



Morphogenesis: a focus on marine invertebrates

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Abstract

Morphogenesis is a process describing how the shapes of living tissues and bodies are created during development. Living and fossil organisms exhibit enormously diverse tissue architecture and body forms, although the functions of organs are evolutionally conserved. Current knowledge reveals that relatively conserved mechanisms are applied to control development among different species. However, the regulations of morphogenesis are quite diverse in detail. Animals in the ocean display a wide range of diversity of morphology suitable for their seawater environment. Nevertheless, compared with the intensive studies on terrestrial animals, research on marine animal morphogenesis is still insufficient. The increasing genomic data and the recently available gene editing methods, together with the fast development of imaging techniques, quantitative analyses and biophysical models, provide us the opportunities to have a deeper understanding of the principles that drive the diverse morphogenetic processes in marine animals. In this review, we summarize the recent studies of morphogenesis and evolution at molecular, cellular and tissue levels, with a focus on three model marine animals, namely ascidians, sea urchins and sea anemones.

Keywords Ascidian · Morphogenesis · Sea anemone · Sea urchin

Introduction

Morphogenesis is one of the most exciting phenomena in living organisms, and the mechanisms underlie the formation of diverse morphologies have fascinated biologists for over a century. At the cellular level, morphogenesis is achieved by cell division and death, cell shape change, cell migration and cell rearrangement. The cells get the instructions for what should be done from a signaling cascade starting from morphogens. Morphogens are diffusible molecules that specify

cell fates in a concentration-dependent manner, thereby setting up the patterning of a given tissue (Lawrence 2001). The term “morphogen” was coined by Alan Turing in his seminal paper (Turing 1953). Decades later, genes encoding morphogens were identified by Christiane Nüsslein-Volhard and Eric Wieschaus in the famous “Heidelberg screen” (Nüsslein-Volhard and Wieschaus 1980), which was a large-scale genetic screen for the genes essential for *Drosophila* embryogenesis. Further genetic cloning and molecular characterizations revealed that most morphogens are secreted signaling proteins, such as Wnt, fibroblast growth factor (FGF) and Hedgehog (Lawrence 2001). The morphogens bind to specific receptors, thereby activating or deactivating certain genetic circuits in defined domains of the developing tissue. Those genetic circuits do not control cell shape directly. Rather, cell and tissue shape change is executed by cellular mechanical machinery (including actomyosin networks, adherens junctions, cell polarity complexes) in the cell cortex (“Box 1”). The genetic circuits activate or suppress the expression of proteins, such as guanine nucleotide exchange factors (RhoGEF) (Kölsch et al. 2007) and phosphatase and tensin (PTEN) (Bardet et al. 2013), which then modulate the activities or properties of the cellular machinery spatiotemporally, to determine exactly when, where and how a specific tissue shape forming will take place (Fig. 1a).

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Upon the arrival of instructions, the cells undergo remodeling of their cytoskeletons and junctions, leading to the coordination among the cells at tissue level by chemical–mechanical signals. As a result, the tissue achieves the designated shape. This indicates that morphogenesis can be analyzed at different levels: the molecular level, the cellular level and the tissue level (Fig. 1b). The collective behavior of the lower level in the hierarchy leads to the emergence of new properties. These emergent properties and their functions, however, are not seen at the lower level.

Mechanical forces generated by the cellular machinery propagate through transmembrane receptors to neighboring cells and the extracellular matrix. The integration of these

forces on tissue level drives morphogenetic events, such as tissue invagination, stratification and elongation. There is growing evidence that the information flow from morphogen to tissue architecture is not unidirectional. Rather, a feedback loop exists between morphogens and the tissue shaping process. After forming a certain shape, the tissue in turn affects both the distribution of morphogens and cell fate (Averbukh et al. 2014; Shyer et al. 2015). However, the feedback mechanisms remain elusive.

Oceans provide 71% of the living space on our planet, and an estimated 243,000 accepted marine species in higher taxa are found in oceans (Costello and Chaudhary 2017), most of which are invertebrates. Marine invertebrates exhibit a wide range of developmental procedures and diverse forms of body plan, from the simple, soft sac-like animal Cnidaria to Urochordates (tunicates), the closest living invertebrate relative of the vertebrates. Marine invertebrates provide a great source of interesting morphogenic cases, for example, the remarkable morphological mollusk shells (McDougall and Degnan 2018) and the intricate polychaete germline morphogenesis (Brubacher and Huebner 2009). Different developmental modes are found in marine invertebrates: some animals undergo direct development without feeding larval stage in the life cycle, and other species contain a feeding larval stage and instead undergo indirect development. The morphologies and the wide range of tissue remodeling (Johnson et al. 2019) during the metamorphosis are also of great interest. To develop a comprehensive understanding of the mechanism underlying morphogenesis in marine invertebrates, it is important to focus on major marine model organisms, which are genetically and visually trackable in vivo. Recent advances in live imaging techniques, genetical perturbation, and quantitative analysis have facilitated a better understanding of marine invertebrate morphogenesis at molecular, cellular and tissue levels. In this review, we discuss these progresses, focusing on the recent discoveries and open questions in the areas of cellular dynamics during ascidian, sea urchin and sea anemone morphogenesis (see “Box 2”).

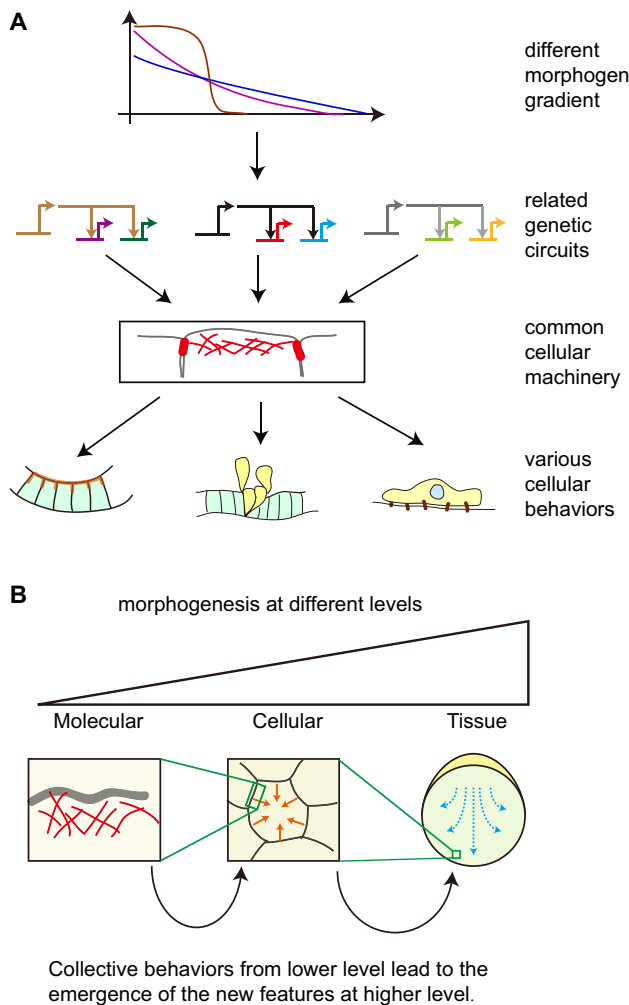
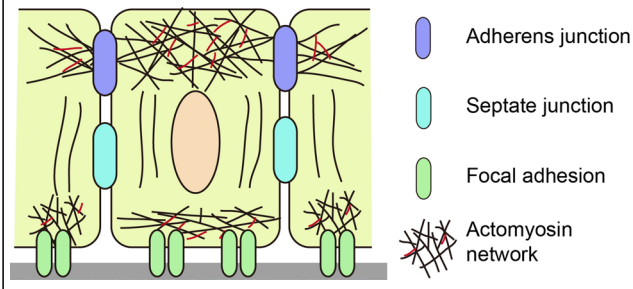


Fig. 1 Overview of morphogenesis. **a** Morphogen concentration determines the morphogenesis in certain regions of embryos via activating or inactivating related genetic circuits, which control the cellular mechanical machinery at the cell cortex. Cell cortex functions as a hub, promoting cellular apical constriction, epithelia–mesenchymal transition, or migration to sculpture the tissue, depending on the developmental contexts. **b** Morphogenesis occurs at different levels. The interactions of the elements at lower level lead to the emergence of new features at higher level

Box 1. The molecular machinery of morphogenesis

Beneath the plasma membrane is a thin network made of actin filaments, cross-linkers and motor protein nonmuscle myosin II. The motor activity generates the contractile force in the cortical network under the regulation of several signals. The cortex is attached to the cell membrane by ERM proteins (Fehon et al. 2010); therefore, the structures and the properties of the cortex determine the cell shape. Cell polarity refers to spatial difference in cellular components in one cell. Cell polarity plays an important role

during morphogenesis. Epithelial cells display apical–basal polarity. The establishment and maintenance of cell polarity involve positive and negative feedback between multiple polarity molecules, in which the Par-3/Par-6/aPKC complex plays a fundamental role. Adherens junctions (AJs) mediate cell–cell junction via homophilic trans-interaction of cadherins molecules from neighboring cells. Inside the cell, AJs bind to actin filaments, therefore the actomyosin contraction can be transmitted via AJs across multiple cells.



Box 2. Sea urchins, sea anemones and ascidians

Compared with their terrestrial and freshwater counterparts, the molecular and cellular biology of marine animals is poorly known, despite their large number of species and high diversity of morphology. Owing to the development of genome sequencing and the live imaging techniques, the marine animals, especially sea urchins (McClay 2011),

sea anemones (Technau and Steele 2011) and ascidians (Lemaire 2011), have emerged as powerful model organisms for research on evolutionary developmental genetics and morphogenesis. Sea anemones belong to the phylum Cnidaria, which have only two germ layers, the outer ectoderm and the inner endoderm or gastrodermis which lines the gut cavity. This prominent feature makes the starlet sea anemone, *Nematostella vectensis*, an ideal model for studies on the evolution of the mesoderm (Steinmetz et al. 2017) and bilaterians (He et al. 2018). Sea urchins belong to the phylum Echinodermata. Their embryos have been used as a model system to address many questions in developmental biology since the 1800s. Sea urchins can be maintained easily in laboratories. Their optical transparency makes sea urchins an attractive model for in vivo imaging. The launch of its genome sequence has made the sea urchin a popular model organism for the study of developmental biology (Sea Urchin Genome Sequencing Consortium et al. 2006). Ascidians, known as sea squirts, are invertebrate chordates, which belong to the subphylum Urochordata/Tunicata. They have a notochord at the larval stage, but they lack vertebrae in their life cycle. Ascidian larvae are free-swimming tadpoles, whereas, after metamorphosis, the tail undergoes regression associated with notochord degeneration, and the animal settles on a hard surface. The genome had been sequenced by 2002 (Dehal et al. 2002). Research on ascidians has shed light on notochord evolution (Reeves et al. 2017) and the origin of important vertebrate tissues/organs, such as the neural crest (Abitua et al. 2015; Stolfi et al. 2015).

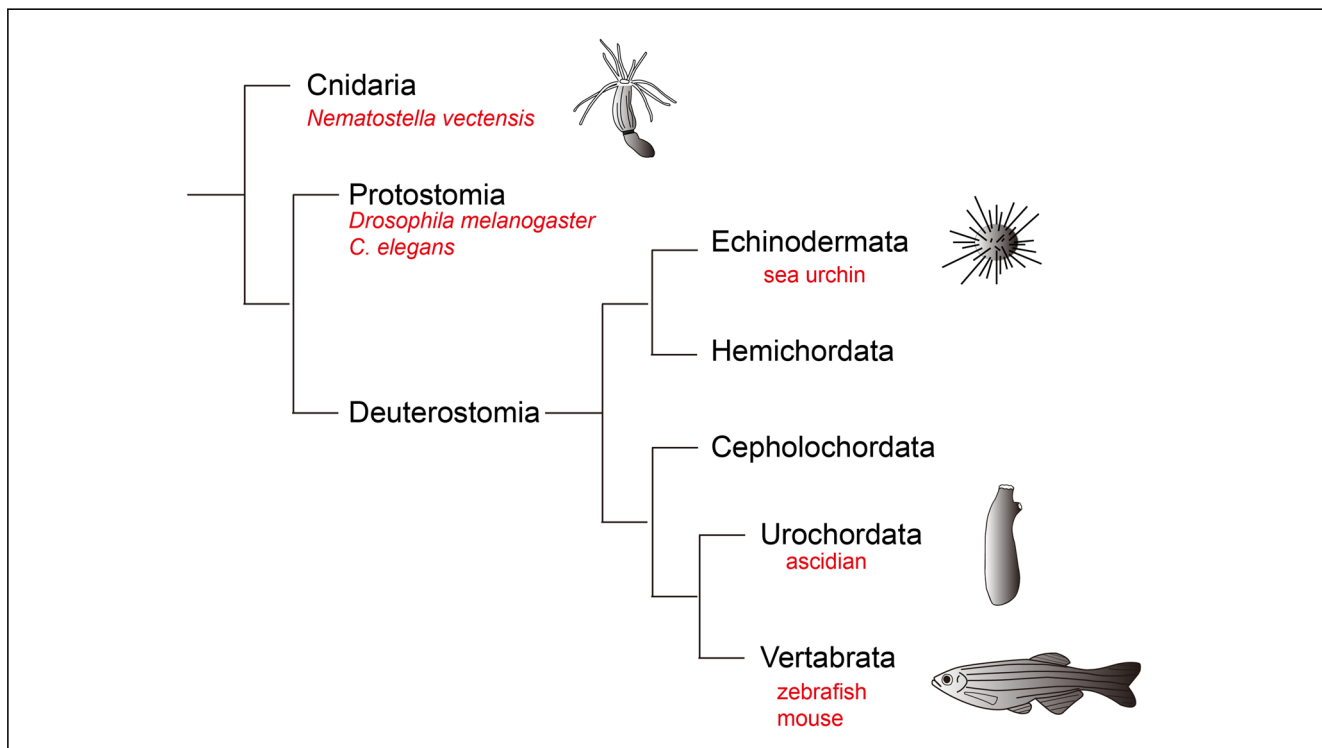
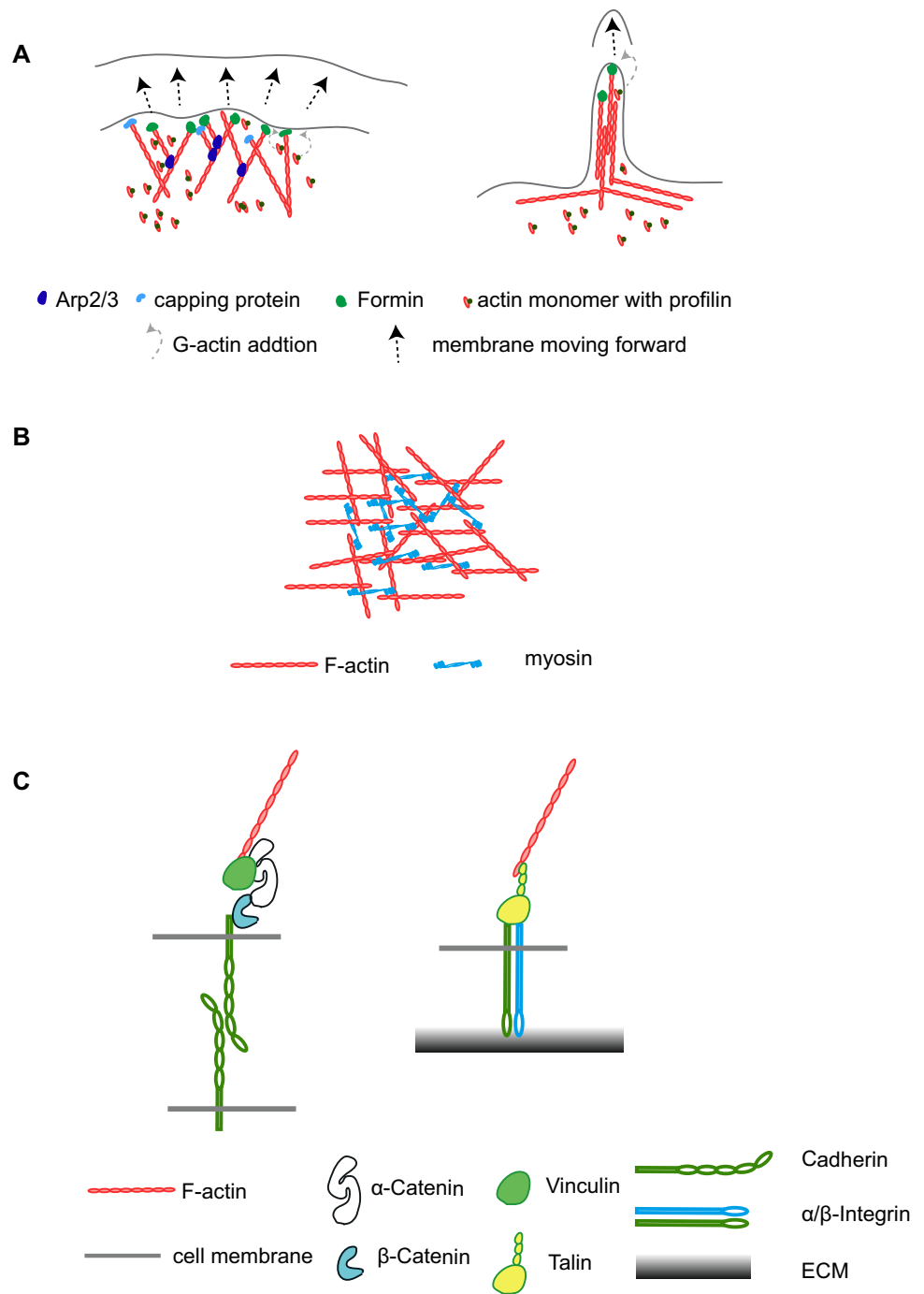


Fig. 2 Actin-based force generation apparatus. **a** Actin polymerization leads to protrusive activity, i.e., lamellipodia and filopodia. **b** Actomyosin networks have the ability to contract via different mechanisms (see main text). **c** The F-actin attaches to cell–cell or cell–ECM contacts



F-actin-based force generation at molecular level

Morphogenesis is a well-known self-organized process, driven by forces that are generated and integrated within tissues (Gilmour et al. 2017; Heisenberg and Bellaïche 2013). These forces can be generated by motor activity on polymers, elongation of actin and microtubule filaments, osmotic pressure and other mechanisms (Mammoto and Ingber 2010). Two different strategies are utilized for actin-based force

generation: ① actin filament assembly beneath the plasma membrane and ② actomyosin network contraction (Fig. 2a, b). Although actin monomers can spontaneously polymerize into filaments in vitro (Pollard and Cooper 2009), living cells control actin filament assembly tightly. Profilin binding to actin monomers hampers actin self-assembly (Theriot and Mitchison 1993), and the capping proteins bind to barbed ends of existing actin filaments to restrict filament growth (Borisy and Svitkina 2000). Only in the presence of actin nucleator and elongation factors such as Arp2/3, formin and

Ena/VASP protein can actin filaments grow. At the cellular level, actin filament-based forces are widely used for cell shape change, migration and division. The growth of actin filaments pushes the plasma membrane to form protrusive structures at the leading edges of cells, such as lamellipodia and filopodia (Rottner and Schaks 2019). Lamellipodia are thin-layer protrusive structures at the front of spreading and migrating cells, which contain two-dimensional actin networks. Lamellipodia are found in motile cells in various contexts, including wound healing in sea anemones (Kamran et al. 2017), archenteron elongation in sea urchins (Kominami and Takata 2004) and notochord morphogenesis in ascidians (Munro and Odell 2002). Filopodia are thin, tubular extensions of the plasma membrane with parallel actin bundles inside. Filopodia structures were observed in the second mesenchymal cells of sea urchins (Gustafson and Wolpert 1961; Miller et al. 1995), in the tunic of the ascidian *Botryllus schlosseri* (Izzard 1974) and in the endoderm of sea anemones (Kraus and Technau 2006). Cytoneme is a type of filopodium that can deliver signaling proteins to target cells or receive signaling proteins from other cells (Kornberg and Roy 2014). It is highly likely that filopodia play a role as cytonemes in the developmental processes mentioned above. However, further molecular characterization is required.

The second strategy is the usage of actomyosin networks. Nonmuscle myosin II (referred to hereafter as myosin) molecules assemble into bipolar minifilaments with two motor domains at the ends, embedded in actin networks that can be organized monoscopically in 2D networks (Chugh and Paluch 2018), rings (Abreu-Blanco et al. 2012) or linear bundles (Monier et al. 2010). The motor activity allows myosin to move on F-actin, thereby generating contractile forces. Actomyosin contractility plays an indispensable role in numerous biological processes, such as apical constriction, body axis formation in *Caenorhabditis elegans* (Munro et al. 2004) and cell junction remodeling (Rauzi et al. 2010). Cross-linkers such as α -actinin (Laporte et al. 2012) and plastin (Ding et al. 2017) determine the material properties of actomyosin networks. Therefore, the activity, turnover rate and specificity of cross-linkers must be under precise regulation (Levayer and Lecuit 2012). Actomyosin networks are highly dynamic. F-actin severing and depolymerization are essential for the maintenance of actomyosin networks (Chugh and Paluch 2018). The contractility of actomyosin networks is achieved mainly by myosin motor activity, which is under the control of the Rho GTPase signaling pathway, including Rho, Rac and Cdc42 (Guilluy et al. 2011). In addition, F-actin turnover has been suggested to contract actomyosin networks in a myosin-independent manner. For example, in starfish oocytes the chromosomes are transported to the cortex of the egg by actomyosin networks, in

which F-actin depolymerization and turnover are indispensable (Bun et al. 2018).

The forces generated by actomyosin networks cannot work alone. To change cell shape and drive cell motion, the actomyosin networks have to anchor to the plasma membrane via adapter molecules (Fig. 2c). For example, during cytokinesis, the actomyosin network is coupled to plasma membrane by ERM proteins and anillin, which is essential for cleavage furrow ingression (Hiruma et al. 2017). In the epithelium, cells are connected by cell–cell junctions, including adherens junctions (AJs). AJs are composed of the transmembrane protein cadherin and the cytosolic protein catenin. Cell–cell adhesion is mediated by Ca^{2+} -dependent homophilic interactions of extracellular cadherin domains. The intracellular domain of cadherin binds to the catenin complex, thus linking to actin filaments. Vinculin binding to α -catenin proteins enhances the connection of the actin cytoskeleton to AJs. In this way, AJs transmit forces generated by actomyosin from one cell to another. AJs are not only passive force adopters, but also involved in coordinating cells actively (Rübsam et al. 2018). The actomyosin networks attach to integrins, and therefore the forces generated by actomyosin are exerted against the extracellular matrix (ECM), resulting in cell migration and cell shape change (Schwartz 2010). In some cases, the ECM undergoes remodeling owing to the forces being exerted on them (Haigo and Bilder 2011).

This raises two important questions: How are the AJs and cellular polarity established? How do actomyosin networks drive tissue morphogenesis?

Polarity establishment

Cells in developing blastula develop distinctly in the apical and the basolateral domains. In parallel, cellular junction complexes are assembled. Generally, the cell–cell contact region is in the basolateral domain, whereas cell contact-free region is in the apical domain (Fig. 3a). Extensive research on *Drosophila*, *C. elegans* and mammalian cultured cells has provided us a picture of the molecular regulation of polarity formation and maintenance. Three groups of molecules are required in polarity formation. ① Par-3/Par-6/aPKC and ② Crumbs complex, which localize at the apical domain, whereas ③ Scribble complex and Par-1 localize at the basolateral domain. The Scribble complex and Par-1 inhibit the apical proteins from localizing at the basolateral domain. Likewise, Par-3/Par-6/aPKC and Crumbs complex restrict the basolateral localization of Scribble complex and Par-1. Such mutually antagonistic interactions between apical and basolateral proteins lead to the formation of polarity (Fig. 3b). The emergence of cell polarity during early embryogenesis in several species has been documented

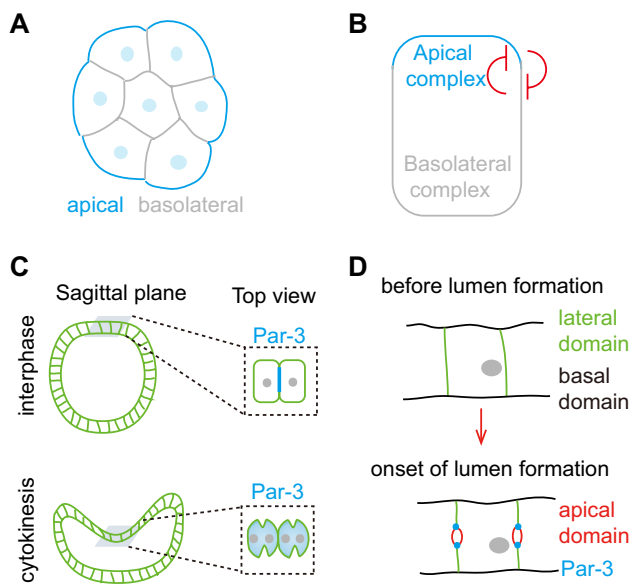


Fig. 3 Polarity establishment. **a** The apical and basolateral domain of cell in blastula. **b** Mutually antagonistic interactions between apical and basolateral protein complex leads to the polarity formation and maintenance. **c** The polarity undergoes “re-establishment”–“de-establishment” cycle along with the cell cleavage cycle in *Nematostella*. **d** The polarity emerges during lumen formation in ascidian notochord cells

(Nance 2014). The molecular mechanisms are conserved. However, the regulatory details may differ among species.

The sea anemone *Nematostella vectensis* displays a chaotic cleavage without a pattern (Fritzenwanker et al. 2007), implying that cell polarity is not yet established at this developmental stage (Salinas-Saavedra et al. 2015). Antibody immunostaining and mRNA injections indicate that different Par proteins show distinct localizations in the gastrula, but not in the cleavage stage (Salinas-Saavedra et al. 2015). Ragkousi et al. (2017) proposed that cell polarity was obtained by the four-cell stage and a primary epithelium was formed by the 16-cell stage based on the localization of Par proteins at the cell–cell contacts.

How is cellular polarity establishment embedded in the fast cleavage cycle in early embryogenesis? A recent study has shown that cell polarity undergoes a re-establishment–de-establishment cycle along with the cell cleavage cycle in *Nematostella* (Ragkousi et al. 2017). Despite the high variability of cleavage patterns, a coeloblastula with a single layer of epithelial cells is formed after 11 rounds of cleavage. The prominent feature during *Nematostella* cleavage cycles is that the whole embryo shows interphasic compaction and mitotic decompaction cycles, and such cyclic behavior parallels the re-establishment and de-establishment of apical polarity marked by Par-3 and Par-6 (Ragkousi et al. 2017) (Fig. 3c). These compaction–decompaction cycles are more pronounced later in the blastula, showing a pulsating

behavior which occurs between the 128-cell stage and gastrulation. Blastoderms display a spherical shape when the cells are in interphase, whereas they become flattened when the cells are in mitosis. The invagination from a single pole leads to the blastula flattening. Upon the completion of cell cytokinesis, the spherical shape of blastoderms reforms. After several rounds of the spherical–flattened reshaping cycles, the blastula stops pulsating and remains as a spherical shape until the beginning of gastrulation (Fritzenwanker et al. 2007). Pharmacological inhibition of the cell cycle and of the cytoskeletal structure leads to loss of the re-establishment–de-establishment cycle of apical polarity (Ragkousi et al. 2017) and stops the pulsating of the blastula in later stages (Fritzenwanker et al. 2007). It is reasonable to consider that the cellular material properties, e.g., stiffness and tension (Kasza et al. 2007) differ between interphase and mitosis, which leads to the blastula flattening. Alternatively, cytokinesis releases the tension and thus the blastula flattens, like a balloon leaking air. Nevertheless, the molecular details of how the cell cycle couples to tissue shape change need to be addressed in future. Another remaining question is how the invagination point is determined. The point of the invagination will be the position where gastrulating invagination takes place. This implies the existence of a genetic or physical constraint in this particular region.

The early embryogenesis of ascidians starts with a series of stereotypical cleavages, giving rise to a compact monolayer ball of cells, which is polarized by expression and localization of Par proteins. In later stage, the AJs assemble and a mature epithelium is organized (Patalano et al. 2006). A striking feature of ascidians is the de novo formation of the apical domain during notochord morphogenesis (Fig. 5d) (Dong et al. 2009). When the notochord cells reach a certain volume, the center of contact between neighboring cells differentiates and acquires apical domain properties. This process is typical polarity establishment. Like in *Drosophila* embryos and cells in a Petri dish, Par-3/Par-6/aPKC components in the ascidian notochord show polarized distribution, and Par-3 is the key molecule controlling the formation of polarity (Denker et al. 2013) (Fig. 3d). However, the upstream signal regulating RAP-3 is unknown.

Tissue invagination: apical constriction and beyond

The contraction of apical actomyosin networks, attached to AJs, drives the shrinking of the apical area of the cell, resulting in a cell shape change from columnar to wedge or bottle shape. Apical constriction is widely used in different developmental contexts, especially in the initiation of gastrulation (Fig. 4). Tissue invagination mediated by apical constriction is a key step of transition from a flat 2-D cell sheet to 3-D

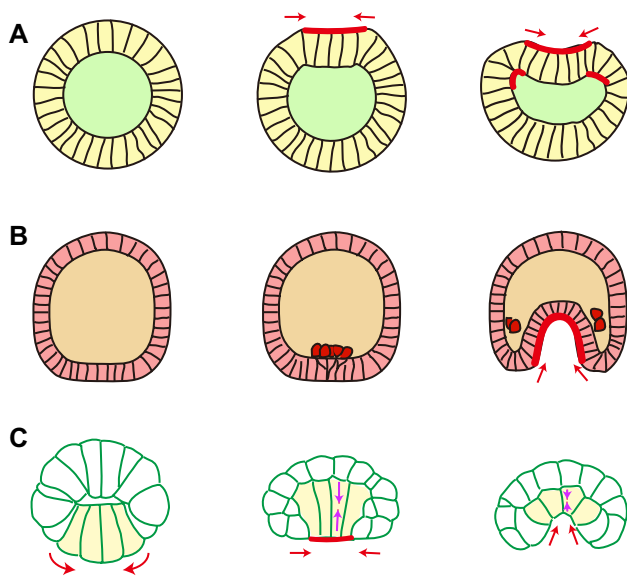


Fig. 4 The apical constriction initiates the gastrulation. **a** Sea anemone. **b** Sea urchin. **c** Ascidian

structure, such as pits and tubes. Being a very widespread process, tissue invagination takes place in all developmental processes, from gastrulation, neurulation to eye cups and renal tubule formation (Martin and Goldstein 2014).

The molecular regulation of apical constriction has been extensively studied in ventral furrow formation during *Drosophila melanogaster* gastrulation (Coravos and Martin 2016; Martin et al. 2009, 2010; Mason et al. 2013; Vasquez et al. 2014; Xie and Martin 2015). These studies provide a detailed understanding of how apical constriction is achieved and regulated spatially and temporally, and how apical constriction drives tissue to reshape on a multiple cellular level.

At the onset of gastrulation in the sea anemone *Nematostella*, the animal pole of cells forms a pre-endodermal plate, which then invaginates into the blastocoel via typical apical constriction with the strong accumulation of F-actin at the apical regions of constricting cells (Magie et al. 2007; Tamulonis et al. 2011). Surprisingly, inhibition of myosin activity cannot completely eliminate apical constriction, which suggests the existence of additional mechanisms for this constriction (Pukhlyakova et al. 2018). Shortly after, the blastoporal cells, which surround the pre-endodermal plate, constrict on the basal side and promote further invagination of the pre-endodermal plate. The constriction from the blastoporal cells relies on myosin activity (Pukhlyakova et al. 2018); however, the molecular pathway of this process remains unclear.

During gastrulation of sea urchins, the skeletogenic mesenchyme cells, also called primary mesenchyme cells (PMCs), leave the vegetal region of the spherical blastoderm and ingress into the blastocoel under the control of

transcriptional factor *snail* (Wu and McClay 2007). During the detachment, PMCs undergo apical constriction (Sepúlveda-Ramírez et al. 2018) and membrane remodeling via endocytosis (Wu and McClay 2007). The cells in the vegetal region thicken and flatten, resulting in the formation of the vegetal plate and the shape change of the blastoderm. The vegetal plate invaginates about 1/4 to 1/2 the way into the blastocoel via apical constriction, forming the archenteron (Kimberly and Hardin 1998). After a short pause, the archenteron starts elongating, a process that involves different cellular mechanisms (see below).

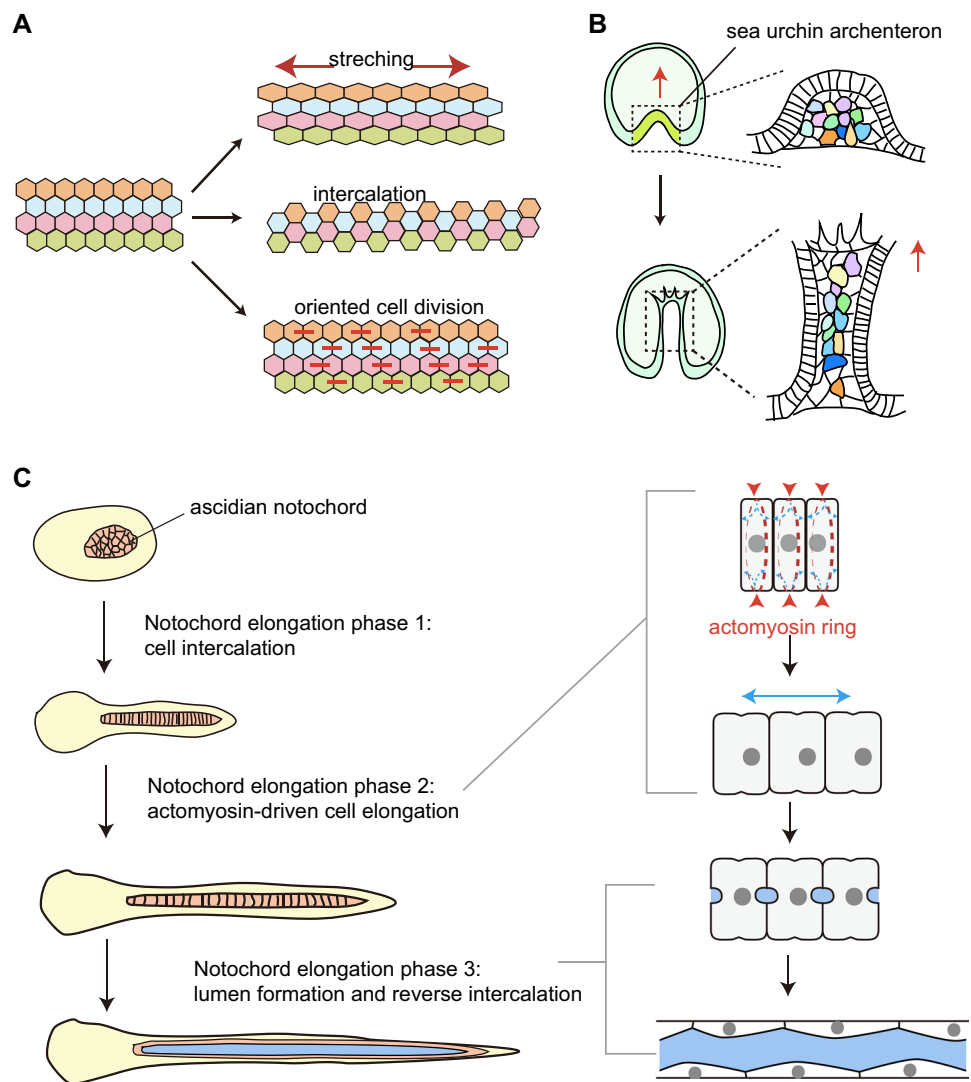
Apical constriction is crucial for tissue bending. In addition, there are other mechanisms that contribute to this process. For example, cell mitotic rounding (Kondo and Hayashi 2013) and apoptosis (Monier et al. 2015) are actively involved in tissue invagination in certain developmental contexts. Endoderm invagination in ascidians utilizes apical constriction as the first step. Interestingly, in the next step the apical–basal shortening mechanism drives the bona fide invagination process, as demonstrated both experimentally and by numerical simulations (Sherrard et al. 2010). Surprisingly, the apical–basal shortening relies on Rho-independent myosin contractility, although the molecular pathway is unknown.

Tissue elongation

Tissue elongation is another important event during embryogenesis and organogenesis. Tissue elongation can be achieved in different ways such as stretching by external forces, cell intercalation and oriented cell division (Fig. 5a). The protrusive lamellipodia and filopodia in the leading cells (Malinda et al. 1995; Miller et al. 1995) and the migration of neighboring cells (Lye et al. 2015) are the commonly used external forces. Normally, tissue elongation by stretching as a result of external forces is transient (Kong et al. 2017). Subsequent cell rearrangement or cell-oriented division compensates for the tissue strain from the external forces (LeGoff and Lecuit 2015).

In the gastrulation stage of sea urchins, following archenteron invagination, there is a pause prior to archenteron elongation. The dominant driving force of archenteron elongation is cell intercalation, as the number of cells around the circumference of the archenteron decreases (Hardin 1989, 1990) (Fig. 5b). The second contributing factor to archenteron elongation is cell shape change with the thickened cells of the vegetal plate become thinner. However, the molecular regulation of this is not clear yet. The third contributing factor is the filopodia from secondary mesenchyme cells (SMCs) located at the tip of the archenteron. These filopodia extend through the blastocoel, searching the proper positions on the wall of the blastocoel by continually

Fig. 5 Tissue elongation. **a** Three mechanisms used in tissue elongation. **b** Sea urchin archenteron elongation involves cell intercalation, as shown in color-coded cells, as well as stretching from SMCs protrusion and oriented cell division. **c** The three phases of ascidian notochord elongation. Cell intercalation is involved in the first phase. In the second phase, the contractile actomyosin ring drives the notochord cell elongating along the A–P axis. In the third phase, luminal pockets enlarge and the notochord cells migrate bi-directionally, leading to the fusion of the luminal pockets, resulting in a long, continuous, single lumen and an elongated notochord



extending and retracting. As soon as the filopodia attach to the right region of the wall, they pull the archenteron inward (Malinda et al. 1995; Miller et al. 1995). A recent study suggested that oriented cell division was also involved in this process (Martik and McClay 2017). These mechanisms work in coordination, driving the archenteron elongation.

The ascidian notochord is an ideal model to study the mechanisms of tissue elongation. Its morphogenesis is well documented (Jiang and Smith 2007; Lu et al. 2019; Smith 2018). The notochord arises from two lineages, the anterior 32 notochord cells derive from blastomeres A7.3, A7.7 and their bilateral partners, and the posterior eight cells derive from blastomere B8.6 and its bilateral partner. After two rounds of mitosis of the blastomeres, the notochord precursor forms a monolayer of 40 cells which will undergo a series of cell shape changes and rearrangements, giving rise to a swimming larva with an elongated tail (Smith 2018). The ascidian notochord elongates in three phases (Lu et al. 2019): ① intercalation of cells drives the notochord to form

a monolayer structure with only one row of cells; ② the cell shapes change leading to the elongation of notochord along the A–P axis; ③ tubulogenesis further lengthens the notochord, resulting in a straight tube that is closed at both ends (Fig. 5c–f).

In the first phase, the notochord precursor cells invaginate into the midline of the embryo via an apical constriction (Munro and Odell 2002). The molecular dynamics in this process is yet to be studied in detail. Subsequently, cells undergo intercalation and deformation in the cylindrical rod, giving rise to an extended notochord along the A–P axis. The 40 cells are disc shaped and aligned end to end with each other giving a “stack of coins” appearance (Fig. 5c). Actin-based protrusions were observed during this process (Munro and Odell 2002). It was subsequently demonstrated that the protrusive activity relies on FGF3 signaling (Shi et al. 2009) and that the orientation of the protrusions depends on PCP signaling (Jiang et al. 2005; Keys et al. 2002).

In the second phase, the coin-shaped cells increase in length along the A–P axis forming a tambour-shaped structure (Fig. 5c). Experimental studies and physical models indicate that the process is driven by the actomyosin contractile ring (Dong et al. 2011; Sehring et al. 2014). The actomyosin contractile ring is reminiscent of the cytokinetic ring in terms of its compositions and architecture. Nevertheless, the consequences of these two similar ring structures are completely different, with one driving cell elongation and the other separating the parent cell into two daughter cells. Nevertheless, several questions remain to be answered, for example: How does functional differentiation occur in different cellular contexts? Is Rho signaling regulated differently in these two scenarios? Does the material properties of cell cortex matter?

Tubulogenesis takes place during the third phase. After the cells form tambour shape, a novel luminal domain (apical domain) emerges at the center of contact between two adjacent notochord cells, which requires the Par-3/Par-6/aPKC system (Denker et al. 2013). This process resembles lumen formation in hepatocytes and MDCK cells. The emergence of the new apical domain requires interplay between RhoA and Rac1 activities in MDCK cells (Overeem et al. 2015). Whether similar mechanisms are used in the ascidian notochord needs to be determined by further studies. The apical domain expands with the increase of the extracellular lumen volume, leading to bi-concave notochord cells (Fig. 5e). After lumen growth, the cell surface increases 1.5-fold (Dong et al. 2009). The molecular nature of how to fuel cell surface growth is unclear. Vesicle trafficking is one potential mechanism. Lumen inflation requires transmembrane transport of fluid into the lumen and the power for such transport is an osmotic gradient that originates from the high amount of glycosaminoglycans and ions in the lumen (Denker and Jiang 2012). Anion transporter Ci-Slc26a α is indispensable for lumen expansion, but is not involved in apical/luminal domain specification and biogenesis (Deng et al. 2013). At the onset of lumen pocket formation, cytoplasmic flow in the notochord cells is observed (Mizotani et al. 2018), which might be interpreted as transportation of material for apical membrane growth and lumen inflation. Such cytoplasmic flow requires the interaction of the scaffold protein 14-3-3 ϵ a with ERM (ezrin/radixin/moesin) at the basal cortex of the cell. 14-3-3 ϵ a and ERM display pulsatile accumulation at the basal cortex, in parallel with the translocation of these two proteins to the luminal domain. Chemical inhibition of 14-3-3 ϵ a leads to the disappearance of cytoplasmic flow, thereby hampering lumen formation. This phenotype can be copied by morpholino knock-down of ERM (Mizotani et al. 2018). However, the physical nature underlying the cytoplasmic flow is unknown.

When the lumen pockets reach a certain volume, a highly coordinated cellular rearrangement is triggered via

an unknown mechanism, leading to the fusion of separate lumens. At the completion of apical domain expansion, the volume of the extracellular lumen increases resulting in bi-concave notochord cells with a deep curvature. At this stage, notochord cells start changing shape dramatically. On one side the anterior and posterior edges retract and move close to each other, whereas on the opposite side the cell extends anteriorly and posteriorly. Neighboring cells undergo the opposite movement. As a result of this collective movement, the anterior and posterior edges meet and adjacent lumens coalesce. New cell–cell contacts are established during this process such that any given notochord cell has six cell–cell contacts instead of the initial two contacts. As a consequence, the number of neighboring cells of a given notochord cell increases from two to four. Due to the similarity with the conventional cell intercalation, this process is called reverse intercalation (Dong et al. 2009). Such coordinated and self-organized cellular behavior prompts the following questions: How do the cells coordinate with each other? Is mechano-transduction process involved? How is the reverse intercalation initiated? Does the reverse intercalation rely on the integrity of tissue? Answering those questions will help us to increase our understanding of tissue elongation and development.

Metamorphosis in ascidians

Morphogenesis can occur in the post-embryonic stage, for example during metamorphosis in insects, amphibians and fish. Metamorphosis, a process that transforms the larva into an adult, is widespread in the animal kingdom ranging from Porifera to Chordata (Werner 1988). This transformation involves complicated morphogenetic movements and physiological changes, which depend on the degree of difference between the two forms. For example, salamanders undergo only minor changes in body plan, whereas insects and tunicates experience more radical change. An intriguing question is how these cellular events, mechanical forces and regulatory gene networks are coordinated in space and time as the “old” organs are destroyed and “new” organs are established. Numerous studies based on several animal phyla have demonstrated that metamorphosis is reliant on conserved endocrine hormone signaling (Laudet 2011), such as ecdysones in insects and thyroid hormones (THs) in cephalochordates (Paris et al. 2008), urochordates (Patricolo et al. 2001), teleost fishes (Schreiber and Specker 1998; Yamano and Miwa 1998) and amphibians (Yaoita 2019). Among these, the tail resorption of *Xenopus* tadpoles is the most spectacular and best-known example of a THs-dependent chordate metamorphic event. Localized morphogenetic and physiological changes in tadpole tissues, such as muscle cell

death and the growth of hindlimbs, are strongly related to their differential sensitivity to THs level.

Compared with terrestrial animals, less is known about metamorphosis in marine organisms. Many marine invertebrates that undergo metamorphosis, including the corals (Grasso et al. 2011), sea urchins (Cameron and Hinegardner 1978; Rahman et al. 2012) and sea squirts (Theriot and Mitchison 1993), possess a biphasic life history with a pelagic larval and benthic adult phase. Although metamorphosis in most marine invertebrates remains to be explored, tunicates have provided an excellent model. One of the best-known metamorphic events in this group is tail regression during the transition from larva to juvenile in ascidians (Borisy and Svitkina 2000). Tail regression of certain ascidian larvae can be completed within 15–20 min after the initiation of metamorphosis (Rottner and Schaks 2019). The morphogenetic mechanism underlying the remarkable speed of this process has fascinated biologists for years. For example, although the cellular events during the tail regression have been well described (Kamran et al. 2017), the mechanical force that drives the withdrawal of the tail into the trunk remains ambiguous. It has been proposed that the mechanical forces are provided by the contractile notochord, epidermis or muscle tissue (Kominami and Takata 2004). Programmed cell death is also involved in ascidian tail regression and contributes to tail shortening in some species (Munro and Odell 2002). To date, however, there is no direct evidence to show that such a mechanism is sufficient to drive tail regression at the speed observed. By live imaging, genetic perturbations and biophysical modeling, it is becoming possible to elucidate the underlying cellular processes and mechanical basis. Ascidian tail regression is emerging as a new model to study the mechanism of morphogenesis, which might provide new insights into how an organism couples mechano-chemical signals to achieve tissue transformation.

Conclusion and perspectives

A handful of cellular mechanisms and biophysical constraints give rise to a broad developmental phenomenon, embedded in different developmental contexts. For example, cell apical constriction mediated by actomyosin contractility is involved in most tissue-bending processes, and cell intercalation occurs in most tissue elongation processes. This prompts an interesting question: How do these animals use similar toolboxes to generate such different forms of life? To answer this question, we need data not only on well-established model organisms such as *Drosophila* and *C. elegans*, but also other, especially marine, animals which show a striking diversity of developmental morphologies. Significant progress on marine animal developmental research in

recent years has expanded our understanding. For example, a recent study found that the *Hox* gene is involved in axial identities in the developing embryo of sea anemones and proposed that the axial *Hox* code may have controlled body patterning before the evolution of the bilaterian A–P axis (He et al. 2018). The second example is the discovery of reverse intercalation in ascidian notochord morphogenesis as a novel strategy to form a tubular organ (Dong et al. 2009). Subsequently, this type of tubulogenesis is also observed in zebrafish vascular anastomosis (Herwig et al. 2011) and gut formation (Alvers et al. 2014), suggesting that this novel mechanism of morphogenesis found in marine animals is highly conserved. Many questions remain unanswered, for example, the mechanisms of cell coordination in ascidian notochord morphogenesis, and the molecular pathway to link tissue invagination and cell cycle in the sea anemone blastula. Knowledge and tools developed in *Drosophila* and other model organisms, together with the striking features of marine animals, will contribute much to our understanding of basic biological processes.

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Compliance with ethical standards

Conflict of interest All the authors declare that they have no conflict of interest.

Animal and human rights statement This article does not contain any studies with human participants or animals performed by any of the authors.

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