



## Review article

## Ascidian notochord elongation

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## ARTICLE INFO

## Keywords:

*Ciona*  
 Notochord  
 Elongation  
 Actomyosin ring  
 Circumferential contraction

## ABSTRACT

The elongation of embryo and tissue is a key morphogenetic event in embryogenesis and organogenesis. Notochord, a typical chordate organ, undergoes elongation to perform its regulatory roles and to form the structural support in the embryo. Notochord elongation is morphologically similar across all chordates, but ascidian has evolved distinct molecular and cellular processes. Here, we summarize the current understanding of ascidian notochord elongation. We divide the process into three phases and discuss the underlying molecular mechanisms in each phase. In the first phase, the notochord converges and extends through invagination and mediolateral intercalation, and partially elongates to form a single diameter cell column along the anterior-posterior axis. In the second phase, a cytokinesis-like actomyosin ring is constructed at the equator of each cell and drives notochord to elongate approximately two-fold. The molecular composition and architecture of the ascidian notochord contractile ring are similar to that of the cytokinetic ring. However, the notochord contractile ring does not impose cell division but only drives cell elongation followed by disassembly. We discuss the self-organizing property of the circumferential actomyosin ring, and why it disassembles when certain notochord length is achieved. The similar ring structures are also present in the elongation process of other organs in evolutionarily divergent animals such as *Drosophila* and *C. elegans*. We hereby propose that actomyosin ring-based circumferential contraction is a common mechanism adopted in diverse systems to drive embryo and tissue elongation. In the third phase, the notochord experiences tubulogenesis and the endothelial-like cells crawl bi-directionally on the notochord sheath to further lengthen the notochord. In this review, we also discuss extracellular matrix proteins, notochord sheath, and surrounding tissues that may contribute to notochord integrity and morphogenesis.

## 1. Introduction

Multicellular organisms develop from a single-celled zygote, which proliferates and differentiates into multiple cells and diverse tissues during embryonic development. Simultaneously, the development of embryo is accompanied by tissue morphogenesis and organogenesis, which are usually self-organizing processes. One of the prominent features of animal development is elongation of body along the anterior-posterior (A-P) axis (Keller, 2002). The mechanisms that regulate this fundamental morphogenesis process remain a central unsolved mystery in developmental biology.

Notochord is one such key embryonic organ to undergo elongation during morphogenesis. It is typically present in the midline region of the embryo tail, where it functions to provide structural support and performs as a signaling center to regulate the development of surrounding tissues (Stemple, 2005). In all three chordate subphyla-cephalochordates, ur-

chordates (tunicates), and vertebrates, the notochord develops into a flexible yet stiff rod tapered at both ends (Veeman and Smith, 2013). These physical and morphological properties allow it to serve as a flexible skeleton, which contributes to the swimming behavior of the larva (Keller, 2006). Notochords in different subphyla are morphologically variable at the cellular level: tunicates have the simplest notochord made of a small number of cells—40 cells in all ascidian species that have been examined and enclosed in a simple extracellular notochordal sheath, while notochord in cephalochordates and vertebrates is much more complex, consisting at least of thousands of cells and possessing abundant extracellular matrix in the notochordal sheath (Annona et al., 2015).

Despite the differences in cellular complexity, the early phase of notochord development and elongation begins with a common and well-studied process of convergent extension driven by cell intercalation (Shindo, 2018). Recently, convergent extension-like behavior was also found in the elongation of an axial structure in the non-chordate,

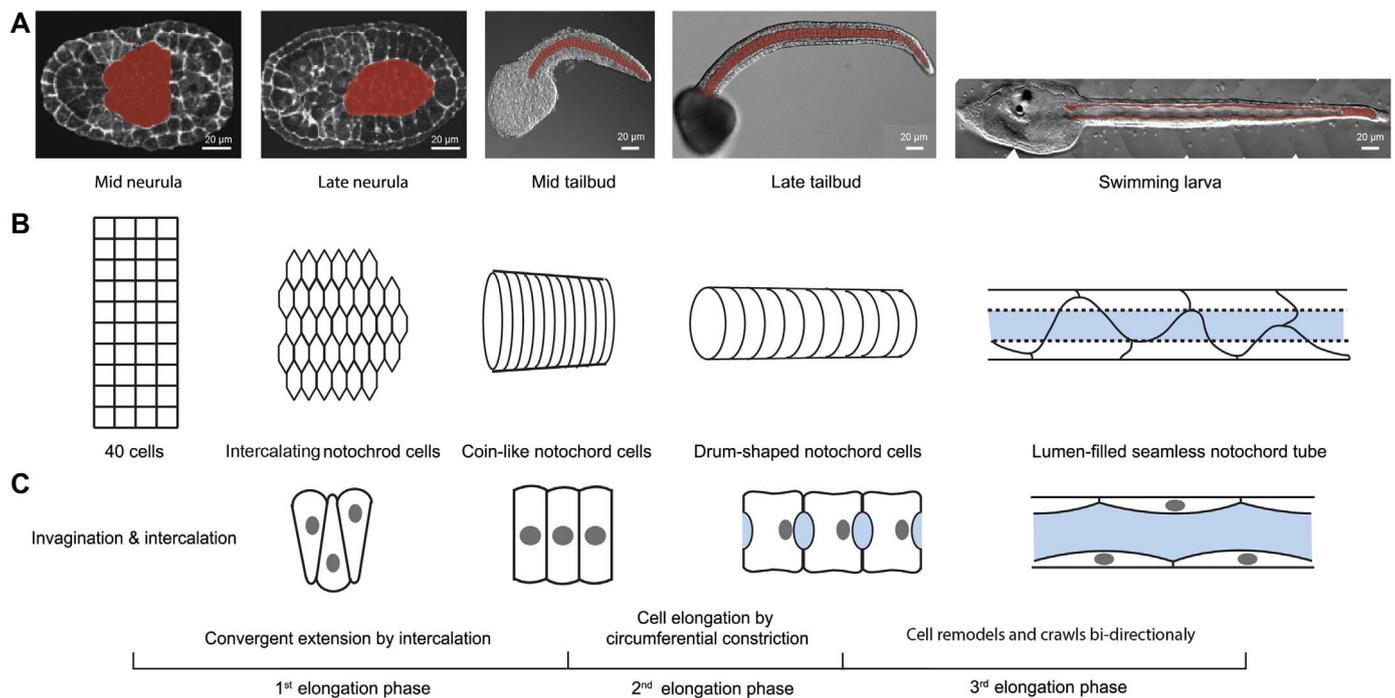
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<https://doi.org/10.1016/j.ydbio.2018.11.009>

Received 10 April 2018; Received in revised form 15 November 2018; Accepted 15 November 2018

Available online 17 November 2018

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**Fig. 1.** Three distinct phases of ascidian notochord elongation. (A) Notochord tissues (indicated by red color) in ascidian embryos at different stages and in a swimming larva. Images were borrowed from (Jiang and Smith, 2007) and (Sehring et al., 2014). (B) Notochord cell shapes and architecture during ascidian embryonic and larval development. Gray blue indicates lumen in the notochord tube. (C) The development of ascidian notochord in three distinct elongation phases.

called axochord, which appears to contribute to the lengthening of the embryo, and the authors proposed that axochord and notochord share a common ancestry (Lauri et al., 2014), which remains controversial (Hejnal and Lowe, 2014). After completion of convergent extension, further elongation of the notochord occurs by various mechanisms in different chordate lineages. For example, the vertebrate notochord elongates by cell proliferation and accumulation of large intracellular vacuoles (Ellis et al., 2013) or by cell migration (Hara et al., 2013). In contrast, proliferation of ascidian notochord cells, as in species *Ciona intestinalis*, stops before convergent extension. Subsequent elongation is driven by the activity of actomyosin contractile ring (Dong et al., 2011; Sehring et al., 2014) and a dramatic remodeling and active migration of notochord cells (Dong et al., 2009). This multiphase process lasts for 24 h (at 16 °C) in *Ciona* embryos and bring about a 3-fold elongation of the notochord.

We have divided the processes of ascidian notochord elongation into three phases based on the distinct cellular mechanisms that take place sequentially (Fig. 1). In the first phase, notochord cells form a single cell line along the A-P axis through invagination and mediolateral intercalation. In the second phase, 40 tightly arranged coin-like notochord cells elongate by approximately 2-fold, which is a significant elongation relative to the first phase, forming a cylindrical, solid cell rod through actomyosin network driven-cell shape change. In the third phase, the notochord cells start to crawl bi-directionally along the notochord sheath and transit into endothelia-like cells, resulting in further notochord elongation by approximately 1.5-fold (Dong et al., 2009).

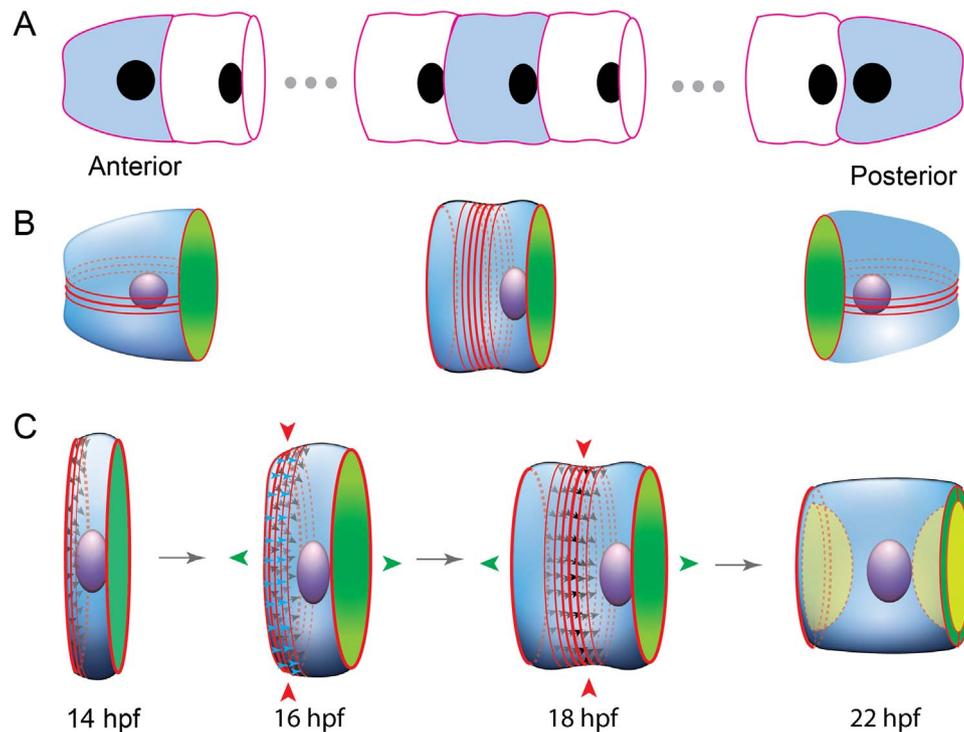
In this review, we summarize the cellular mechanisms used during ascidian notochord elongation and the underlying molecular mechanisms. We focus more on the cellular processes and molecular mechanisms during the second phase, and elaborate on how the contractile ring forms, how it maintains its equatorial position, and how it drives the notochord elongation in ascidian embryo and larva.

## 2. Notochord elongation by convergent extension (Phase 1)

Convergent extension (CE) is a fundamental strategy of tissue elongation during embryogenesis (Keller et al., 2008). Cells that

intercalate along one direction can increase the length of the tissue while reducing its width along the direction of intercalation. Like in ascidians, *Xenopus laevis*, and *Danio rerio* embryos, notochord formation utilizes the mechanism of CE (Glickman et al., 2003; Keller et al., 1989; Miyamoto and Crowther, 1985; Munro and Odell, 2002b). CE in ascidian notochord formation has been described in detail via careful observations of live and/or fixed embryos of *C. intestinalis* (Miyamoto and Crowther, 1985; Munro and Odell, 2002b) and *Corella inflata* (Miyamoto and Crowther, 1985; Munro and Odell, 2002b). After the final cell division, 40 notochord cells that form a monolayer invaginates around the midline of the embryo to form a cylindrical rod. At the same time, notochord cells intercalate mediolaterally and, as a consequence, the notochord transforms into a stack of 40 coin-shaped cells. During intercalation, notochord cells crawl actively among the neighboring cells using polarized filamentous actin-based lamellar protrusions. Mutants that fail to form lamellar protrusions have convergent extension defects, suggesting that cell migration is indispensable for this process in notochord (Shi et al., 2009).

Although CE in the ascidian embryo is cell-autonomous (Keller, 2002; Munro and Odell, 2002a), several intercellular and intracellular signaling pathways such as the fibroblast growth factor (FGF) pathway, the Wnt, the activators and target of notochord-specific transcription factor *Brachyury*, suppressor of *Hairless*, *leprecan*, and the planar cell polarity (PCP) pathways are required for the formation of lamellar protrusions and subsequent cell migration during CE (Corbo et al., 1998; Dunn and Di Gregorio, 2009; Jiang et al., 2005; Niwano et al., 2009; Shi et al., 2009). The formation of polarized lamellar protrusions is suppressed by introducing a dominant negative form of FGF3, which results in defective notochord intercalation (Shi et al., 2009). Notochord cells in *Ciona* embryo mutant for *Prickle*, a gene in the PCP pathway, can still form lamellipodia but fail to intercalate mediolaterally (Jiang et al., 2005). In addition to planar cell polarity, it has also been revealed that polarity along the dorso-ventral axis of the notochord may also contribute to CE. The disruptions of the asymmetric accumulation of aPKC and laminin, by introducing a dominant negative form of Eph4 lead to defective intercalation (Oda-Ishii et al., 2010). The role of extracellular matrix (ECM) plays in CE has been



**Fig. 2.** Mechanism of the second phase of notochord elongation in *Ciona*. (A) An overview of the solid cylindrical rod-shaped structure of the notochord. (B) Highlights of the actomyosin structure (red) of notochord cells (grayish-blue cells in A). Median notochord cells (cell 2–39) form circumferential actomyosin rings at cell equators, whereas semi actomyosin rings parallel to A-P axis are formed in both cells at the anterior (cell 1) and posterior (cell 40) ends of the notochord. (C) The formation and positioning of the equatorial actomyosin rings. At the beginning of the second phase of notochord elongation (14 h post fertilization, hpf), coin-shaped notochord cells form circumferential actomyosin ring at the anterior pole of the cells. As the development proceeds, the actomyosin ring moves posteriorly (blue arrowheads) and finally localizes at the cell's equators (18 hpf). During this process, the actomyosin ring is contractile and produces a circumferential constriction (dark arrows) but do not divide the cells into two. Transverse loading generated by these constrictions presumably drives the notochord to narrow and thus elongates along the A-P axis (green arrowheads).

increasingly recognized (Shindo, 2018), including in ascidian notochord. For example, chondroitin 6-O-sulfotransferase, an enzyme that is responsible for sulfated polysaccharide chain binding to core proteins to form proteoglycans, is required for CE of the *Ciona* notochord (Nakamura et al., 2014). Moreover, in the mutant of *laminin*, which encodes the protein that is the main component of the basement membrane, the notochord cells initiate normal intercalation but fail to organize into a stack of 40 coin-shaped cells, which likely caused by the disruption of the integrity of the notochord sheath (Veeman et al., 2008). Similarly, when fibronectin, a key matrix glycoproteins, was deleted by CRISPR-mediated mutagenesis, the medio-lateral polarity of individual cells was maintained, and the notochord sheath was intact, but intercalation of the notochord cells was abnormal (Segade et al., 2016).

Mechanosensing and mechanoprotection are also essential for proper embryogenesis (Fernandez-Sanchez et al., 2015). Morphogenesis generates forces, which if were not counteracted may damage the embryo. During CE, notochord cells experience forces due to both internal and external cues and need mechanoprotection (Hamada, 2015). Various mechanosensors such as ion channels, stretch receptors, and caveolae reside on the plasma membrane. Caveolae are microdomains that act as membrane reservoir to protect the cells from mechanical stress (Cheng and Nichols, 2016). Previously, a large number of caveolae were found in the zebrafish notochord cells (Nixon et al., 2007). Recently, it was found when the number of caveolae was reduced experimentally, the tail length became shorter and lesions were observed in the notochord (Garcia et al., 2017; Lim et al., 2017). This highlights the importance of mechanoprotection during notochord elongation. Another study conducted in mouse embryo showed that notochord elongation coincides with the expansion of amniotic cavity expansion (Imuta et al., 2014). When a glass capillary insertion, the expansion of amniotic cavity was prevented by

draining the amniotic fluid, notochord elongation is disrupted. Subsequent observation showed that notochord became wider due to a failure in CE. The authors concluded that the force exerted by the expansion of the amniotic cavity drives notochord elongation in mouse embryos (Imuta et al., 2014). However, in a study carried out in *Xenopus* embryos, a similar global force provided by the surrounding tissue does not elongate the embryo. In this case, the primary force generated by cell migration was responsible for the elongation of the notochord (Hara et al., 2013). In *Ciona* embryos, when the *Xenopus* homeobox gene *bix* is expressed in the surrounding muscle cells in *Ciona*, the elongation of notochord is compromised due to failure of muscle cell differentiation (Di Gregorio et al., 2002). And the most probable reason is that less force is contributed by the undifferentiated muscle cells during tail morphogenesis. These examples show that mechanical forces are also important for notochord elongation.

### 3. Notochord elongation by equatorial circumferential constriction (Phase 2)

After CE, the ascidian notochord elongates further (Dong et al., 2009; Miyamoto and Crowther, 1985) by a mechanism that is unique to ascidians. In this second phase, notochord cells generate an equatorial cytokinesis-like ring, which is for cell elongation rather than cell division (Dong et al., 2011; Sehring et al., 2014). In the following sections, we will discuss our current understanding of this novel mechanism for notochord elongation in *Ciona*.

#### 3.1. Architecture of circumferential rings in *Ciona* notochord

During the second phase, the shape of ascidian notochord cells changes from a coin shape to a drum shape by the basal constriction localized at the equator of the cells (Fig. 2A), whose molecular

architecture has begun to emerge (Dong et al., 2011; Sehring et al., 2014). The principle components are actin filaments and myosin filaments containing phosphorylated myosin II light chains (pMLC) forming a structure like a contractile ring in the cleavage furrow of dividing cells. Indeed, several observations can prove this point. First, time-lapse movies of elongating notochord cells expressing fluorescence-tagged protein markers show that F-actin and pMLC filaments move toward the equator, which are derived from the both anterior and posterior lateral domain of the notochord cells (Sehring et al., 2014). Similarly, the cortical actomyosin flows are also observed in cytokinetic cells (Yumura, 2001), which are thought to be tension dependent. The two poles of the cell are relaxed, while the surface stiffness remains high tense in the median region of the cell leading to the cortical flows from low tension to regions of high tension regions and thus form the cytokinetic ring (Bray and White, 1988). However, whether the actomyosin flows in *Ciona* notochord are relevant to the cell surface tension, and why the actomyosin flows arise at both the poles of the cell remain unknown. Second, the quantitative results from fluorescence recovery after photobleaching experiments also indicate that F-actin in the equatorial plane is highly dynamic with high turnover in the notochord (Sehring et al., 2014). Indeed, during the cytokinesis in many eukaryotes, the actomyosin rings are highly dynamic, the F-actin and other ring components such as myosin and actin-binding proteins are observed in high turnover during contraction (Chew et al., 2017; Guha et al., 2005; Srivastava and Robinson, 2015). Third, multiple actin-binding proteins such as cofilin,  $\alpha$ -actinin, tropomyosin, IQGAP, anillin, septin 2, talinA, and talinB are colocalized with the actomyosin ring in the notochord cell, resembling remarkably as in the case of the dividing cells (Mukhina et al., 2007; Sehring et al., 2014). Taken together, the architecture of the circumferential rings in *Ciona* notochord shares the common features with the cytokinetic ring in the furrow of the dividing cell, though some differences might exist.

### 3.2. Mechanics and principle of *Ciona* notochord elongation driven by equatorial actomyosin ring

During cytokinesis, the dividing cell elongates along the longitudinal axis as the furrow deepens at the equatorial region of the cell (Spira et al., 2017). The physical basis of this observation is that the transverse loading generated by the circumferential constrictive force on a compression-resisting system is converted into pushing force that is especially higher along the longitudinal axis, thus driving the cell to elongate. Since the relative cellular arrangement and cell volume remain unchanged during the second phase of notochord elongation (Dong et al., 2009), the notochord cell can be treated as a compression-resisting system, suggesting the possibility that the notochord cell uses the same principle to drive cell elongation as that used by the cytokinetic cell. Recent evidence based on genetic and drug manipulation has confirmed this possibility (Sehring et al., 2014).

The constriction and elongation of the notochord cells cease after treatment with myosin II ATPase inhibitor or F-actin inhibitor, suggesting that the equatorial ring exert contractile force around the cell circumference (Dong et al., 2011; Sehring et al., 2014). Spatiotemporal coupling of the retractions of bleb-like membrane deformations and the recruitment of actomyosin contractile elements are detectable during notochord cell elongation. Additionally, blebbing at the basal membrane and the displacement of apical membrane also reveal a strong temporal correlation, suggesting that the contraction at the equatorial contractile ring drives the elongation of notochord cells. By contrast, the first and 40th cells of the notochord normally do not assemble at the equatorial contractile ring and do not elongate during the second phase (Fig. 2B). Furthermore, genetic analyses also show that the equatorial actomyosin rings are essential for notochord cell elongation. For example, cells expressing the dominant negative form of cofilin, which interferes with the actin dynamics, are unable to elongate and are squeezed by the flanking wild type cells, resulting in a dumbbell shaped notochord cell

(Sehring et al., 2014). In the mutant expressing  $\alpha$ -actininROD, which disrupts the connection between actin filaments and their connection with the equatorial membrane, actomyosin ring is mislocalized, thus inhibiting cell elongation (Sehring et al., 2014).

### 3.3. Positioning of the contractile ring during notochord cell elongation

Where and how the contractile apparatus is positioned in the cell have been some of the major questions complicating our understanding of the cytokinesis process (Oliferenko et al., 2009). The current view of the timing and positioning of the contractile ring during cytokinesis is based on mechanisms related to microtubule spindle. For example, in some cell types, astral microtubules reduce the local tension produced by actin-myosin bundles at the cell cortex and increase the tension at the spindle equator, thus promoting cortical contraction at equatorial site (Alberts et al., 2014). However, neither central spindle nor astral microtubules are found during notochord cell elongation (Dong et al., 2011). Moreover, ring positioning and cell elongation are not affected, as microtubule is depolymerized by nocodazole (Sehring et al., 2014). These data suggest that the microtubules do not contribute to ring positioning in *Ciona* notochord. Therefore, other mechanisms must exist for the positioning the ring correctly in ascidian notochord cells.

The phenotype of dominant negative of  $\alpha$ -actinin, which bridges actomyosin ring localization and cell elongation, suggests that the equatorial position of the ring is pivotal for the cell elongation (Sehring et al., 2014). Recently, studies involving live cell imaging and physical modeling have provided new insights into the positioning of the contractile actomyosin ring in *Ciona* notochord. A weak cortical actomyosin ring is generated close to the anterior pole during cell intercalation, which gradually relocalizes toward the equatorial region of notochord cells (Fig. 2C). This re-localization is actomyosin contraction dependent. Previous study showed that the cortical actomyosin ring fails to localize the midway of the notochord cells when contraction is blocked by blebbistatin (Sehring et al., 2015). Furthermore, when the elongated notochord cells are treated with blebbistatin for a prolonged time, the equatorial actomyosin rings move toward the anterior pole of notochord cells (Sehring et al., 2015). This observation also verifies that the formation of the actomyosin rings is highly dynamic and related with contraction. A simple and appealing explanation for this observation is that the actomyosin cortex is a viscous contractile gel undergoing continuous turnover by actomyosin flow, which is initially generated from both ends of the notochord cell and then moves toward the equator, resulting in a higher contraction compared with its surroundings. The small furrow created as a result of the contraction possibly leads to more accumulation of actomyosin in this region, making it denser and thus more contractile. A physical modeling based on this idea correctly predicts the movement and positioning of the actomyosin rings (Sehring et al., 2015). However, the relocalization and positioning of actomyosin rings is not simply regulated by constriction but rather by the precise interaction between the PCP signaling and contractility. In the mutant of one of PCP genes, *prickle*, the actomyosin rings are normally localized at the equator of notochord cells. However, when the actomyosin contraction are abolished in *prickle* mutant, the rings do not migrate toward anterior pole of the cells as that in blebbistatin-treated embryos. Instead, they randomly distribute either in equator, anterior, or posterior pole of the cells (Sehring et al., 2015). Therefore, the position of notochord actomyosin ring is maintained at the cell equatorial region by a tug-of-war between the actomyosin contractility and planar cell polarity.

### 3.4. Novel mechanism of cell elongation and unanswered questions

Exploiting the cytokinetic-like contractile elements and regulatory proteins to drive cell elongation rather than cell division is a novel mechanism of cell elongation, which was first demonstrated in *Ciona* notochord cells (Dong et al., 2011; Sehring et al., 2014). Continued

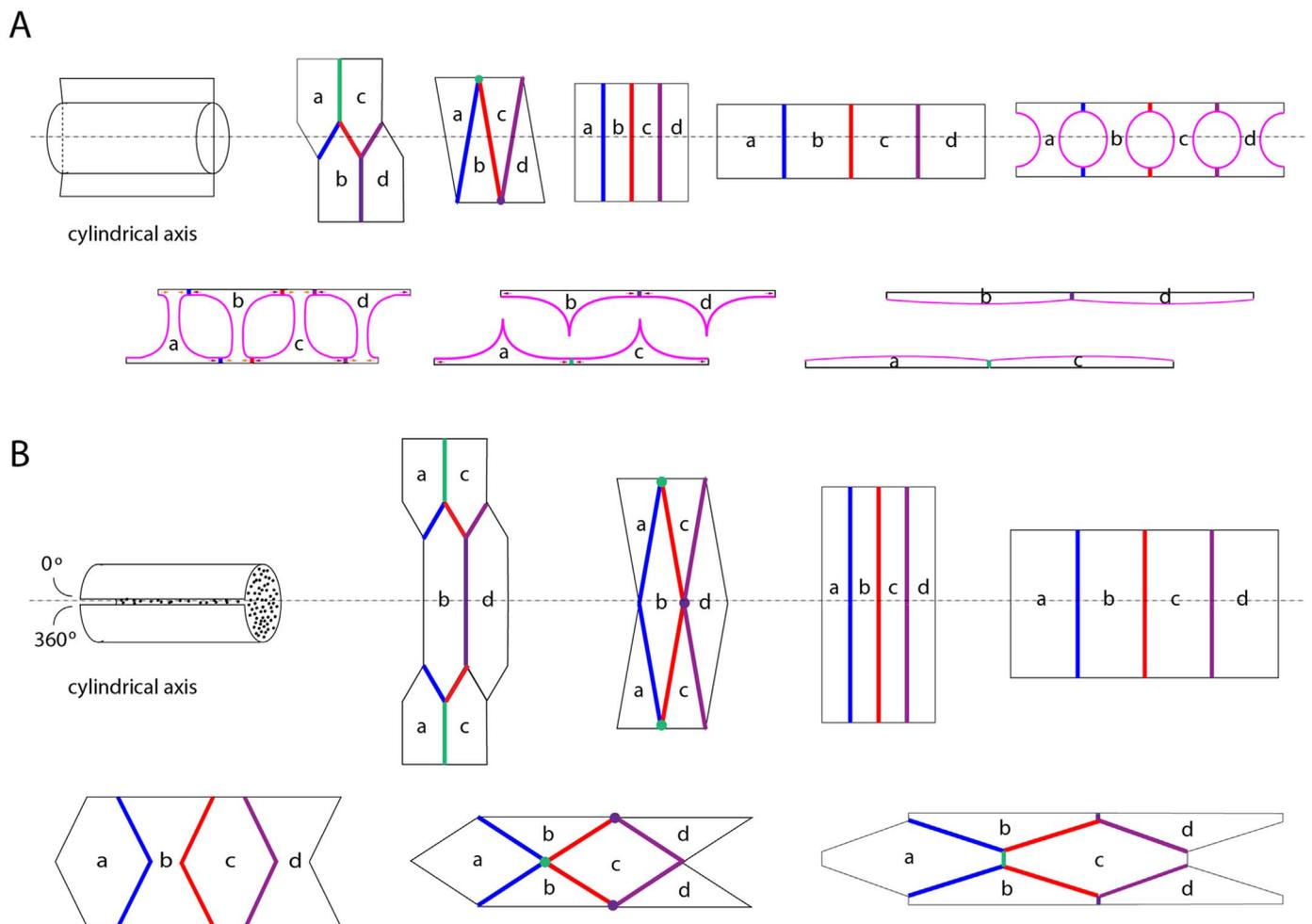
investigation of this novel mechanism would further deepen our understanding of its underlying mechanics and regulation. How the contractile ring disassemble as contraction progresses during notochord cell elongation is the most intriguing. This notochord-lengthening function is also found in another tunicate, *Oikopleura dioica*; however, after a period of non-divided stage, the notochord cell somehow begins to undergo cell division (Soviknes and Glover, 2008). It is, therefore, important to identify why the ascidian notochord does not divide whereas the *Oikopleura* notochord does. In situ hybridization experiments reveal that several cell cycle regulators, such as *Ciona* homologs of CDC45, CDC23 and CDK2AP1 are expressed in the notochord (Hotta et al., 2000). Among these, CDC45, an essential protein required for the initiation of DNA replication (Hotta et al., 2000; Zou et al., 1997), is highly expressed in post-mitotic notochord cells, which contradicts the notion that CDC45 is present in dividing cells. The signaling pathways underlying this contradiction remain elusive.

Additionally, the *Ciona* notochord exhibits dynamic cellular polarities during morphogenesis (Veeman and McDonald, 2016). For example, nuclei of notochord cells, except for the 40th cell, always position at the posterior of the cell cortex (Jiang et al., 2005). This positioning of nuclei is PCP dependent. The apical marker, aPKC, accumulates along the ventral side of the notochord, whereas the basal marker, laminin, is enriched on the dorsal side (Oda-Ishii et al., 2010). Moreover, the cytoskeleton also appears to be polarized during the 2nd

phase of the notochord cell elongation. The MLC localizes equally on the perinotochordal surfaces during the intercalation, however, it is polarized anteriorly in the second phase of notochord elongation (Newman-Smith et al., 2015). Whether these dynamic cell polarities contribute to the ring formation and position are the main challenges that need to be addressed in future studies.

#### 4. Notochord elongation via bi-directional cell migration (Phase 3)

After the completion of notochord cell elongation via the actomyosin ring, the tail still continues to elongate (Dong et al., 2009; Miyamoto and Crowther, 1985), indicating that there must be additional mechanisms for further elongation of the notochord during this period. Previous studies have shown a *de novo* mechanism of tubulogenesis in the *Ciona* notochord, which is thought to be primarily responsible for the 3rd phase of notochord elongation (Dong et al., 2009). During the second phase of notochord elongation, notochord cells undergo a mesenchymal-epithelial transition (Jiang and Smith, 2007). In this process, apical extracellular lumen begins to form at both ends of the notochord cell while transitioning into an unusual epithelial-like cell type with two opposing apical domains (Denker et al., 2013; Dong et al., 2009). As the luminal pockets enlarge, notochord cells attain a bi-concave shape from a drum shape (Fig. 3A). During this time, the apical domains increase in



**Fig. 3.** Schematic representation of the intercalation of cell-cell junctions during the three phases of notochord elongation in *Ciona*. (A) Median views of cell-cell junctions of four consecutive notochord cells (a, b, c, and d). (B) Same views as those shown in (A) flattened onto a 2-D space. At the beginning of convergent extension (CE), cell a develops cell junctions with both cells b (a/b, blue) and c (a/c, green), whereas cells b and c share one junction (b/c, red). The a/c junction is perpendicular to the anterior-posterior (A-P) axis at this time point but disappears with the completion of CE. Remodeling of cell-cell junction is required for the emergence and expansion of extracellular lumens (magenta), which connect and form a tube during the 3rd phase of notochord elongation. The cell-cell junction between cells a and c appears again at the beginning of the 3rd phase, whereas the a/b junction shortens along the Dorsal-ventral (D-V) axis and elongates along the A-P axis. The main results of this reverse intercalation lead the notochord elongation and tube formation in *Ciona*.

surface areas at the expense of the lateral domains. Once the apical extracellular lumens reach a defined volume, the bi-concave shaped notochord cells become motile via unknown mechanisms and migrate bi-directionally on the notochord sheath. At this stage, the two trailing edges of notochord cells retract and move toward the midway along the inner surface of the notochord sheath, whereas the two leading edges extend in the opposite direction. As a consequence, this concerted crawling behavior within the notochord cells tilts the adjacent luminal pockets, finally fusing them into a single lumen (Dong et al., 2009). Recent studies have shown that the mechanism observed in the ascidian notochord is also present in zebrafish, specifically in vascular anastomosis in embryo (Herwig et al., 2011) and lumen formation in the gut of zebrafish (Alvers et al., 2014), suggesting that this is a novel mechanism involved in biological tube formation. During notochord cells crawl in the 3rd phase, the cell shape and cell adhesion junction undergo striking remodeling in a process termed as reverse intercalation (Fig. 3B). Although the molecular mechanism underlying reverse intercalation in notochord cells has not been reported yet, it is likely that the cytoskeleton participates in the regulation of bi-directional crawling of notochord cells. For example, the microtubule forms straight and parallel bundles extending to the leading edges at this period. When microtubules are disrupted by nocodazole, bi-directional crawling becomes inconsistent and fails to connect the tube (Dong et al., 2011). Whether the microtubule generates polarized signals that establish two protrusions at both leading edges or not, and the nature of those signals is essential questions for understanding notochord tubulogenesis, and thus notochord elongation.

### 5. Evolutionary conservation of tissue elongation mechanisms found in the ascidian notochord

Although the common underlying mechanism of notochord elongation is currently unknown, notochord of all chordates possess an evolutionarily conserved property of elongating the body along A-P axis, regardless of their origin. It appears that embryos of most of chordates utilize multiple different mechanisms to drive notochord elongation. For example, after the completion of CE, amphibians and fish notochord elongates by principle of the hydraulic cylinder as it is surrounded by a fibrillar ECM (Keller, 2006). The notochord of amphibians and fish forms vacuoles containing proteoglycans, which hydrate and swell, consequently elongating the notochord in the A-P direction. Because radial expansion is inhibited by the ECM, a cylindrical-shaped elongated notochord is formed (Mookerjee, 1953). However, the most basal chordate, amphioxus (Lemaire, 2011), utilizes cell proliferation as the mechanism for notochord elongation. Studies involving *Branchiostoma lanceolatum* reveal that the amphioxus notochord also contains transverse contractile actin and myosin filaments (Bocina and Saraga-Babic, 2006; Flood et al., 1969); however, the function of these contractile elements have not been determined in notochord morphogenesis. By contrast, the tunicates, which are phylogenetically positioned between the cephalochordates and vertebrates, exploit a distinct mechanism of notochord elongation that is different from other chordates, indicating that the notochord structure and function have changed during chordate evolution. According to the second phase elongation of the ascidian notochord, the circumferential actomyosin network plays an important role in equatorial constriction. Interestingly, a similar notochord-lengthening actomyosin ring is also present in another tunicate, *Oikopleura dioica*. In addition to the ascidian notochord, the non-cytokinetic circumferential actin ring has also been found in other cell types and tissues, including the neural plate cells in amphibians (Holtfreter, 1946), eyes in certain fish species (Lin-Jones et al., 2009; O'Connor and Burnside, 1981), axons in mammals (Xu et al., 2013), ovaries in flies (He et al., 2010), tracheal tube in *Drosophila* (Hannezo et al., 2015; Matusek et al., 2006), and hypodermal cells in the embryo of *C. elegans* (Priess and Hirsh, 1986). Thus, it appears that the lengthening mechanism of

non-cytokinetic actomyosin contractile apparatus is evolutionarily conserved from individual cells to entire tissues (embryos). Another feature of the ascidian notochord morphogenesis is the luminal tube formation, which takes place in the second and third phases of elongation. The elongation of *Ciona* notochord during tubulogenesis is mainly due to dynamic cell remodeling characterized by reverse intercalation. It is expected that polarized cell intercalation can lead to the elongation of a developing organ or embryo, such as that shown in the germ-band elongation in *Drosophila* embryo (Bertet et al., 2004). Therefore, we suggest that mechanisms of elongation exploited by the ascidian notochord are evolutionarily conserved tissue elongation mechanisms found in diverse animal embryos.

### 6. Concluding remarks

The evolutionary conservation of notochord elongation mechanisms remains an open question. Among the chordates, the ascidians have particularly evolved with a distinct mechanism of notochord cell elongation. This knowledge has been available because of recent advances in imaging techniques and transgenic approaches developed for studying the ascidian embryo. These tools have allowed us to investigate the morphogenesis of key tissues, for example, the notochord in the chordate embryo (Stolfi and Christiaen, 2012). This review focuses on our current understanding of how the ascidian notochord cell elongates during embryogenesis, including the dynamic cellular behaviors and novel underlying molecular mechanisms. Multifaceted processes of ascidian notochord elongation provide insights for us to understand the complex but highly coordinated tissue morphogenetic processes.

### Acknowledgements

We would like to thank Di Jiang for his critical reading and comments on the manuscript. B.D was supported by the National Natural Science Foundation of China (Grant no. 31572352, 31771649), Pilot National Laboratory for Marine Science and Technology (Qingdao), the Marine S&T Fund of Shandong Province (No. 2018SDKJ0302-1), and the Taishan Scholar Program of Shandong Province, China (201502035).

### References

- Alberts, B., Johnson, A., Walter, P., Lewis, J., Raff, M., 2014. Molecular Biology of the Cell 6 ed.. Garland Science, New York.
- Alvers, A.L., Ryan, S., Scherz, P.J., Huisken, J., Bagnat, M., 2014. Single continuous lumen formation in the zebrafish gut is mediated by smoothed-dependent tissue remodeling. *Development* 141, 1110–1119.
- Annona, G., Holland, N.D., D'Aniello, S., 2015. Evolution of the notochord. *Evodevo* 6, 30.
- Bertet, C., Sulak, L., Lecuit, T., 2004. Myosin-dependent junction remodelling controls planar cell intercalation and axis elongation. *Nature* 429, 667–671.
- Bocina, I., Saraga-Babic, M., 2006. Immunohistochemical study of cytoskeletal and extracellular matrix components in the notochord and notochordal sheath of amphioxus. *Int. J. Biol. Sci.* 2, 73–78.
- Bray, D., White, J.G., 1988. Cortical flow in animal cells. *Science* 239, 883–888.
- Cheng, J.P.X., Nichols, B.J., 2016. Caveolae: one function or many? *Trends Cell Biol.* 26, 177–189.
- Chew, T.G., Huang, J., Palani, S., Sommese, R., Kamnev, A., Hatano, T., Gu, Y., Oliferenko, S., Sivaramakrishnan, S., Balasubramanian, M.K., 2017. Actin turnover maintains actin filament homeostasis during cytokinetic ring contraction. *J. Cell Biol.* 216, 2657–2667.
- Corbo, J.C., Fujiwara, S., Levine, M., Di Gregorio, A., 1998. Suppressor of hairless activates brachyury expression in the *Ciona* embryo. *Dev. Biol.* 203, 358–368.
- Denker, E., Bocina, I., Jiang, D., 2013. Tubulogenesis in a simple cell cord requires the formation of bi-apical cells through two discrete Par domains. *Development* 140, 2985–2996.
- Di Gregorio, A., Harland, R.M., Levine, M., Casey, E.S., 2002. Tail morphogenesis in the ascidian, *Ciona intestinalis*, requires cooperation between notochord and muscle. *Dev. Biol.* 244, 385–395.
- Dong, B., Deng, W., Jiang, D., 2011. Distinct cytoskeleton populations and extensive crosstalk control *Ciona* notochord tubulogenesis. *Development* 138, 1631–1641.
- Dong, B., Horie, T., Denker, E., Kusakabe, T., Tsuda, M., Smith, W.C., Jiang, D., 2009. Tube formation by complex cellular processes in *Ciona intestinalis* notochord. *Dev. Biol.* 330, 237–249.

- Dunn, M.P., Di Gregorio, A., 2009. The evolutionarily conserved leprecan gene: its regulation by Brachyury and its role in the developing *Ciona* notochord. *Dev. Biol.* 328, 561–574.
- Ellis, K., Bagwell, J., Bagnat, M., 2013. Notochord vacuoles are lysosome-related organelles that function in axis and spine morphogenesis. *J. Cell Biol.* 200, 667–679.
- Fernandez-Sanchez, M.-E., Brunet, T., Roeper, J.-C., Farge, E., 2015. Mechanotransduction's impact on animal development, evolution, and tumorigenesis. In: Schekman, R. (Ed.), *Annual Review of Cell and Developmental Biology* 31, 373–397.
- Flood, P.R., Guthrie, D.M., Banks, J.R., 1969. Paramyosin muscle in the notochord of *Amphioxus*. *Nature* 222, 87–88.
- Garcia, J., Bagwell, J., Njaine, B., Norman, J., Levic, D.S., Wopat, S., Miller, S.E., Liu, X., Locasale, J.W., Stainier, D.Y.R., Bagnat, M., 2017. Sheath cell invasion and trans-differentiation repair mechanical damage caused by loss of caveolae in the zebrafish notochord. *Curr. Biol.* 27, 1982–1989.
- Glickman, N.S., Kimmel, C.B., Jones, M.A., Adams, R.J., 2003. Shaping the zebrafish notochord. *Development* 130, 873–887.
- Guha, M., Zhou, M., Wang, Y.L., 2005. Cortical actin turnover during cytokinesis requires myosin II. *Curr. Biol.* 15, 732–736.
- Hamada, H., 2015. Role of physical forces in embryonic development. *Semin. Cell Dev. Biol.* 47–48, 88–91.
- Hannezo, E., Dong, B., Recho, P., Joanny, J.-F., Hayashi, S., 2015. Cortical instability drives periodic supracellular actin pattern formation in epithelial tubes. *Proc. Natl. Acad. Sci. USA* 112, 8620–8625.
- Hara, Y., Nagayama, K., Yamamoto, T.S., Matsumoto, T., Suzuki, M., Ueno, N., 2013. Directional migration of leading-edge mesoderm generates physical forces: implication in *Xenopus* notochord formation during gastrulation. *Dev. Biol.* 382, 482–495.
- He, L., Wang, X., Tang, H.L., Montell, D.J., 2010. Tissue elongation requires oscillating contractions of a basal actomyosin network. *Nat. Cell Biol.* 12, 1133–1142.
- Hejnol, A., Lowe, C.J., 2014. Animal evolution: stiff or squishy notochord origins? *Curr. Biol.* 24, R1131–R1133.
- Herwig, L., Blum, Y., Krudewig, A., Ellertsdottir, E., Lenard, A., Belting, H.G., Affolter, M., 2011. Distinct cellular mechanisms of blood vessel fusion in the zebrafish embryo. *Curr. Biol.* 21, 1942–1948.
- Holtfreter, J., 1946. Structure, motility and locomotion in isolated embryonic amphibian cells. *J. Morphol.* 79, 27–62.
- Hotta, K., Takahashi, H., Asakura, T., Saitoh, B., Takatori, N., Satou, Y., Satoh, N., 2000. Characterization of Brachyury-downstream notochord genes in the *Ciona intestinalis* embryo. *Dev. Biol.* 224, 69–80.
- Imuta, Y., Koyama, H., Shi, D., Eiraku, M., Fujimori, T., Sasaki, H., 2014. Mechanical control of notochord morphogenesis by extra-embryonic tissues in mouse embryos. *Mech. Dev.* 132, 44–58.
- Jiang, D., Munro, E.M., Smith, W.C., 2005. Ascidian prickle regulates both mediolateral and anterior-posterior cell polarity of notochord cells. *Curr. Biol.* 15, 79–85.
- Jiang, D., Smith, W.C., 2007. Ascidian notochord morphogenesis. *Dev. Dyn.* 236, 1748–1757.
- Keller, R., 2002. Shaping the vertebrate body plan by polarized embryonic cell movements. *Science* 298, 1950–1954.
- Keller, R., 2006. Mechanisms of elongation in embryogenesis. *Development* 133, 2291–2302.
- Keller, R., Cooper, M.S., Danilchik, M., Tibbetts, P., Wilson, P.A., 1989. Cell intercalation during notochord development in *Xenopus laevis*. *J. Exp. Zool.* 251, 134–154.
- Keller, R., Shook, D., Skoglund, P., 2008. The forces that shape embryos: physical aspects of convergent extension by cell intercalation. *Phys. Biol.* 5, 015007.
- Lauri, A., Brunet, T., Handberg-Thorsager, M., Fischer, A.H.L., Simakov, O., Steinmetz, P.R.H., Tomer, R., Keller, P.J., Arendt, D., 2014. Development of the annelid axochord: insights into notochord evolution. *Science* 345, 1365–1368.
- Lemaire, P., 2011. Evolutionary crossroads in developmental biology: the tunicates. *Development* 138, 2143–2152.
- Lim, Y.-W., Lo, H.P., Ferguson, C., Martel, N., Giacomotto, J., Gomez, G.A., Yap, A.S., Hall, T.E., Parton, R.G., 2017. Caveolae protect notochord cells against catastrophic mechanical failure during development. *Curr. Biol.* 27, 1968–1981.
- Lin-Jones, J., Sohlberg, L., Dose, A., Breckler, J., Hillman, D.W., Burnside, B., 2009. Identification and localization of myosin superfamily members in fish retina and retinal pigmented epithelium. *J. Comp. Neurol.* 513, 209–223.
- Matusek, T., Djiane, A., Jankovics, F., Brunner, D., Mlodzik, M., Mihaly, J., 2006. The *Drosophila* formin DAAM regulates the tracheal cuticle pattern through organizing the actin cytoskeleton. *Development* 133, 957–966.
- Miyamoto, D.M., Crowther, R.J., 1985. Formation of the notochord in living ascidian embryos. *J. Embryol. Exp. Morphol.* 86, 1–17.
- Mookerjee, S., 1953. Experimental dissociation of cells from chick embryos. *Nature* 171, 796.
- Mukhina, S., Wang, Y.L., Murata-Hori, M., 2007. alpha-actinin is required for tightly regulated remodeling of the actin cortical network during cytokinesis. *Dev. Cell* 13, 554–565.
- Munro, E.M., Odell, G., 2002a. Morphogenetic pattern formation during ascidian notochord formation is regulative and highly robust. *Development* 129, 1–12.
- Munro, E.M., Odell, G.M., 2002b. Polarized basolateral cell motility underlies invagination and convergent extension of the ascidian notochord. *Development* 129, 13–24.
- Nakamura, J., Yoshida, K., Sasakura, Y., Fujiwara, S., 2014. Chondroitin 6-O-sulfotransferases are required for morphogenesis of the notochord in the ascidian embryo. *Dev. Dyn.* 243, 1637–1645.
- Newman-Smith, E., Kourakis, M.J., Reeves, W., Veeman, M., Smith, W.C., 2015. Reciprocal and dynamic polarization of planar cell polarity core components and myosin. *Elife* 4, e05361.
- Niwano, T., Takatori, N., Kumano, G., Nishida, H., 2009. Wnt5 is required for notochord cell intercalation in the ascidian *Halocynthia roretzi*. *Biol. Cell* 101, 645–659.
- Nixon, S.J., Carter, A., Wegner, J., Ferguson, C., Floetenmeyer, M., Riches, J., Key, B., Westerfield, M., Parton, R.G., 2007. Caveolin-1 is required for lateral line neuromast and notochord development. *J. Cell Sci.* 120, 2151–2161.
- O'Connor, P., Burnside, B., 1981. Actin-dependent cell elongation in teleost retinal rods: requirement for actin filament assembly. *J. Cell Biol.* 89, 517–524.
- Oda-Ishii, I., Ishii, Y., Mikawa, T., 2010. Eph regulates dorsoventral asymmetry of the notochord plate and convergent extension-mediated notochord formation. *PLoS One* 5, e13689.
- Oliferenko, S., Chew, T.G., Balasubramanian, M.K., 2009. Positioning cytokinesis. *Genes Dev.* 23, 660–674.
- Priess, J.R., Hirsh, D.I., 1986. *Caenorhabditis elegans* morphogenesis: the role of the cytoskeleton in elongation of the embryo. *Dev. Biol.* 117, 156–173.
- Segade, F., Cota, C., Famiglietti, A., Cha, A., Davidson, B., 2016. Fibronectin contributes to notochord intercalation in the invertebrate chordate, *Ciona intestinalis*. *Evodevo* 7, 21.
- Sehring, I.M., Dong, B., Denker, E., Bhattachan, P., Deng, W., Mathiesen, B.T., Jiang, D., 2014. An equatorial contractile mechanism drives cell elongation but not cell division. *PLoS Biol.* 12, e1001781.
- Sehring, I.M., Recho, P., Denker, E., Kourakis, M., Mathiesen, B., Hannezo, E., Dong, B., Jiang, D., 2015. Assembly and positioning of actomyosin rings by contractility and planar cell polarity. *Elife* 4, e09206.
- Shi, W., Peyrot, S.M., Munro, E., Levine, M., 2009. FGF3 in the floor plate directs notochord convergent extension in the *Ciona* tadpole. *Development* 136, 23–28.
- Shindo, A., 2018. Models of convergent extension during morphogenesis. *WIREs Dev. Biol.* 7, e293.
- Soviknes, A.M., Glover, J.C., 2008. Continued growth and cell proliferation into adulthood in the notochord of the appendicularian *Oikopleura dioica*. *Biol. Bull.* 214, 17–28.
- Spira, F., Cuylen-Haering, S., Mehta, S., Samwer, M., Reversat, A., Verma, A., Oldenbourg, R., Sixt, M., Gerlich, D.W., 2017. Cytokinesis in vertebrate cells initiates by contraction of an equatorial actomyosin network composed of randomly oriented filaments. *Elife* 6, e30867.
- Srivastava, V., Robinson, D.N., 2015. Mechanical stress and network structure drive protein dynamics during cytokinesis. *Curr. Biol.* 25, 663–670.
- Stemple, D.L., 2005. Structure and function of the notochord: an essential organ for chordate development. *Development* 132, 2503–2512.
- Stolfi, A., Christiaen, L., 2012. Genetic and genomic toolbox of the Chordate *Ciona intestinalis*. *Genetics* 192, 55–66.
- Veeman, M.T., McDonald, J.A., 2016. Dynamics of cell polarity in tissue morphogenesis: a comparative view from *Drosophila* and *Ciona*. *F1000Res* 5.
- Veeman, M.T., Nakatani, Y., Hendrickson, C., Ericson, V., Lin, C., Smith, W.C., 2008. Chongmague reveals an essential role for laminin-mediated boundary formation in chordate convergence and extension movements. *Development* 135, 33–41.
- Veeman, M.T., Smith, W.C., 2013. Whole-organ cell shape analysis reveals the developmental basis of ascidian notochord taper. *Dev. Biol.* 373, 281–289.
- Xu, K., Zhong, G., Zhuang, X., 2013. Actin, spectrin, and associated proteins form a periodic cytoskeletal structure in axons. *Science* 339, 452–456.
- Yumura, S., 2001. Myosin II dynamics and cortical flow during contractile ring formation in *Dictyostelium* cells. *J. Cell Biol.* 154, 137–145.
- Zou, L., Mitchell, J., Stillman, B., 1997. CDC45, a novel yeast gene that functions with the origin recognition complex and MCM proteins in initiation of DNA replication. *Mol. Cell Biol.* 17, 553–563.