氏　　名	飯岡　英和
学位（専攻分野）	博士（理学）
学位記番号	総研大甲第875号
学位授与の日付	平成１７年３月２４日
学位授与の要件	生命科学研究科　分子生物機構論専攻 学位規則第６条第１項該当
学位論文題目	Cytoskeletal regulation by Wnt signaling in Xenopus gastrulation movements
論文審査員	主　査　教授　髙田　慎治
	教授　上野　直人
	教授　諸橋　憲一郎
	教授　黒岩　厚（名古屋大学）

論文内容の要旨

Gastrulation is one of the most important developmental events for many multicellular organisms. In the amphibian embryos, mesodermal cells involute to the inside of the embryo and migrate along the blastocoel roof (BCR) to establish the three germ layer structure and body axes. These processes involve several morphogenetic cell movements including convergent extension. In convergent extension, cells are polarized, elongated mediolaterally, and then intercalated each other.

It is known that Wnt signaling pathways play an essential role in the regulation of convergent extension movements. Wnts are a family of secreted proteins that regulate many biological processes. Functional analyses in *Xenopus* suggest that the Wnt family can be divided into two functionally distinct groups. The first group of Wnts induces a secondary axis when ectopically expressed in *Xenopus* embryos. They activate the canonical Wnt/β-catenin pathway and induce transcription of target genes such as *siamois* and *Xnr3*. The second group of Wnts, which include Wnt5a and Wnt11, activates the noncanonical Wnt signaling pathway that controls morphogenetic cell movements. The noncanonical Wnt pathway branches into two cascades. One is the PCP (Planar Cell Polarity) pathway, and the other is the Wnt/Ca^{2+} pathway. Previous data showed that Wnt/PCP pathway has been implicated in the regulation of convergent extension. One of the PCP signaling components Dishevelled (Dsh) is essential for convergent extension. However, it is largely unknown how the PCP pathway regulates convergent extension.

Because active cell movements occur during convergent extension, the regulation of cytoskeletal dynamics may be important for the regulation of this process. It has been shown that Dsh activates Rho family small GTPases, suggesting that the PCP pathway may be involved in the cytoskeletal regulation. In order to investigate the regulatory mechanisms of actin cytoskeleton during gastrulation, He cloned a gene encoding an actin-binding protein MARCKS (Myristoylated Alanine Rich C-Kinase Substrate) from a *Xenopus* embryonic cDNA library. MARCKS was first identified as a PKC (Protein Kinase C) substrate in mammalian cells. It attaches with the plasma membrane through N-terminal myristoylation. He showed that loss of MARCKS function by MO (Morpholino oligonucleotide) in *Xenopus* embryo induced a gastrulation defect phenotype without affecting mesoderm induction. To elucidate why *MARCKS* MO caused gastrulation defect, cell biological analyses were conducted. During convergent extension, *MARCKS* MO inhibited polarization and intercalation of mesodermal cells. He performed further observation at the cellular level. As a result, cell adhesion on fibronectin, protrusive activity of mesodermal cells and cortical actin formation in the cells were also inhibited by *MARCKS* MO. Furthermore, He found that activation of the PCP pathway promoted formation of filopodia- and lamellipodia-like structures in ectoderm explant cells, and *MARCKS* MO inhibited this activity. These results indicate that MARCKS regulates cortical actin dynamics, and it is requisite for the morphological processes regulated by the PCP signaling pathway. In addition, *MARCKS* MO also severely impaired neural tube closure without affecting the neural induction. It is consistent with the phenotype of mice deficient in *MARCKS*. MARCKS function may be conserved in vertebrates. Taken together, MARCKS is an essential molecule not only for gastrulation movements but also neural tube closure through controlling the cortical actin formation. These results are shown and discussed in Chapter 2.

It is known that Dsh is translocated to the plasma membrane in response to Wnt signaling in animal cap cells. In this thesis, He showed the bipolor localization of Dsh in mesodermal cells during convergent extension. These data indicate that the regulation of Dsh localization is important for the regulation of convergent extension.

But its regulatory mechanism is unknown. Thus, He analyzed molecular mechanism to regulate Dsh localization and identified three proteins involved in the PCP pathway, PKC δ, $G_{\alpha 11}$ (G_{11}) and $G_{\alpha i1}$ (G_{i1}). First He identified PKC δ as an essential factor to regulate Dsh localization and showed that it physically interacted with Dsh. Loss of PKC δ function induced a gastrulation defective phenotype without affecting mesoderm induction. Confocal microscopic analyses revealed that both PKC δ and Dsh were translocated from the cytoplasm to the plasma membrane by Fz7 signaling. In addition, loss of PKC δ function reduced the signal-dependent Dsh translocation. These results indicate that PKC δ regulates Dsh localization under the control of Wnt signaling. Next, He focused on heterotrimeric G protein α subunits. Injections of antisense MOs against G_{11} or G_{i1} caused a phenotype in the body axis elongation and/or gastrulation defect. In addition, these MOs inhibited elongation of DMZ explants. These results suggested these G proteins might be required for convergent extension. Thus, He investigated functions of these G proteins in the PCP pathway and found that G_{i1} and G_{11} are necessary for the membrane localization of Dsh. G_{11} MO reduced both hyperphosphorylation of Dsh and the protrusive activity induced by the PCP pathway, whereas G_{i1} MO did not. These data indicate that both G_{i1} and G_{11} are required for the Dsh translocation, but these molecules may play distinct roles. These results are shown and discussed in Chapter 3.

This work has demonstrated that the PCP pathway regulates convergent extension movements through cytoskeletal regulation, and identified molecules essential for the intracellular signaling components in this pathway. These findings may contribute to understand the mechanisms of convergent extension movements and the other developmental processes in which the PCP pathway is involved.

論文審査結果の要旨

脊椎動物の発生過程において、受精卵はまず卵割により細胞数を増やして胞胚を形成した後、細胞・組織レベルでのダイナミックな再編成を伴う原腸形成運動を行う。この原腸形成運動は、予定中胚葉が予定外胚葉と予定内胚葉の間に滑り込むことにより体の基本構造である三胚葉構造を形作る必須の過程である。この運動の中心的な役割を果たす背側中胚葉組織では、紡錘状に極性化した細胞同士が互いに滑り込み運動を起こした結果として組織の伸長が起きることが分かっており、この現象は収斂伸長運動（Convergent Extension）と呼ばれている。この運動に関しては、分泌タンパク質であるWnt11、その受容体タンパク質Frizzled7、その下流で働く細胞内タンパク質Dishevelledを介したシグナル伝達経路の働きが必須であることがわかっているが、そのシグナル経路がどのような作用機序で組織の運動を制御しているかは明らかではない。申請者は、アフリカツメガエルの胚を用いて収斂伸長運動の制御メカニズムについて解析を行った。まず、この過程が細胞の形態変化や移動を伴うことからアクチン細胞骨格に着目し、アクチン結合タンパク質の機能的なスクリーニングを行った。その結果 MARCKS (Myristoylated Alanine Rich C Kinase Substrate)というアクチン結合タンパク質が収斂伸長運動に必須の役割を果たしていることを見いだした。さらに細胞生物学、発生生物学両面からのアプローチにより、MARCKSが細胞膜に局在すること、細胞表層アクチンの形成に必須であること、細胞の形態や移動性、接着性などアクチン繊維の制御に基づくと考えられる細胞の様々な性状の変化に重要であることを示した。また、Wntシグナルとの関連を追求し、Dishevelledの局在がアクチン繊維の細胞内局在と一致することから、MARCKSはアクチンを介したDishevelledの局在決定に重要であること、さらに、Wntシグナルによる細胞の形態変化にはMARCKSの機能が必須であることを明らかにした。また、Wntシグナルが中胚葉細胞における細胞突起形成に必須であることを示し、Wntシグナルによる原腸形成運動制御がアクチン細胞骨格を介して行われている可能性を強く示唆した。

本研究は、アクチン結合タンパク質MARCKSやWntシグナル経路の機能解析により、原腸形成運動におけるアクチン細胞骨格制御の重要性を示した重要な研究であり、学位授与にふさわしいものであると判断した。