanocytes, on the other hand, occur across all sponge clades and appear to maintain pluripotency, enabling contribution to tissue regeneration via transdifferentiation. For instance, it has been reported that choanocytes can dedifferentiate into archeocytes upon tissue damage in the demosponge *Suberites massa* (Diaz 1977),

which, in turn, presumably replenish lost cell types. Moreover, electron microscopic evidence suggests that choanocytes transdifferentiate into exopinacocytes during regeneration in another demosponge *Halisarca dujardini* (Borisenko et al. 2015) and in the encrusting homoscleromorph *Oscarella lobularis* (Ereskovsky et al. 2015). These comparative data are consistent with the last common ancestor of sponges having used choanocytes to regenerate lost tissues by transdifferentiation.

In summary, while generation of postembryonic cells during metamorphosis and regeneration from the segregated stem cell type, archeocytes, seems common among demosponges, abundant morphological and cell lineage tracing data support trans-differentiation of somatic cells as a general mechanism of generating postembryonic cells in sponges. In particular, evidence for the capacity for ciliated epithelial cells of larvae and choanocytes of juvenile/adult sponges to transdifferentiate is found across major sponge clades. This supports an argument for deep ancestry of cell-type switching within sponges.

4.4 CTENOPHORA

Ctenophores, commonly known as comb jellies, are a group of gelatinous marine carnivores whose phylogenetic position relative to sponges is currently debated; recent phylogenomic studies place them as the earliest or the second earliest diverging animal lineage (Dunn et al. 2008; Moroz et al. 2014; Ryan et al. 2013; Simion et al. 2017).

Ctenophora is traditionally classified into six orders: Platyctenida, Lobata, Thalassocalycida, Cestida, Beroida, and polyphyletic Cydippida (Mertensiidae, Pleurobrachiidae, and Haeckeliidae). Phylogeny reconstruction based on 18S rRNA sequence data indicates that Mertensiidae is the earliest branching group, followed by Platyctenida, which is sister to a group consisting of [Pleurobrachiidae, [Beroida, Haeckeliidae], [Lobata, Thalassocalycida, Cestida]] (Podar et al. 2001). Platyctenes are the only benthic ctenophores; the rest are pelagic.

Ctenophores are characterized by a rotational (biradial) symmetry along the oral-aboral axis, eight longitudinal rows of ciliary comb plates (or ctene plates) used for locomotion, a pair of tentacles bearing sticky cells, referred to as colloblasts, that are employed to capture prey. An aboral apical organ composed of a gravity-sensitive statocyst is the only identifiable sensory structure. Ctenophores are diploblastic, consisting of an outer ectodermal epithelium and an inner endodermal epithelium separated by an extracellular matrix, the mesoglea. The mesoglea contains various cell types, including muscle cells. Ctenophore development is direct; a fertilized egg undergoes a stereotyped cleavage pattern that generates a free-swimming cydippid form with features of adult body plan (Martindale and Henry 1999). New cell types do not appear to develop postembryonically, but "postregeneration" can generate missing structures postembryonically (see below).

Most ctenophores (except for Beroida) can regenerate lost body parts. In the lobate warty comb jelly *Mnemiopsis leidyi* and the platyctene *Vallicula multiformis*, this includes the apical organ, comb plates, and tentacles (Coonfield 1936; Martindale 1986; Freeman 1967). Thus, somatic stem cells and/or differentiated cells capable of transdifferentiation must exist in ctenophores. The ctenophore phylogeny described

above suggests that the last common ancestor of ctenophores was capable of regeneration and that regenerative potential was lost in Beroid ctenophores (Martindale 2016).

The mechanism of tissue regeneration in ctenophores is enigmatic. In *M. leidyi*, comb plates normally develop from e_1 and m_1 micromeres of the 16-cell stage embryo (Farfaglio 1963; Reverberi and Ortolani 1963; Martindale and Henry 1997a). Each quadrant of the 16-cell stage embryo contains two M cell descendants—a small m_1 micromere and a large 1M macromere—and two E cell descendants—an e_1 micromere and a 1E macromere.

When e_1 micromeres are experimentally removed, comb plates fail to develop during embryogenesis but develop from m_1 micromeres after several days (Martindale 1986; Martindale and Henry 1996). The process of generating a structure that was never present during the course of development is referred to as "*postregeneration.*" When both of the cell lineages (e_1 and m_1) that normally generate comb plates are deleted, however, postregeneration does not occur (Henry and Martindale 2000). These manipulations argue that cell lineages that are not e_1 or m_1 do not generate pluripotent stem cells or somatic cells capable of forming comb plates via transdifferentiation. Instead, developmental potential to generate comb plates appears restricted to the e_1 and m_1 cell lineages. Thus, during postregeneration of comb plates upon removal of e_1 micromeres, the m_1 cell lineage must give rise to comb plate progenitor cells, transdifferentiation-competent somatic cells, or both, which enable the formation of comb plates.

Not all cell lineages are as restricted in fate as e_1 and m_1 cell lineages, however. In *M. leidyi*, 2M blastomeres at the 32-cell stage embryo normally give rise to the muscular core of the tentacles, but when they are deleted, tentacles form normally without defects in contractility (Martindale and Henry 1997a,b). Thus, non-2M cell lineages must be able to generate the muscular core in the absence of the 2M cell lineage, presumably through transdifferentiation of somatic cells or differentiation of pluri- or multipotent stem cells. The source of regenerated muscular core tissues remains unresolved.

Adult ctenophores appear to have tissue-specific (i.e. non-toti- or pluripotent) somatic stem cells that contribute to tissue homeostasis, however. Cell labeling experiments in adult sea gooseberries *Pleurobrachia pileus* indicate that fate-restricted somatic stem cells occur in localized regions at the tentacle bases, the comb rows, and aboral apical organ (Alie et al. 2011). In particular, proliferative cells in tentacle bases and comb rows were shown to differentiate into somatic cell types, colloblasts and ciliated polster cells, respectively; the fate of proliferative cells associated with the apical organ remains unclear. These data are consistent with a role for the ctenophore stem cell system in regulating tissue homeostasis. Thus, contribution of these tissue-specific somatic stem cells to replenishing missing cells during regeneration and postregeneration appears likely, although this remains to be confirmed.

In contrast to Cnidaria and Porifera, there is currently no clear evidence for transdifferentiation of somatic cells during development or regeneration in Ctenophora. Neither is there any report of i-cell- or archeocyte-like pluripotent stem cell types that are segregated early in development. However, lineage-restricted somatic stem cells seem to regulate tissue homeostasis in tentacles, comb rows, and the aboral apical organ.

4.5 CHOANOFLAGELLATA

Choanoflagellates, marine and freshwater protists, are the closest relative to animals (Steenkamp et al. 2006; Carr et al. 2008; Ruiz-Trillo et al. 2008; King et al. 2008). Comparative studies of choanoflagellates and animals therefore may provide insights into the biology of early animals (King 2004). Molecular phylogenetic analyses suggest that choanoflagellates can be divided into three major clades: Clade 1, Clade 2, and Clade 3, with the relationship [Clade 3, [Clade 1, Clade 2]] (Carr et al. 2008). Choanoflagellates are characterized by an apical flagellum surrounded by a collar of microvilli, referred to as a collar complex. These morphologically resemble choanocytes of sponges. Choanoflagellates beat flagella to generate water flow, which propels the cell and allows the collar of microvilli to capture and phagocytose bacteria.

Interestingly, some choanoflagellate taxa such as *Salpingoeca rosetta* display temporal cell differentiation depending on environmental conditions (Fairclough et al. 2010; Dayel et al. 2011). For instance, *S. rosetta* can transdifferentiate from a free-swimming form to a sessile thecate form attached to a substrate via theca, or vice versa, and can form multicellular colonies of different morphological types (rosettes and chains) via mitosis (Fairclough et al. 2010; Dayel et al. 2011). Transcriptome data from *S. rosetta* show that distinct solitary and colonial forms are characterized by differential gene expression (Fairclough et al. 2013). Thus, transformation of cellular states is under genetic control. Also, in *S. rosetta*, haploid solitary cells can directly transdifferentiate into gametes, both small and large flagellated cells (Levin and King 2013; Woznica et al. 2017). Ancestral character state reconstruction based on the molecular phylogeny of choanoflagellates suggests that multicellular colony development predated the divergence of Clade 1 and 2 or evolved independently in different choanoflagellate lineages multiple times (Carr et al. 2008). The former scenario leaves open the possibility that colony formation, and thus temporal differentiation of cellular states, is an ancestral trait of choanoflagellates.

4.6 EARLY ANIMALS WERE CAPABLE OF REPROGRAMMING SOMATIC CELLS

The data summarized above can be mapped onto metazoan phylogeny. This allows preliminary inference of the evolutionary history of postembryonic mechanisms that generate new or lost cell types (Figure 4.2).

As mentioned above, it is currently debated whether Ctenophora or Porifera is sister to the rest of the animals (e.g., Dunn et al. 2008; Moroz et al. 2014; Ryan et al. 2013; Simion et al. 2017), and thus, we assume polytomy at the base of the animal phylogeny (Figures 4.1 and 4.2A). Phylogenetically widespread instances of cellular reprogramming across early-evolving animal groups—Porifera and Cnidaria, in particular—are consistent with the hypothesis that early animals were capable of reprogramming somatic cells during postembryonic development or regeneration (Figure 4.2B). Furthermore, although it remains to be addressed whether transdifferentiation is an ancestral trait in Choanoflagellata or a derived trait of *S. rosetta* (and other colony-forming choanoflagellates), the evidence of alteration of differentiated cellular states in a choanoflagellate raises the possibility that cellular
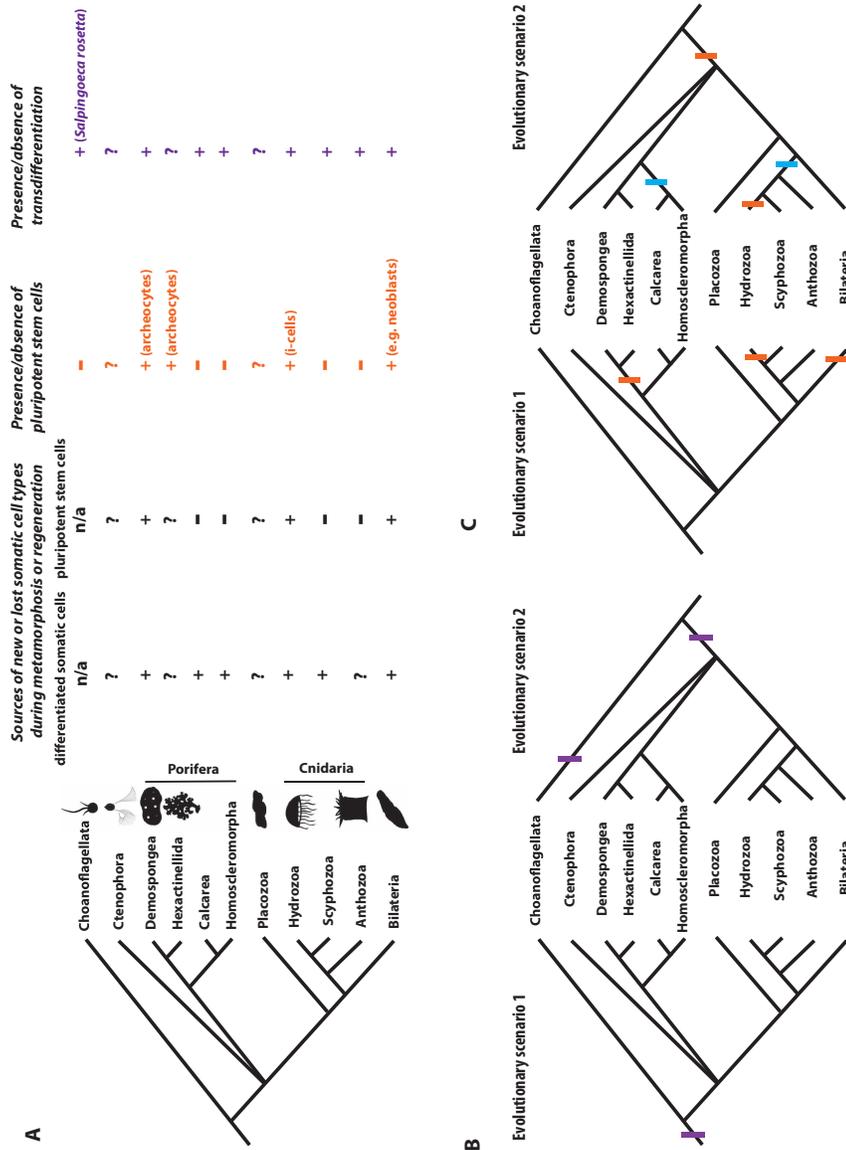
FIGURE 4.2 Possible evolutionary histories of transdifferentiation and pluripotent stem cells in animals. (A) Animal phylogeny (left) and a character matrix (right) used for reconstructing ancestral character states. Characters were scored for taxa where information was available (see text for details). (B) Alternative evolutionary scenarios for the origin of transdifferentiation. Purple lines indicate emergence. (C) Alternative evolutionary scenarios for the origins of pluripotent stem cells. Orange lines indicate emergence; blue lines indicate evolutionary losses. Silhouette images in (A) are from phylopic.org and are under the Public Domain.

reprogramming mechanisms may even predate animal origin (evolutionary scenario 1 in Figure 4.2B). Alternatively, the ability to change differentiated cellular states may have evolved independently in *S. rosetta* and the metazoan stem lineage (evolutionary scenario 2 in Figure 4.2B).

In contrast, segregated populations of pluripotent stem cells are found sporadically in divergent taxa, indicative of more complex evolutionary histories than previously assumed. This suggests multiple gains or losses of pluripotent stem cells consistent with the unique attributes of stem cells in different groups.

As discussed above, archeocytes are found only in demosponges and its sister group hexactinellids within Porifera, and interstitial stem cells (i-cells) are restricted to hydrozoans within Cnidaria. Although these stem cell populations express an orthologous set of germ line determinants (*piwi, vasa*, *bruno*, and *pl-10*) as discussed above, the limited occurrence across metazoan phylogeny, combined with the lack of segregated pluripotent stem cells in the outgroup taxa (e.g., choanoflagellates), leaves the ancestral state ambiguous. One possibility is that pluripotent stem cell populations evolved independently in divergent lineages (evolutionary scenario 1 in Figure 4.2C). This scenario implies that gametes were generated by transdifferentiation of somatic cells in early animals, similar to the condition encountered in sponges where a differentiated somatic cell type, the choanocyte, is thought to give rise to gametes—both eggs and sperm, but sperm only in some demosponges—reviewed by Harrison and De Vos (1991). Consistent with this hypothesis, it has been reported that striated muscle cells of a hydrozoan cnidarian have the potential to generate gametes via transdifferentiation (Schmid et al. 1988). Interestingly, both sponge choanocytes and hydrozoan striated muscle cells express *piwi* (Funayama et al. 2010; Seipel et al. 2004), indicating that "germ line" determinants can function in differentiated somatic cells, possibly, to maintain genome integrity and cellular potency (van Wolfswinkel 2014). Also noteworthy is that under this evolutionary scenario, it must be assumed that the Weismann barrier, in which genetic information flows from germ line cells to somatic cells but not vice versa, was absent in early animals, in disagreement with the primordial stem cell hypothesis that assumes otherwise (Solana 2013). Alternatively, the last common ancestor of animals may have developed pluripotent stem cells during embryogenesis, which were subsequently lost in Homoscleromorpha/Calcarea and Cnidaria independently, followed by a reversal in Hydrozoa (evolutionary scenario 2 in Figure 4.2C).

4.7   FUTURE DIRECTIONS

A number of problems remain unresolved.

First, sources of cells during metamorphosis or regeneration are unknown in some phylogenetically informative taxa. For instance, in ctenophores, although tissue-specific somatic stem cells do seem to exist, it is unclear whether and how they contribute to tissue regeneration and whether transdifferentiation has any role in the process. In anthozoan cnidarians, segregated somatic stem cells in the form of i-cells appear absent, but this does not necessitate that transdifferentiation generates postembryonic cell types during metamorphosis or regeneration; postembryonic cells could come from reserve somatic stem cells that have yet to be discovered.

Second, the molecular basis of transdifferentiation is poorly understood in early-diverging animal groups. Relevant data are currently limited to the hydrozoan cnidarian *Podocoryne,* where *bmp2/4, bmp5/8, msx,* and *piwi* have been found to be differentially expressed during transdifferentiation of striated muscle cells, indicative of their role in regulating transdifferentiation (Seipel et al. 2004; Galle et al. 2005; Reber-Muller et al. 2006). The precise roles of BMP signaling, *msx,* and *piwi* in striated muscle transdifferentiation remain to be established by gene function perturbation approaches.

Third, an understanding of how cellular states are maintained in early-diverging animal groups is lacking. Some somatic cell types such as neurons appear to be stably differentiated across Bilateria and Cnidaria, although the possibility of transdifferentiation under specific conditions (e.g., during regeneration) remains to be investigated. The knowledge of the molecular mechanisms that maintain differentiated cellular states in early-evolving animal groups is key to gaining insights into how cellular plasticity was regulated—to prevent malignant cellular reprogramming that leads to the formation of cancer—in early animals.

4.8   CONCLUSIONS

In this chapter, we have reviewed developmental origins of postembryonic cell types that arise at life cycle transition and/or regeneration in early-branching animal lineages—Cnidaria, Ctenophora, and Porifera. Based on these data, we propose that *transdifferentiation is likely to be an ancestral mode to postembryonically generate new or lost cell types in animals.* It is possible that cellular reprogramming mechanisms even predated animal origin, as temporal alteration of differentiated cellular states occurs in unicellular relatives of animals. However, an alternative possibility of independent evolutionary origins of cellular reprogramming in choanoflagellates and Metazoa cannot be ruled out. Within Metazoa, the evolutionary history of pluripotent stem cells that are capable of generating new or lost cell types postembryonically appear more complex. They may have emerged independently in Demospongea/Hexactinellida (archeocytes), Hydrozoa (interstitial stem cells), and Bilateria. Alternatively, the last common ancestor of animals may have had pluripotent stem cells, which were later lost in some lineages—Homoscleromorpha/Calcarea and Cnidaria—followed by a reversal in Hydrozoa. We infer that regulation of cellular reprogramming was integral to the biology of early animals, and so a more comprehensive understanding of transdifferentiation is critical to an understanding of evolutionary history of stem cells and the evolution and diversification of animals.

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