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学位論文題目	XRab40 and XCullin5 form a ubiquitin ligase complex essential for the noncanonical Wnt pathway
論文審査委員	主　査　教授　髙田　慎治 教授　上野　直人 教授　大隅　良典 教授　小林　悟 教授　菊池　章（広島大学）

論文内容の要旨

Gastrulation is the first major morphogenetic process in *Xenopus* development that governs the formation of three germ layers – ectoderm, mesoderm and endoderm. During *Xenopus* gastrulation, convergent extension movement takes place in order to establish the anterior-posterior axis of the body plan (Shih and Keller, 1992; Wallingford, 2002). At the onset of gastrulation, protein complexes might need to deliver to their corrected compartments to regulate dramatic cell movements. It is know that membrane trafficking plays a crucial role in the delivery of protein complexes between organelles and it might be involved in the regulation of the gastrulation movement. A number of proteins have been identified to control the membrane trafficking process, including Rab GTPases. In the present study, she sought to elucidate the roles of Rab40 GTPase in *Xenopus* gastrulation. Rat Rab40c has shown to be expressed in oligodendrocytes and localized in the recycling compartment (Rodriguez-Gabin et al., 2004), however, its function remains unknown. She found that a *Xenopus* homolog of Rab40 (XRab40) is required for normal gastrulation.

XRab40 encodes a protein containing a Rab GTPase and a SOCS box domains. Rab GTPases family proteins belong to the Ras superfamily of small GTPases and function as a key regulator for membrane trafficking. Besides the Rab domain, Rab40 contains a SOCS box. The SOCS family proteins recruit substrates to the ElonginB/C-Cul2/Cul5-SOCS-box (ECS) complex and comprise a large family of ubiquitin ligases.

XRab40 is ubiquitously expressed in *Xenopus* embryos by whole mount *in situ* hybridization and RT-PCR. It localizes at the Golgi apparatus. Both overexpression of *Rab40* mRNA and knockdown of Rab40 by using specific morpholino (Rab40 Mo) caused gastrulation defects in *Xenopus* embryos. She demonstrated that XRab40 is required for cell polarization and migration during convergent extension.

In order to further understand the roles of Rab40, a yeast-two hybrid screen and mass spectrometric analysis were carried out to identify Rab40 interacting partners. ElonginB, ElonginC, Cullin5 and Rap2 were identified as XRab40-interacting proteins. Elongin B/C and Cullin 5 were found to interact with the SOCS domain of Rab40 to form a ubiquitin ligase complex whereas Rap2 interacts with the GTPase domain of XRab40. She then examined the correlation between XRab40 and XCullin5 by analyzing the roles of XCullin5 in *Xenopus* development. She found that loss of XCullin5 function phenocopies and synergizes with the effect of XRab40 Mo. XCullin5 is required for the Golgi apparatus localization of XRab40. Since Cullin5 is known as a ubiquitin ligase component, she then investigated the effect of XRab40/XCullin5 on the ubiquitination of Rap2. She showed that XRap2 is a substrate for XRab40/XCullin5 ubiquitin ligase and the localization of XRap2 is regulated by its ubiquitination.

She attempted to further elucidate how XRab40 and XRap2 is involved in the regulation of *Xenopus* gastrulation and found that one of the Rap2 effector, Misshapen/Nck-interacting kinase (MINK), which is also required for gastrulation and binds to Dishevelled, a cytoplasmic component of Wnt pathway. MINK is translocated from the cytoplasm to the plasma membrane when Rap2 was co-expressed. It also translocates to the plasma membrane in response to Wnt activation.

Next, she investigated the effects of XRab40 in Wnt signaling. She demsonstrated that

XRab40/XCullin5 and XMINK are essential for the punctate localization of Dishevelled. Intriguingly, disruption of the membrane trafficking process by expressing dominant negative dynamin inhibits the punctate localization of Dishevelled, suggesting that membrane trafficking is required for Wnt signaling. Moreover, phosphorylation and membrane localization of Dishevlled are disrupted by Rab40 and MINK knockdowns. Rab40 is required for frizzled, a seven transmembrane receptor of Wnt ligand, to activate JNK pathway suggesting that Rab40 is involved in the non-canonical Wnt pathway. Although Rap2 has been reported as a positive regulator in the canonical Wnt pathway (Choi and Han, 2005), she demonstrated that it also plays a role in the noncanonical Wnt pathway to regulate the convergent extension movement. XRab40 is required for XRap2 function in the noncanonical Wnt pathway but is dispensable for its function in the canonical Wnt pathway.

In conclusion, XRab40 plays an essential role during *Xenopus* gastrulation and it forms a ubiquitin ligase complexed with Cullin5 at the Golgi apparatus. In addition, Rap2 GTPase was identified as a potential substrate for the XRab40/Cullin5 ubiquitin ligase. XRab40 plays an essential role in the noncanonical Wnt pathway by regulating Rap2 and its effector Misshapen/Nck interacting kinase (MINK). XRab40 regulates the membrane localization of Dsh that is necessary to mediate the Wnt signal transduction pathway. She propose a novel signaling cascade including XRab40/Cullin5, Rap2 and MINK to modulate the Wnt signaling pathway.

References:

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論文の審査結果の要旨

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と呼ばれている。申請者は、この複雑な組織の運動を細胞レベルで解明することを目標とし、特に細胞内膜輸送系に関わるRabファミリーGTPaseに注目して研究を行った。Rab GTPaseは数十種ものファミリーメンバーが同定されており、その多くは膜輸送系の様々な場所で重要な役割を果たしていることがわかっている。本研究では、カエル初期胚で発現している約３０種の Rab の機能的なスクリーンを行った。そして細胞機能がこれまでほとんど解析されていないRab40ホモログXRab40が原腸形成運動に必須の役割を果たしていることを示した。細胞観察からXRab40はゴルジ体に局在すること、生化学的な解析からXRab40がXCullin5、XElonginB/Cと結合してユビキチンリガーゼ複合体を形成することを明らかにした。さらに、XRab40のユビキチン化の基質として低分子量GTPase　XRap2、さらにそのエフェクターとして XMINK(Xenopus Missphapen/Nck interacting kinase)を同定した。この結果から、XRab40/XCullin5-XRap2-XMINK はシグナル経路を形成すること、また、これらはすべて原腸形成運動に必須であることを明らかにした。原腸形成運動に関しては、分泌タンパク質である Wnt11、その受容体タンパク質 Frizzled、その下流で働く細胞内タンパク質 Dishevelled を介した非古典的 Wnt シグナルが必須であることがわかっているが、そのシグナル伝達のメカニズムはほとんどわかっていない。申請者は、XRab40/XCullin5-XRap2-XMINK が Wnt シグナルの細胞内シグナル伝達に必須であること、そのターゲットは細胞内小胞様構造にある Dishevelled の局在制御であること、その分子メカニズムとして XMINK と Dishevelled の相互作用が重要であることを明らかにした。

本研究は、Rab40の細胞機能と、ユビキチン化による非古典的Wntシグナル経路の制御メカニズムを初めて明らかに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。