

Deciphering the Cellular and Physical Mechanisms in Spiral Cleavage

Spiral cleavage is the ancestral developmental mode within the Spiralia. It covers a period of the early development characterized by asymmetric cell divisions with alternating division angles giving the embryo a spiral looking appearance. However, the mechanisms controlling spiral cleavage are poorly understood. To elucidate the molecular, cellular and physical mechanisms of spiral cleavage, we use the marine annelid *Platynereis dumerilii* as a model. We unravel the role of the cytoskeleton during spiral cleavage through mRNA injections of fluorescently labeled tubulin and histone into the zygote to label cytoplasmic elements. We imaged the live embryos with selective plane illumination microscopy, processed the data with Fiji softwares, and reconstructed this way early cell cleavages in *Platynereis*. Next, we measured dynamic cellular events such as inclination of the mitotic spindles, transportation of the nuclei within the cells, and membrane deformation during cell division. Furthermore, we monitored the cortical actomyosin dynamics through syn21-lifeact-mKate2 mRNA or protein injection. We described that actomyosin polarizes in the first two asymmetric divisions as well as toward the micromeres prior to each spiral cleavage of the macromeres. A counter chiral flow of the cortical actomyosin is observed in the macromeres during the first spiral cleavage. Moreover, cell orientation remains normal when the embryo develops in an eggshell-free status or when cells are dissociated. Drug treatments showed that membrane deformation and actomyosin dynamics were not interfered with exogenous colchicine or nocodazole. However, actin polymerization inhibition with latrunculin A severely disrupted spindle position, suggesting that the actomyosin plays critical roles in both establishing cell polarity and controlling division pattern. This study provides mechanistic insights into the spiralian development and a base to compare the degree of conservation among spiralian.

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