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[REVIEW]

Cell Lineages and Fate Maps in Tunicates: Conservation and Modification

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Comparison of features of the cell lineages and fate maps of early embryos between related species is useful in inferring developmental mechanisms and amenable to evolutionary considerations. We present cleavage patterns, cell lineage trees, and fate maps of ascidian and appendicularian embryos side by side to facilitate comparison. This revealed a number of significant differences in cleavage patterns and cell lineage trees, whereas the fate maps were found to be conserved. This fate map similarity can be extended to vertebrates, thus representing the fate map characteristics of chordates. Cleavage patterns and cell lineages may have been modified during evolution without any drastic changes in fate maps. Selective pressures that constrain developmental mechanisms at early embryonic stages might not be so strong as long as embryos are still able to generate a chordate-type fate map. Aquatic chordates share similar fate maps and morphogenetic movements during gastrulation and neurulation, eventually developing into tadpole-shaped larvae. As swimming by tail beats, and not by cilia, is advantageous, selective pressure may maintain the basic elements of the tadpole shape. We also discuss the evolutionary origin of the vertebrate neural crest and the embryonic origin of the appendicularian heart to illustrate the usefulness of cell lineage data. From an evolutionary standpoint, cell lineages behave like other characteristics such as morphology or protein sequences. Both novel and primitive features are present in extant organisms, and it is of interest to identify the relative degree of evolutionary conservation as well as the level at which homology is inferred.

Key words: cell lineages, fate map, ascidian, appendicularian, neural crest, heart, homology hypothesis

INTRODUCTION

Description of cleavage patterns and cell lineages as a branching tree in space and time during embryogenesis provides basic information on how embryos develop at the cellular level. Lineal trajectory analyses reveal the progenitors and descendants of a individual cell. This in turn provides information about branching and restriction patterns of cell fates and serves as a basis for experiments involving embryonic perturbations, especially in cases where cell lineages are stereotyped and invariant among individuals. Fate maps are also informative even if cell lineages are not invariant. Comparison of the characteristic features of cell lineages between related species contributes to our understanding of developmental mechanisms, and can provide a fundamental insight into evolutionary processes.

In this review, we compare the early embryogenesis of ascidians and appendicularians, focusing mainly on their cell lineages and fate maps. Ascidians and appendicularians belong to the subphylum Tunicata (or Urochordata) within the phylum Chordata, which also includes Vertebrata and Cephalochordata. The Tunicata include numerous and divergent species that are conventionally divided into three groups: Ascidiacea, Appendicularia, and Thaliacea (e.g., Burighel and Cloney, 1997; Lemaire, 2011), although most recent studies indicate that Thaliacea is more closely related to ascidian subgroups (e.g., Stach and Turbeville, 2002; Tsagkogeorga et al., 2009; Govindarajan et al., 2011). Eggs of most ascidian and appendicularian species, as well as some thaliacean species, develop into tadpole-shaped larvae representing the basic body plan of chordates (Fig.1A, C) (Nishino and Satoh, 2001). This supports the hypothesis that these animals are close relatives of vertebrates. Phylogenetic analyses of genome sequence data support the notion that Tunicata branched from the lineage leading to vertebrates later than cephalochordates (Delsuc et al., 2006). Overall, the data indicate that tunicates are the closest relatives to vertebrates. Appendicularians retain the tail throughout their entire life as pelagic tunicates without

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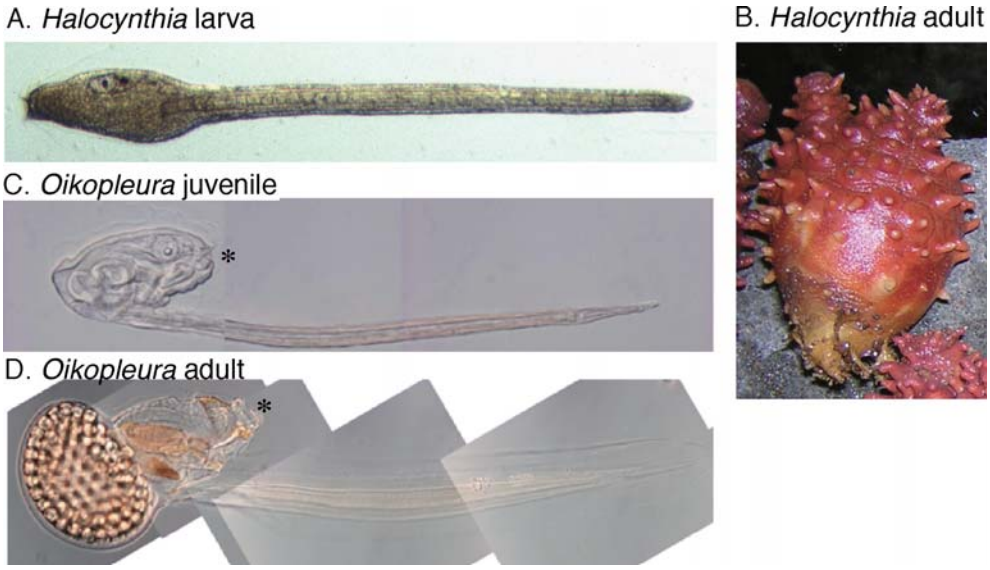

Fig. 1. **(A)** A hatched larva of the ascidian, *Halocynthia roretzi*, at 35 h after fertilization. The length is approximately 1500 μm. **(B)** A sexually mature adult of *H. roretzi*, three-years old. The size is 15 cm. **(C)** A juvenile of the appendicularian, *Oikopleura dioica*, at 10 h after fertilization. The length is approximately 450 μm. **(D)** A sexually mature female of *O. dioica* on the fifth day after fertilization. The length is 3000 μm. Asterisks indicate the position of the mouth. Photos of *O. dioica* have been reproduced from Nishida (2008) with permission.

exhibiting the drastic metamorphosis seen in ascidians, which eventually become sessile (Fig. 1B, D). Approximately 2800 ascidian species have been described (Shenkar and Swalla, 2011), whereas 72 thaliacean (Govindrajan et al., 2011) and 69 appendicularian species have been cataloged so far (Fenaux, 1998).

The embryonic cell lineages of ascidians and appendicularians are well described, whereas details of thaliacean development are scanty at best. Partly due to his intuitive selection of *Cynthia partita* (now: *Styela canopus*) as his experimental species, Edwin G. Conklin (1905) described cell lineages in ascidian embryos in surprising detail. His descriptions of cleavage patterns were later confirmed by scanning electron microscopy (Satoh, 1979), and those of cell fates were confirmed and slightly modified on the basis of studies using microinjection of lineage tracer molecules into the blastomeres (Nishida, 1987). The cleavage patterns and cell fates in ascidians are invariant among individuals in a single species, and are nearly identical in several well-studied and distantly related solitary ascidian species across distinct orders, with only slight modifications (Zalokar and Sardet, 1984; Niermann-Kerkenberg and Kurt, 1989; Hudson and Yasuo, 2008; Nakamura et al., 2012).

Conklin's counterpart researcher of appendicularian species was Hendricus C. Delsman (1910, 1912), who observed living and fixed embryos of *Oikopleura dioica* in detail. His description of the cleavage patterns was confirmed much later by confocal microscopy (Fujii et al., 2008). Recently, cell lineages during the entire process of embryogenesis were recorded and described by tracing nuclear positions and mitoses by time-lapse 4D microscopy using DIC optics, exploiting the fact that the embryos of this animal are small and transparent (Stach et al., 2008). The entire embryonic cell lineage is invariant in *O. dioica*, and the cleavage pattern, at least, is conserved in the two congeneric species *O. dioica* and *O. longicauda* (Nishino and Satoh, 2001).

CLEAVAGE PATTERNS AND BLASTOMERE NOMENCLATURE

As in many animals, the first and second cleavage planes are vertical along the animal-vegetal axis and perpendicular to each other. The third cleavage plane is horizontal, dividing the animal and vegetal hemispheres, resulting in the 8-cell stage with blastomeres showing a characteristic shape and size in ascidians and *O. dioica* (Fig. 2, top). In both species, the plane of the first cell division coincides with the future median sagittal plane of the larvae. The plane of the second cleavage divides the anterior and posterior blastomeres. Thus, the anterior-posterior axis is traditionally defined as perpendicular to the animal-vegetal axis. The geometries of the subsequent cell division patterns in both species are shown for comparison in Fig. 2. Supplementary Figure S1 online shows a similar diagram with the blastomere names and cell fates superimposed.

In ascidians, cells after the 8-cell stage have traditionally been named in accordance with the nomenclature proposed by Conklin (1905). At the 8-cell stage, animal cells are indicated by a lower case letter and vegetal cells by a capital one. Anterior cells are named "a" and "A", and posterior cells are named "b" and "B". These letters are inherited by every descendant after the 8-cell stage. Cells on the right side of the embryo are underlined (e.g., a5.3 is on the left side, a5.3 is on the right). The first digit following the letter denotes the cell generation, counting the egg as the first (see Fig. 3 and Supplementary Figure S1). For example, cells at the 32-cell stage are the 6th generation. The second digit following the dot gives the cell number, which doubles at each division (e.g., a5.3 divides into a6.5 and a6.6). Cells that lie closer to the vegetal pole are assigned the lower number.

In *O. dioica*, Delsman (1910) applied the nomenclature system used for annelid and mollusk embryos after the 8-cell stage. After each division, a figure 1 or 2 is added, with 1 indicating the daughter cell closer to the animal pole. For example, A divides into A^1 and A^2. Then the A^1 divides into A^{11} and A^{12}, and A^2 generates A^{21} and A^{22}. Similar to the usage in ascidians, cells on the right side of the embryo are denoted by underlined numbers (i.e., A^{21} on the left side corresponds to A^{21} on the right side). Stach et al. (2008) adopted the ascidian nomenclature system for *O. dioica* and supplied a table for translation of one system into the other. These are also indicated in parentheses in Fig. 3 and

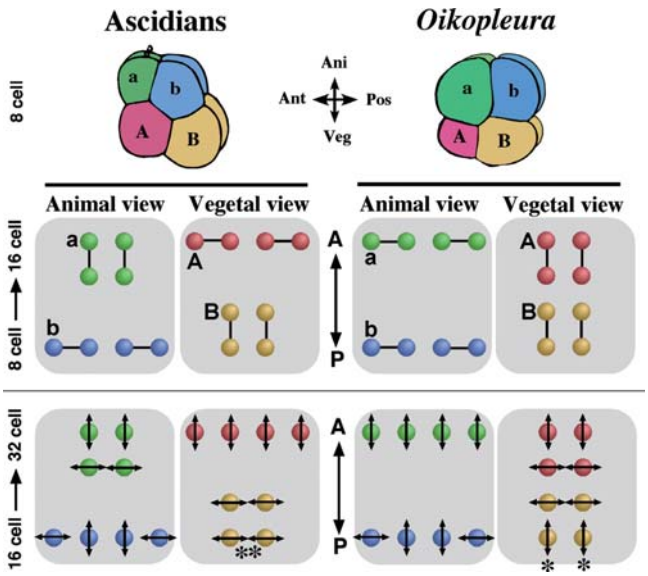

Fig. 2. Cleavage patterns of ascidians and *Oikopleura* embryos. **(Top)** Lateral views of the 8-cell-stage embryos. The names of blastomeres are shared in both. The anterior blastomeres are assigned 'a' (shown in green) and 'A' (red) with lower case for the animal hemisphere. The posterior blastomeres are assigned 'b' (blue) and 'B' (yellow) with lower case for the animal hemisphere. The arrangements of blastomeres show bilateral symmetry. **(Middle)** Orientations of cell division from the 8- to 16-cell stage are shown in the animal and vegetal hemispheres. Each color-coded sphere represents the position of the blastomere in the 16-cell embryo with its origin from the 8-cell embryo shown. Sister cells are connected with bars. Anterior is up. **(Bottom)** Orientations of cell division from the 16- to 32-cell stage. Each sphere represents the position of the blastomere in the 16-cell embryos. Double-headed arrows show the orientation of the next cell divisions. Asterisks indicate germ cell precursors. Note that the orientation of every cell division is projected onto the animal and vegetal hemisphere in this diagram. However, the division axes are also inclined along the animal-vegetal axis. See Supplementary Figure S1 online for a similar diagram with blastomere names and cell fates superimposed. For details of the spatial arrangements of blastomeres, see Satoh (1979), Nishida (2005, 2008), and Fujii et al. (2008). Ani, animal pole. Veg, vegetal pole. Ant and A, anterior. Post and P, posterior.

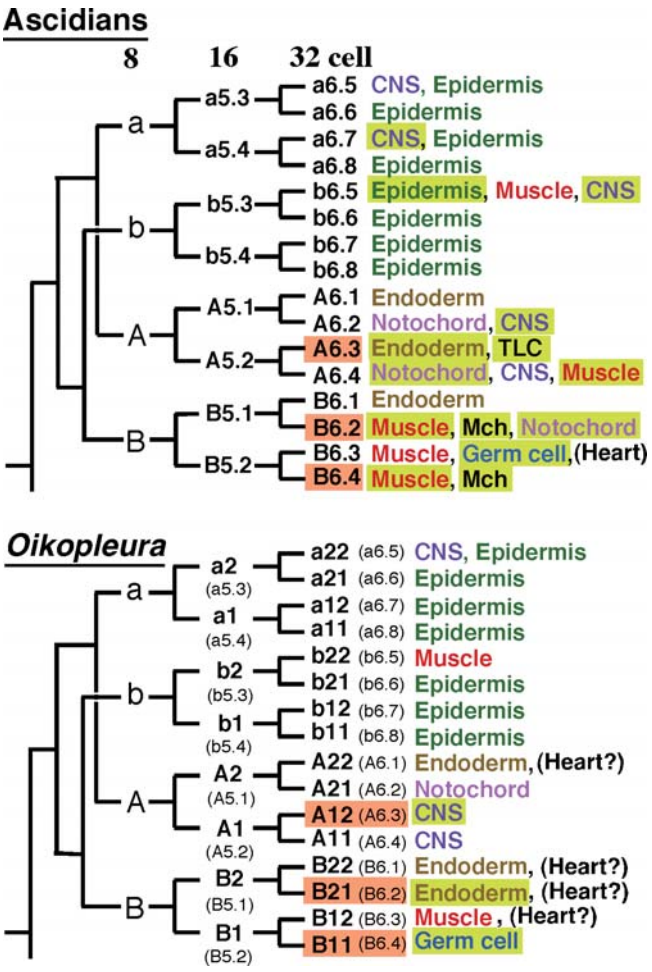

Fig. 3. Cell lineage diagrams of ascidians and *Oikopleura* embryos. The lineage trees are basically bilaterally symmetric, and so the bilateral halves are shown. The only exception is the A^{22}-derived notochord, which originates only from right side A^{22} cell in *Oikopleura*. The A^{22}-derived notochord is not shown in this figure. The nomenclature for ascidian embryos accords with that of Conklin (1905). That in *Oikopleura* follows Delsman (1910), with the ascidian system shown in parentheses according to Stach et al. (2008). Developmental fates of cells at the 32-cell stage are shown. Fates are color-coded. Fates unique to each organism are highlighted with yellow rectangles. The blastomeres that have totally distinct cell fates are highlighted with orange rectangles. The precursors of the heart are also shown in parentheses, although the heart is present in juveniles and adults, but not in larvae. In *Oikopleura*, the origin of the heart is not precisely determined, and is shown with question marks. For further details of lineage trees, see Nishida (1987), Hirano and Nishida (1997), and Stach et al. (2008). CNS, central nervous system. Mch, mesenchyme. TLC, trunk lateral cell.

Supplementary Figure S1. While this translation underscores similarities in the cleavage patterns of the two tunicate species, care is necessary because cells given the same name in ascidians and *O. dioica* are not always located in the same position within the embryo, as the cleavage pattern is not identical.

Figure 2 shows the orientations of cell divisions projected onto the animal and vegetal hemispheres. The patterns are distinct between ascidians and *O. dioica*. This is not surprising, as the two phylogenetic lineages have probably been separated for over 500 million years (Chen et al., 2003; Swalla and Smith, 2008). The anterior blastomeres (a- and A-lines; green and red spheres) divide in different directions in the two species. The animal a-line cells in ascidians and the vegetal A-line cells in *O. dioica* show similar patterns, and A-line cells in ascidians and the a-line cells in *O. dioica* divide similarly. In contrast, the pattern in the posterior blastomeres (b- and B-lines; blue and yellow) is highly similar in the two species. One of the likely reasons is that the cleavage pattern in the B-line cells is regulated by the centrosome-attracting body (CAB), which is a subcellular structure positioned in the posterior pole region. It attracts the posterior centrosome in the posterior-most blastomeres, thereby regulating the orientation of cell divisions and causing successive unequal cell divisions in the B-line descendants. The CAB is present in ascidians (Hibino et al., 1998; Nishikata et al., 1999), and a similar structure was also

observed in *O. dioica* by Delsman (1910). In fact, the posterior blastomeres successively divide unequally in size both in ascidians and *O. dioica*, and eventually the smallest posterior-most blastomeres become germ line cells (Fig. 2, asterisks) (Shirae-Kurabayashi et al., 2006; Stach et al., 2008).

CELL LINEAGES

Cell lineage diagrams (Fig. 3) show relationships between lineal ancestry and prospective cell fates. The tadpole of ascidians and the adult of *O. dioica* each consist of approximately 3000 cells. In ascidians, cell fates of most blastomeres are restricted to give rise to a single cell type by the 110-cell stage (Nishida, 1987) with the completion of cell fate restriction in some blastomeres starting at the 16-cell stage. In contrast, cell fate restriction of most blastomeres completes as early as the 32-cell stage in *O. dioica* (Stach et al., 2008). This makes the 32-cell embryo of *O. dioica* an extremely simple chordate embryo in which cell-fate restriction is largely completed. In comparison, the prospective cell fate of each blastomere of 32-cell ascidian embryos is more complex. In Fig. 3, the unique fates in each organism are highlighted with yellow rectangles. Blastomeres that have a completely distinct cell fate are highlighted with orange rectangles. The lineage diagrams are similar, but there are also conspicuous differences, especially in the vegetal hemispheres (A- and B-lines).

Cells in the animal hemisphere mostly develop into epidermis in addition to the central nervous system (CNS) (Supplementary Figure S1). The only exception is muscle cells derived from b6.5 blastomeres and their counterparts, b^{22}, in both cases. These cells are located in the lateral region within the embryo. Therefore, this particular characteristic is evolutionarily conserved. Germ cells originate from B6.3 cells in ascidians, whereas in *O. dioica* they are derived from B^{11} (the B6.4 counterpart). However, these cells are similarly located at the posterior pole in both species (Fig. 2, asterisks). Thus, the difference is due to the distinct cleavage patterns and nomenclature systems.

Each of the fundamental tissues, such as the CNS, notochord, muscle, and endoderm, are derived from various separated branches in the lineage tree, making the tree intricately organized. The tree only shows lineal relationships of progenitors and descendants; information on spatial relationships, i.e., the spatial position of each cell within the embryo, is not depicted. As shown in the next section, the territories that give rise to a single type of tissue in fate maps are actually contiguous even if the cells originate from various separated branches in the lineage trees. Therefore, it is plausible that mechanisms of cell fate specification utilize information on the geographic positions of the blastomeres to a greater degree than information on their respective genealogical origins of cells, although the relationship between lineal ancestry and cell fate is fixed and invariant in these animals.

COMPARISON OF FATE MAPS BETWEEN ASCIDIANS AND APPENDICULARIANS

Using information on cleavage pattern and cell fate, fate maps can be generated for the blastula stage prior to the complex movements that occur during gastrulation (Fig. 4). A similarity of topography in presumptive tissue territories is recognizable between the fate maps of ascidians and *O. dioica*, despite their distinct cleavage patterns and cell lineages. The differences are that *O. dioica* does not have cells corresponding to ascidian mesenchyme cells and trunk lateral cells, which generate the cells that are embedded in the outer tunic of ascidians after metamorphosis, and adult

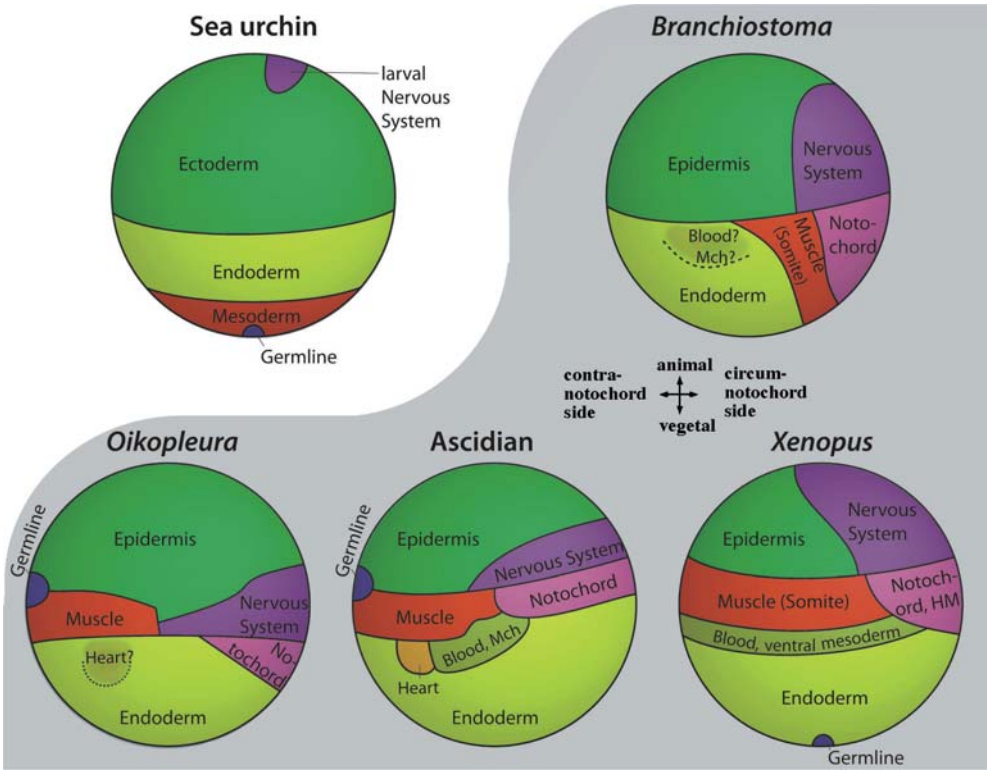

Fig. 4. Comparison of fate maps of ascidians, *Oikopleura*, *Xenopus*, amphioxus *Branchiostoma*, and sea urchin at the blastula stage. Lateral views. Ectoderm, mesoderm, and endoderm form in this order from the animal pole in chordates (gray background). The circum-notochord side, from which notochord cells form, is to the right in chordate fate maps. This side corresponds to Spemann's organizer side in the frog. Note the similarity of topography in the presumptive tissue territories in chordate fate maps. HM, head mesoderm; Mch, mesenchyme. Fate maps were drawn based on the sea urchin (Angerer et al., 2011), *Branchiostoma* (Holland and Holland, 2007), *Oikopleura* (Stach et al., 2008; Nishida, 2008), ascidian (Nishida, 1987), and *Xenopus* (Lemaire et al., 2008).

blood cell and body wall muscle cells, respectively (Hirano and Nishida, 1997). The similarity of the fate maps despite the differences in cleavage patterns further supports the idea that cell fate specification also depends on the geographic positions of the blastomeres. This has been confirmed by extensive analyses of fate specification mechanisms in ascidians involving localized maternal factors and short-range inductive cell interactions, as will be discussed below.

It has been shown that two types of mechanism are involved in the spatial arrangement of tissues forming distinct territories in ascidians. First, several maternal factors are prelocalized within the eggs before the first cleavage takes place. These factors are inherited by specific blastomeres during successive cleavages. For example, the presence of yet unknown cytoplasmic factors that play crucial roles in the determination of vegetal hemisphere identity has been revealed by egg cytoplasm deletion and transfer experiments (Nishida, 1993). Macho-1 is a muscle determinant that is localized to the posterior region in ascidian eggs (Nishida and Sawada, 2001). PEM, localized to the posterior pole, is a germ cell factor that mediates zygotic transcriptional quiescence in germ cells (Kumano et al., 2011; Shirae-Kurabayashi et al., 2011). In addition to macho-1 and PEM, dozens of posteriorly localized maternal mRNAs have been found using various high-throughput approaches, and these are referred to as postplasmic/PEM mRNAs (Makabe and Nishida, 2012).

Second, when embryos become multicellular, embryonic induction starts on an intercellular level. This is an important step at the 32- to 64-cell stage for patterning of the equatorial regions in ascidians (Nishida, 2005: Kumano and Nishida, 2007; Lemaire, 2009). Fibroblast growth factor (FGF) secreted from endoderm precursors located at the vegetal pole induces brain, notochord, and mesenchyme fates depending on the competence factor within the signal-receiving cells. The effect of FGF in ascidians is limited to a short range, affecting only the fates of cells adjacent to the FGF-emitting cells (Miyazaki et al., 2007). Thus, the equatorial region (Fig. 4) is patterned along the animal-vegetal axis. Cell fate specification by localized maternal factors and short-range inductive cell interactions provides the basis for cell fate specification depending on the geographic positions of the cells. Invariant cleavage patterns play crucial roles in the precise partitioning of localized maternal factors and in fixed spatial arrangements of interacting blastomeres within embryos. It is likely that appendicularians also utilize similar processes for cell fate specification, although almost nothing is known about how cell fates are determined in appendicularian embryos.

COMPARISON OF TUNICATE FATE MAPS WITH THOSE OF VERTEBRATES

Figure 4 shows fate maps across different deuterostome taxa. The fate maps of the appendicularian *Oikopleura* and ascidians look similar. The topographical similarity of presumptive tissue territories on the fate maps can also be extended to that of the vertebrate *Xenopus*, and to some extent the cephalochordate *Branchiostoma*. However, the fate map of the sea urchin, an echinoderm, is significantly different in that, for example, the mesoderm originates from the vegetal pole region (Fig. 4). Inferences about the basic features of embryogenesis in chordates can be deduced from these fate map similarities. Chordates share similar fate maps, show similar morphogenetic movements during gastrulation and neurulation (Nishida, 2005), and eventually develop into tadpole-shaped larvae with similarly organized tissues and organs. They commonly have a dorsal neural tube and central notochord flanked by bilateral muscle in the tail. The chordate body plan of the larvae and primarily aquatic adults is specialized for swimming by the tails although the adult forms of ascidians and terrestrial vertebrates have diverged. However, even in adults there are some shared features that have been conserved between tunicates and vertebrates, such as the endostyle/thyroid and gill slits. Non-chordate deuterostomes develop larvae that swim with cilia, such as the pluteus larvae of sea urchins and the tornaria of acorn worms (Satoh, 2009). Tail-swimming is thus an evident innovation of chordates that enables them to swim effectively (Stach, 2014).

In spite of these conserved features among chordates, the majority of the mechanisms involved in embryonic axis determination and cell fate specification in early embryos differ markedly between tunicates and vertebrates (Nishida, 2005: Lemaire et al., 2008; Lemaire, 2009). The identity of localized maternal molecules and their localization are fundamentally disparate, and tunicates and vertebrates utilize the same signal molecules in different ways during embryonic induction. In addition, ascidian embryos do not possess an organizer region on the circum-notochord side, which is critical for the development of vertebrate embryos. On the other hand, cephalochordate embryos also have an organizer on the circum-notochord side secreting a BMP antagonist (Yu et al., 2007; Onai et al., 2010). Thus, the molecular strategies leading to the similar fate maps appear to be more diverse than was suspected hitherto, suggesting that organisms could generate the same fate map and eventually the same body plan while developing along divergent routes. Selective pressures that constrain developmental mechanisms at the early embryonic stage might not be so strong as long as the evolutionarily modified mechanisms are still able to generate a similar chordate-type fate map and a tadpole-like body plan. On the other hand, modifications of cell fate specification during early embryogenesis are frequently thought to have a significant and often deleterious impact on later embryogenesis. However, as is evidenced by the differences in cell lineages and fate specification mechanisms between chordate species, early embryogenesis could have been modified and evolved as long as they still generate the roughly conserved fate map and body plan, given the enormous time period of at least 500 million years and the vast numerical opportunities for trial and error.

Why, then, was the tadpole body plan so strictly conserved during the diversification of chordates? A possible explanation from an adaptive viewpoint is that the tadpole structure is highly suitable for effective swimming (see also Stach, 2014) and therefore the selective pressures on an energy-efficient mode of local dispersal and feeding with an active and sophisticated mechanism for swimming behavior would maintain the basic elements of the chordate body plan.

The early development of tunicates is characterized by an invariant cleavage pattern and cell lineage. Why do these organisms develop in such a stereotyped manner? The invariance of early embryogenesis may be correlated with the fact that it involves a small number of cells. Cell fate restriction of most blastomeres completes as early as the 32-cell stage in appendicularians and the 110-cell stage in ascidians. The strictly invariant cleavage pattern facilitates appropriate segregation of localized maternal factors into specific blastomeres, and spatial arrangement of cells for stereotyped short-range intercellular interactions between neighboring blastomeres in tunicate embryos. Therefore, cell fate restriction in the early embryonic stages when the embryo consists of relatively few cells and the invariant cleavage pattern would be linked to each other.

SOME CONSIDERATIONS FROM CELL LINEAGE AND FATE MAP DATA

Precise cell lineage data have proven pivotal in suggesting correspondences of evolutionarily conserved features between different species. Several regulatory genes (*HNK-1*, *zic-2* and others) expressed in the trunk lateral cells that originate from A6.3 at the 32-cell stage and its daughter A7.6 cells in the ascidian, *Ciona intestinalis*, are also expressed in the neural crest cells of vertebrates. Based on this, Jeffery et al. (2008) have suggested that these cells and their descendents are homologous to vertebrate neural crest cells. Like some vertebrate neural crest cells, the cells of the A7.6 lineage in ascidians migrate and differentiate into adult body pigment cells in the ascidian *Ecteinascidia turbinata*, although in the ascidian, *Halocynthia roretzi*, the trunk lateral cells give rise to adult blood cells and body wall muscle, which are considered to be mesodermal in origin. Abitua et al. (2012) have challenged this homology hypothesis and suggested that descendant cells of a6.7 and later a9.49 cells are homologous to vertebrate neural crest cells. This latter homology hypothesis is also based on similarities of gene expression (*Wnt7*, *Tcf*/*Lef*), and gene regulatory network logic (Wnt signaling activates *FoxD*, which in turn represses melanogenesis through *Mitf* inhibition). In addition, Abitua et al. (2012) have suggested a possible evolutionary mechanism whereby these cells could have acquired the ability to migrate through co-option of the gene *Twist*. In their experiments, mis-expression of *Twist* in a9.49 cells resulted in migratory behavior by these cells. Although there are currently two alternative homology hypotheses regarding the origin of the neural crest, the detailed data on cell lineages in tunicates support the latter hypothesis of Abitua et al. (2012). The a9.49 cells are derived from a6.7 progenitors, which are located adjacent to the developing neural tube and have fates that give rise to both the ectodermal nervous system, sensory pigment cells, and epidermis, thus showing similarities to what is known about the vertebrate neural crest (e.g., Bronner-Fraser, 2008; Betancur et al., 2010). However, it is worth pointing out that the cell lineages of vertebrates and the cell lineages and fate maps of cephalochordates are not known in as much detail as those of tunicates, although newly developed techniques show some promise in filling these gaps in our knowledge (e.g., Mikut et al., 2013; Rizzi and Peyrieras, 2014; Loulier et al., 2014).

In the embryo of the ascidian, the cell lineage of the heart[1] can be traced back to B6.3 at the 32-cell stage and its daughter B7.5 cells (Hirano and Nishida, 1997). The cells that develop into the ascidian heart express a set of core transcription factors that are also involved in heart formation in vertebrates (e.g., *Nkx2*/*tinman*, *GATA4*,*5*,*6*/*pannier*, *Hand*, *Mesp*, and *T-box* factors; e.g., Pérez-Pomares et al., 2009; Kokubo et al., 2010) and cephalochordates (Holland et al., 2003; Pascual-Anaya et al., 2013). Whereas adults of sedentary ascidians possess numerous blood cells of different types (Burighel and Cloney, 1997), *Oikopleura dioica* blood is devoid of cells (Lohmann, 1956). Nevertheless, *O. dioica* possesses a heart similar to that of ascidians (Stach, 2009). While molecular developmental mechanisms have been well studied in ascidians, corresponding information for *O. dioica* is almost entirely lacking. However, cell lineage comparison between ascidians and *O. dioica* (Fig. 3) suggests that experiments designed to elucidate heart development can be concentrated on lineages of only a few cells, B^{12} (= B6.3 in ascidian nomenclature) being the most promising.

CONCLUSIONS AND PERSPECTIVES

Descriptions of cell lineages provide basic information for functional and experimental studies involving micromanipulative and molecular perturbations of embryos, especially in cases where cell lineages are invariant among individuals. Fate maps are also informative even if cell lineages are not invariant. Comparisons of cell lineage characteristics between related species are useful for deducing the mechanisms of development and is beneficial in evolutionary considerations. Comparisons of ascidians and appendicularians have suggested that cleavage patterns and cell lineages can be modified during evolution without any dramatic change in the fate map. The mechanical basis of this is that embryonic axes and cell fates are specified by spatial localization of maternal factors and spatially regulated cell interactions in early embryos.

Even from the brief comparisons in this article, it becomes evident that cell lineages behave like other characteristics, such as morphology or molecular sequences, in evolution: some aspects change while others are retained. This leads to a mix of both novel and primitive features in extant organisms, and the scientific task at hand is to identify the relative degree of evolutionary conservation as well as the level at which homology can be inferred (e.g., Hennig, 1979; Wiley and Liberman, 2011; see Wray, 1994 and Stach et al., 2008 for specific suggestions of how cell lineage data can be analyzed within a phylogenetic paradigm). Some examples can be found in the previous descriptions. The cleavage directions indicated in Fig. 2 show similarities and differences. The congruencies in the posterior cells in the animal and the vegetal hemispheres can be interpreted as homologous. Or to formulate it differ-

[1] We use the term “heart” according to common usage in the field of developmental biology (e.g., Davidson, 2007). The anatomically correct term for the pulsating element of the ascidian circulatory system is pericardium. The inner wall of the pericardium is differentiated as myocardium (e.g., Oliphant and Cloney, 1972, Stach, 2008).

ently: the similarity of the b4.2 cells of 8-cell embryos dividing laterally and B4.1 cells dividing along the anteroposterior axis can be seen as evidence that these cell division planes were already present in the last common ancestor of the two tunicate species, which lived more than 500 million years ago. On the other hand, the plane of division in this last common ancestor in the anterior a- and A-cells cannot be hypothesized based on the available evidence. However, it can be concluded that at least in one of the evolutionary routes leading to the two tunicate species the plane of cell division was altered. Thus, in order to postulate homology, the specific similarity supporting the proposition has to be clearly presented.

Again as for other characteristics, conflicts between several homology hypotheses are to be expected. It might be the case that cell position within the embryo supports a homology hypothesis, unlike the position in the lineage tree. For example, according to Fig. 3, several cells in the two tunicate species could be homologized based on similarities of fate and position in the cell lineage tree (e.g., a6.5, a6.6, a6.8, etc.), in other cases there are slight differences in cell fate (e.g., a6.7, b6.5, A6.2 etc.), or there may be conspicuous discrepancies in cell fate. For example, the fate of A6.3 is restricted to endoderm and trunk lateral cells in ascidians, but it becomes the central nervous system in *Oikopleura* (see also Supplementary Figure S1). Such cases provide reasons to double check the results, but might also highlight interesting areas of research in which the mechanism responsible for an obvious evolutionary novelty could be deduced using a comparative approach (Scholtz, 2005, 2010). Thus, careful and transparent argumentation in relation to homology hypotheses will clearly advance the burgeoning field of comparative cell lineage studies and identify interesting and relevant evolutionary novelties for future analysis.

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