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Review

Ciona intestinalis notochord as a new model to investigate the cellular and molecular mechanisms of tubulogenesis

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ABSTRACT

Biological tubes are a prevalent structural design across living organisms. They provide essential functions during the development and adult life of an organism. Increasing progress has been made recently in delineating the cellular and molecular mechanisms underlying tubulogenesis. This review aims to introduce ascidian notochord morphogenesis as an interesting model system to study the cell biology of tube formation, to a wider cell and developmental biology community. We present fundamental morphological and cellular events involved in notochord morphogenesis, compare and contrast them with other more established tubulogenesis model systems, and point out some unique features, including bipolarity of the notochord cells, and using cell shape changes and cell rearrangement to connect lumens. We highlight some initial findings in the molecular mechanisms of notochord morphogenesis. Based on these findings, we present intriguing problems and put forth hypotheses that can be addressed in future studies.

Contents

Abbreviations: A/P, anterior/posterior; MET, mesenchymal–epithelial transition; A/B, apical/basal.

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1. Introduction

1.1. Biological tubes and tubulogenesis

Tubular structures are essential for living organisms as they allow the transport of gases, fluids, and cells throughout tissues and the whole organism, and provide structural support. Biological tubes are invariably composed of an epithelium that encloses a central lumen. The tubular epithelium can be complex, consisting of multiple layers of cells, and the lumens can branch extensively. Some tubes, including capillary vessels [1] and certain segments of the *Drosophila* trachea system [2], are considerably simple and constructed by a single chain of cells. Tubulogenesis involves a highly orchestrated set of cellular processes, including cell shape change, cell proliferation, mesenchymal–epithelial transition (MET), cell polarization, lumen formation, and cell migration [3–9]. Disruption of any of these processes can lead to devastating human diseases such as polycystic kidney disease [10] and vascular stenoses [11].

1.2. Diverse modes of tubulogenesis

Tube formation is the assembly of a polarized epithelium around a central lumen. The epithelium can be generated de novo or reorganized from a pre-existing epithelium [7]. A pre-established epithelium constructs a tube either by wrapping itself to enclose lumen, as observed during the primary neurulation in vertebrates, or by budding from a pre-existing tube through a branching process that occurs in the development of *Drosophila* trachea, mammalian lung, and kidney [7]. In other cases, tube formation involves a MET to create an epithelium with nascent apical/basal (A/B) polarity and produce a lumen at the apical side [7]. The luminal epithelium can also be created by eliminating cells through apoptosis and/or cellular autolysis, as in mammary gland development [12] and the construction of tubular structures in plants [13].

An essential process during tube formation is the creation of lumen [14]. With the exceptions in which extracellular matrix is enclosed as the consequence, for example, of the folding of an epithelial sheet, lumen formation is initiated by secretion from tubular epithelium at the apical site. Depending on the different tubulogenesis models currently studied, this can involve either a simple exocytosis of secretive vesicles or the accretion of small vesicles into intracellular vacuoles that are then delivered across the apical membranes. Whereas secretory trafficking is responsible for the expansion of the apical membrane domain, additional mechanisms involving transmembrane transport are required to enhance lumen expansion [14]. One mechanism enlists ion channels at the apical membrane to create osmotic pressure that drives water flow into the lumen.

1.3. Ascidian notochord

As part of the phylum of the chordates, *Ciona intestinalis* (Fig. 1Aa and b) possesses a transient embryonic and larval structure called the notochord (Fig. 1Ac). *Ciona* notochord at the larval stage is a straight tube closed at both ends. It consists of 40 endothelial-like cells surrounding a single large lumen. The cells are bounded by a notochordal sheath of connective tissue fibrils. The notochord lies in the center position of the tail, flanked on both sides by muscles. It functions presumably as a hydrostatic "skeleton" essential for the locomotion of the swimming larva [15,16]. The tension-resisting fibrils of the notochordal sheath allow the notochord to become rigid as the lumen inflates. To aid in swimming, this stiff yet flexible rod recoils the tail after each alternating stroke of the lateral muscles. The use of a hydrostatic skeleton as the primary support and for locomotion is a common strategy, as it occurs in many soft-bodied animals, including cnidarians, flatworms, nematodes and annelids [17]. Other animals display hydrostatic skeletal structures for part of their body, for example in arthropods, and the echinoderm tube-feet. These structures are generally based on fluid-filled body cavities (coelom-based in bilateral animals) surrounded by muscle walls. In this aspect, *Ciona* notochord is an original system because it is a specialized tubular organ, not a body cavity.

1.4. Early development and initial status before tubulogenesis

The development of ascidian notochord can be divided into distinct phases. Notochord cells are first induced at the blastula stages and become committed before the onset of gastrulation [18]. Concurrent with gastrulation, the notochord cells divide twice in an oriented fashion and the resulting 40 cells organize into a monolayered sheet (Fig. 1B, stage I). No additional cell division occurs during subsequent development, when morphogenetic events only involve cell shape changes, lumen formation, and tissue reconfiguration. The sheet of notochord cells invaginates to form a rod of cells (Fig. 1B, stage II). Subsequently, cells intercalate radially and medio-laterally through a process called convergent extension during neurula and early tailbud stages (Fig. 1B, stage III). This results in the formation of a columnar notochord of 40 cells in length and a single cell in diameter, in a "stack of coins" configuration [19,20]. This arrangement is the initial status before tubulogenesis. At the neurula stage, notochord cells also begin to build a sheath of extracellular matrix, to which they are mechanically anchored throughout the rest of the development.

1.5. Tubulogenesis (Fig. 1B stages IV–VI, detailed in Fig. 1C)

During stage IV, individual notochord cells elongate along the A/P axis. The diameter of each cell decreases, while the length increases, transforming the initial coin-shaped cell into a cylinder. This elongation is accompanied by an A/B polarization process. At stage V, apical/luminal domains appear and extracellular lumen pockets emerge at the opposite ends of each cell (beginning at the center of both the anterior and the posterior surface of the cylindrical cell). Subsequently at stage VI, each notochord cell initiates a bidirectional crawling movement, which results in the merging of two apical domains, the conversion of the cell to an endothelial-like morphology, and the fusion of the neighboring lumen pockets [19,21,22].

1.6. Notochord tubulogenesis as a cord hollowing process

The overall process of *Ciona* notochord tubulogenesis can be compared to the cord hollowing process that occurs in MDCK cell tube formation, zebrafish gut development, and *C. elegans* intestine formation (Fig. 1D). Both processes start with the same initial configuration: a rod or "cord" of cells that is subsequently hollowed by the formation of a central lumen through extracellular lumen delivery, without apoptosis. However, in the case of *Ciona* notochord, the rod is only one cell in diameter and luminal pockets are initially isolated from each other. As a consequence, additional morphogenetic processes are required to produce a through lumen.

At a more detailed level, we will next show how each of the temporally distinct phases of morphological events (cell elongation, lumen formation, cell migration and lumen fusion) involves unique and characteristic cell biological processes. We will also compare them in further detail to other tubulogenesis processes.

2. Individual cell elongation: a contractile ring leading to an incomplete cytokinesis?

After the convergent extension and before the first appearance of extracellular luminal pockets, individual notochord cells