氏　　名	進藤麻子
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第1169号
学位授与の日付	平成２０年３月１９日
学位授与の要件	生命科学研究科　基礎生物学専攻 学位規則第６条第１項該当
学位論文題目	Coordination of Cell Polarity during Xenopus Gastrulation
論文審査委員	主　査　教授　大隅　良典 教授　上野　直人 准教授　野中　茂紀 教授　上村　匡（京都大学）

論文内容の要旨

Morphogenesis of all animals is driven by well-orchestrated cell behaviors. During embryonic development, cells not only undergo cell differentiation, but also change their shapes and migrate in a deterministic way to give rise to a various tissues and organs at appropriate times and spaces. In these processes, cells acquire cell polarities so that they exert their specific cellular functions by positioning cellular components such as organelles, cytoskeltons and cell protrusions in an asymmetrical manner.

During embryonic development, Planar Cell Polarity (PCP) pathway acts as a key regulator for cellular polarizing process, for example, *Drosophila* wing cell, and mesoderm cells in zebrafish and *Xenopus*. During *Xenopus* gastrulation, it is known that chordamesoderm cells are polarized and intercalate each other allowing anterior-posterior elongation of the embryo proper by convergent extension (CE). Although it is well known that the cellular protrusions at both ends of polarized cells exert tractive force for intercalation and that the components of PCP pathway are known to be essential for the cell polarity, little is known about what triggers the cell polarization and what the polarization causes to control intracellular events enabling the intercalation that leads to the CE. These facts implied that only the analyses of single molecule are not sufficient to solve the critical issue of embryogenesis, which prompted me to introduce an approach that can provide information on the timing of cell polarity formation as earliest as possible.

In this study, she used EB3 (end-binding 3), a member of +TIPs that bind to the plus end of microtubule (MT), to visualize the intracellular polarity of chordamesoderm cells during CE to investigate the trigger of the establishment of cell polarity.

She found that EB3 movement is polarized in chordamesoderm cells and that the notochord-somite tissue boundary plays an essential role in generating the cell polarity, because EB3 movements were strongly attracted by notochord-somite boundary. The EB3 polarity was generated before the change of cell morphology. These results suggest that EB3 polarity must sense the polarity cue in early phases, and it must be useful approach to unveil the polarity cue.

The polarized EB3 movement was also observed near the boundary between the chordamesoderm tissue and naïve ectoderm tissue or lateral mesoderm tissues induced by a low concentration of nodal mRNA, showing that distinct property between the two tissues are necessary for generating the EB3 polarity in chordamesoderm cells. The polarized MT elongation may be involved in sensing the mediolateral polarity, because the coordinated cell alignment in relation to the tissue boundary was disrupted when the EB3 movement was randomly. She also found that non - physiological material, such as a block of agarose gel, conferred the polarity on the EB3 movements in chordamesoderm cells. This result suggests that the polarity cue may be not solely chemical cues secreted from juxtaposing tissue, and that mechanical effects generated within the boundary may be involved.

To identify the target of EB3 movements, She investigated about the role of extracellular matrices

(ECM) because they have been known to accumulate at the notochord-somite boundary. From the results of antibody staining, fibrillin, one of the ECM accumulating at the boundary, was not likely a polarity cue because the timing of its accumulation was relatively late as a polarity cue.

The Components of PCP pathway, essential for regulating cell polarity, was not required for the MT polarity in chordamesoderm cells. This result implies the existence of other mechanisms to control and generate the cell polarity.

Despite these attempts to clarify the polarizing signal(s) and cell responsiveness, exactly how the actual triggering signal is generated between two different tissues and how the tissue differences are sensed currently remains unknown. However, this study raised the possibility that definitive tissue separation established by the distinct levels of nodal signaling is essential, and it is possible that the polarity of chordamesoderm cells is coordinated along the mediolateral axis by sensing the tissue separation. It is worthwhile to investigate that the interaction of these distinct two tissues might generate some mechanical forces.

論文の審査結果の要旨

細胞極性は細胞に付与される形態や物質局在、そして機能の非対称性であり、発生過程では細胞の非対称分裂や方向付けられた細胞移動に必要な性質である。
とくに、脊椎動物胚に必須のリモデリング過程である原腸形成に見られる細胞運動「収斂と伸長」には平面内細胞極性の形成が必要であることが分かっている。申請者はアフリカツメガエル胚を用いて「収斂と伸長」運動における細胞極性の形成機構について研究を行った。申請者は細胞極性形成の指標として微小管伸長に焦点をあてその可視化を試みた。微小管伸長端（プラス端）に結合する EB3 (End binding 3)タンパク質と GFP との融合タンパク質を胚に発現させ、原腸形成に寄与する中胚葉細胞群を切除して共焦点顕微鏡で観察することにより微小管伸長の極性化をリアルタイムで可視化することに成功した。同手法を用いて、平面内細胞極性を獲得した中胚葉細胞内では EB3-GFP の追跡から予測される微小管伸長にも極性が見られることを見出した。また、微小管の伸長は細胞形態が極性化し紡錘形に変化する前から極性化していること、その伸長は中軸中胚葉である脊索とその側方に隣接する体節との境界に向かって単極性を示すことを明らかにした。さらに、ノーダル等の細胞増殖因子を用いて分化誘導した二つの外植体を並列に接触させ共培養することによって、組織間相互作用が細胞極性へ与える影響を観察するアッセイ系を構築し、組織間の相互作用による極性形成について詳細に解析した。その結果、中胚葉と外胚葉など、細胞分化状態が異なる組織間の相互作用によって、その境界に極性化開始シグナルが生ずること、また共培養する二組織間の分化状態の差異が、細胞の整列方向に大きな影響を与えることを示した。さらに、平面内細胞極性を制御することが知られている PCP 経路と組織間境界がもつ極性開始シグナルとの関係について検討し、PCP 経路を特異的に遮断した場合、細胞形態は極性を失うものの境界方向に極性化した微小管伸長は影響を受けないことを示し、形態の極性と微小管伸長にみられる細胞内の機能的極性は分離できることを明らかにした。

最後に、中胚葉に分化した外植体にアガロースを並置した場合にも細胞が極性化することから、異なる組織間の相互作用は物理的な障壁で代替できることを示し、細胞極性開始メカニズムに物理的要因を考慮することの必要性を提示した。

本研究は、斬新なアプローチにより細胞極性開始機構の解明に向け新しい知見を与えた重要な研究成果であり、学術的な意義も大きく博士号授与にふさわしい研究と判断した。