

## Research Highlight in Developmental Biology

### Cell Elongation by the Cytokinetic Machinery – Pinching without Dividing

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#### Abstract

Cell elongation is an integral component of cell shape changes that occur during morphogenesis of tissues and organs during embryogenesis. A common morphogenetic process accomplishes tissue elongation through intercalation of proliferating cells. Meanwhile, tissues could also elongate as a result of elongation of the constituent cells. A recent study by Sehring et al., identifies a role for the cytokinetic machinery of non-dividing cells in tissue elongation. Focal actomyosin at the cell equator, similar to the apparatus used during cytokinesis, drives this cell-scale elongation. It is possible that the strategy outlined in this study for cell elongation is utilized by a variety of cell types, in various developmental contexts, to direct cell elongation and consequently tissue/organ elongation.

**Key words:** Cell Elongation, Notochord, Morphogenesis, Actomyosin

Elongation of the midline tissue along the anterior-posterior (A-P) axis is a hallmark of early embryogenesis in bilateria (Keller, 2006). Early embryonic cells utilize various strategies to promote midline assembly and tissue elongation. Cell intercalation which drives tissue convergence and extension, along mutually

orthogonal axes, is a common strategy utilized by embryos to elongate tissues and organs (Keller et al., 2008). Meanwhile, a recent publication by Sehring et al. (Sehring et al., 2014) in PLoS Biology reveals yet another strategy for cell elongation during embryogenesis. The investigations were conducted in the notochord cells of the simple chordate sea squirt *Ciona intestinalis* during axis elongation. The results indicate a role for the equatorial actomyosin ring, which drives cytokinesis in dividing cells (Green et al., 2012), as the driver of cell elongation in the *Ciona* notochord (Figure 1). The results are intriguing since the machinery that drives cytokinesis is also utilized for cell elongation in non-dividing cells, thus revealing the role of equatorial actomyosin contractile ring in a novel morphogenetic context.



Figure 1: *Ciona intestinalis* embryo at 19 hours post fertilization (hpf). The notochord cell-stack containing 40 cells (yellow outline) undergoes approximately 2.5 fold elongation in 5 h, without cell division, resulting in a notochord that is approximately 550  $\mu\text{m}$  long at 19 hpf. Anterior is to the left. Image courtesy of Di Jiang.

Sehring et al. began by verifying the source of *Ciona intestinalis* notochord cell elongation as the equatorial constriction of cells mediated by a circumferential actomyosin ring reminiscent of the cytokinetic cleavage furrow in dividing cells. Since cleavage furrow is typically associated with mitosis, the authors checked for the signs of cryptic cell cycle events in the

elongating notochord. The notochord cells were negative for bromodeoxyuridine (BrdU) – a marker for DNA synthesis (Dolbeare, 1996) – and phosphorylation of histone H3 (pH3) – a marker for mitotic prophase (Hendzel et al., 1997) – thus demonstrating that the notochord of *Ciona* elongates in the absence of cell division by forming a cell-scale equatorial constriction of the constituent cells.

Using immunohistochemistry and fluorescent fusion proteins, Sehring et al. was also able to verify the localization of cytoskeletal regulatory proteins such as cofilin (actin depolymerizing factor),  $\alpha$ -actinin (anchors actin to the equatorial cell membrane), tropomyosin (stabilizes actin filaments and regulates access to myosin), talin (bridges actin and the cell adhesion apparatus), IQGAP (organizes actin cytoskeleton), anillin (binds actin at the cytokinetic furrow), and septin 2 (functions during cytokinesis) at the equatorial region of the elongating notochord cells. Most importantly, constriction at the cell equator requires the force driven by the reversible phosphorylation of serine 19 at the myosin regulatory light chain (MRLC). Antibodies directed against pS19 of MRLC demonstrated its colocalization with the equatorial actin thus suggesting contractile functionality. These initial investigations suggested the possibility of cell elongation in the notochord by the contractile forces of an equatorial actomyosin ring.

In order to observe the cell-scale dynamics during A-P axis elongation, Sehring et al. used live imaging and analysis of notochord cells expressing lifeact-mEGFP. Their analysis revealed that basal membrane blebbing was associated with contractions of the actomyosin network at the cell equator. Interestingly, the temporal profiles of transverse loading (dorsal-ventral axis) that resulted locally from basal membrane contractions during bleb retraction highly correlated with the longitudinally occurring axial strain (A-P axis), which translates as cell elongation. These results demonstrated that tissue-scale elongation in *Ciona* embryos is

made possible by locally occurring membrane deformations and cell-scale contractions. Moreover, live imaging analyses at the cell cortex also showed that both actin and myosin were brought to the cell equator by a highly dynamic non-oscillatory cortical flow from the cell periphery.

To explore the specific requirements for actin dynamics in elongating notochord cells, Sehring et al. electroporated the early *Ciona* embryo with a phosphomimetic mutant – dominant negative – cofilin construct that resulted in a mosaic expression. The function of wildtype cofilin is to sever actin filaments. In cells expressing the mutant cofilin, longitudinal elongation was severely compromised, due to the defects in actin depolymerization and turn over. Actin localization was also shown to be affected in the equatorial region thus affecting the assembly of the constriction ring in cofilin mutant cells. Meanwhile, in notochord cells in which mutant  $\alpha$ -actinin was expressed, the circumferential actin ring failed to anchor at the equator. The failure of  $\alpha$ -actinin mutant notochord cells to elongate suggested that the stabilization of actin filaments by  $\alpha$ -actinin is essential for the formation of the circumferential actin ring that directs cell elongation. Together, these results indicated a role for notochord-specific cytoskeletal factors in mediating the formation and stabilization of the equatorial contractile ring that directs cell elongation.

Sehring et al. made an intriguing observation that the end-cells of the notochordal stack of 40 cells (cell 1 and cell 40 in Figure 1) do not elongate and they also do not assemble the equatorial contractile ring. To verify the correlation between the equatorial actomyosin contractile ring and the ability of cells to undergo elongation, the authors ablated the notochord at various positions thereby creating “end-cells” at the nicks. The newly created “end-cells” not only failed to assemble the equatorial actomyosin ring, but also did not elongate. These observations cemented their

hypothesis that the cytokinetic ring was indeed deployed for the purpose of cell elongation in the *Ciona* notochord. To verify whether cell elongation by the equatorial circumferential actin ring is a conserved evolutionary feature, Sehring et al. examined the notochord elongation in *Oikopleura dioica*, an appendicularian. Despite the difference in the utilization of a drastically different genetic tool kit for its development, the morphogenetic mechanism for notochordal cell elongation in *Oikopleura* involved a circumferential basal constriction comprising an actin ring.

In summary, the study by Sehring et al. demonstrates the utility of the circumferential actomyosin ring for cell elongation without division. The investigation not only showed the localization of the key components driving cytokinesis at the equatorial region of notochordal cells during the elongation phase, but also verified the essential role played by some of those components in mediating cell elongation by mutant analysis. It was also shown that the A-P axis elongation by cell-scale actomyosin contractile rings is an evolutionarily conserved morphogenetic strategy that is utilized by a variety of forms during development. This study, utilizing *Ciona*, also highlights the advantages of using a relatively simple system to determine the intricacies of morphogenesis in the early embryo.

Although, the utility of similar cell elongation strategies during the morphogenesis of the vertebrate axis remains an open question, the study raises intriguing questions about the temporal dynamics of the contractile ring that drives cell elongation in simple chordates, and other invertebrates such as the *C.elegans* embryo. The maintenance of the elongated state by notochord cells would presumably require adhesive substrates in addition to the dynamics of the equatorial cytoskeletal machinery. Future studies may also shed light on the signals that prevent the flux of the contractile ring from continuing through cell division.

## Acknowledgements

I would like to thank Dr. Deborah Andrew for supporting my work, and the two anonymous reviewers for their comments to improve this article.

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