氏　　名	兼崎　琢麿
学位（専攻分野）	博士（理学）
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学位論文題目	Roles of Ric-8 dependent targeting of heterotrimeric G proteins in early embryogenesis of Drosophila
論文審査委員	主　査　教授　広海　健 教授　桂　勲 教授　徳永　万喜洋 助教授　鈴木　えみ子 助教授　小嶋　徹也（東京大学）

論文内容の要旨

Heterotrimeric G protein is one of the most characterized signal transducers in eukaryotic cells. It consists of Gα, Gβ, and Gγ subunits and is highly conserved from yeast to human, and acts as intracellular messenger for external information. In animal development, G protein signaling is involved in various pathways including cell-fate determination, cell-cell interaction, or asymmetric cell division. During *Drosophila* gastrulation, Gα12 (Cta)-mediated signaling controls the ventral cellular movements of epithelia. Although it is only known that the G protein induces change of myosin localization and control the cell shape-change, recently it has been revealed that G protein has multiple functions and targets in this morphogenetic movement. Here, I show that G protein signaling regulates the organization of cortical actin cytoskeleton underlining plasma membrane in the epithelial cells. I found it by mainly focusing on Ric-8 protein, which is critical factor for plasma membrane-targeting of G proteins. Ric-8 protein is expressed ubiquitously in early embryo, and is required for proper localization of all the G proteins expressed in epithelial cells. Entire G protein signaling activity is lost in maternal *ric-8* mutant, and the mutant shows severe defect in cellular movements during gastrulation. Then, the *ric-8* mutant shows the disorganization of cortical actin. From mutant analyses, I found that the two Gα subfamilies, Gα12 and Gαi, are involved in the actin organization, but a known ligand of Gα12, Fog, is not required. When *ric-8* is mutated, the cell surfaces become unstable and show blebs, which is a hallmark of the destabilization of cellular surface. The mutants for the common cofactors of the Gα's, Gβ13F and Gγ1, also show the similar phenotypes. When the cells are treated with inhibitors of actin polymerization, ventral cells show blebs like the *ric-8* mutant. Although the disruption of cortical actin in the mutants for G proteins is seen in all epithelial cells, the disorganization of the surface occurs only in the moving ventral cells. This suggests that the organization of cortical actin seems to be required to endure the stress, which the

ventral cells receive when they move during gastrulation.

Besides, I also show evidence that heterotrimeric G protein signaling is involved in the control of the actin dynamics in syncytial blastderm stage, which is earlier step of embryogenesis than gastrulation. In this stage, nuclear division is carried out with the assistance of two architectures of actin cytoskeleton; actin caps in interphase and pseudocleavage furrow-assembled actin network in mitosis. So far, a lot of genes have been identified as the factors which help morphological change of actin cytoskeleton during each nuclear division. However, most of them encode just actin polymerization factors and therefore it is unclear how the timing of the actin dynamics is controlled. Here, I show that G protein is colocalized with the caps and network of actin throughout this stage. In the *ric-8* mutant, this localization pattern is lost and actin cytoskeleton shows abnormality. Using live-imaging technique, it is revealed that some *ric-8* mutant embryos show the delay of the change of actin morphology and died before gastrulation. These results indicate that G protein is involved in temporal control of the dynamic change of actin morphology during mitosis, and provide new insight into the role of G protein signaling in early embryogenesis of *Drosophila*.

In contrast to these two embryonic stages, the *ric-8* mutant does not show disruption of actin in cellularization stage, suggesting that G protein signaling has multiple but limited roles in actin regulation. Based on these findings, I discuss the mode of activation, the possible downstream element, and the significance of G protein signaling in actin regulation of early embryogenesis of *Drosophila*.

In addition, here I introduce a novel mutant of *ric-8* gene, *ric-8*^{atx}, in which 408th E is substituted to K. The *ric-8*^{atx} mutant embryo shows *cta-* or *fog*-like phenotype of gastrulation, which is milder than that of the *ric-8 null* mutant. Consistently, the *ric-8*^{atx} mutation results in the Gα12-specific defect of targeting. Although the role of Ric-8 has been inferred from the results of genetic analyses and of some *in vitro* assays in past studies, the true molecular function of this protein is still unclear. The findings from the analyses of *ric-8*^{atx} promote elucidating the

mysterious function of Ric-8 protein *in vivo*.

論文の審査結果の要旨

３量体Ｇタンパク質は細胞内の様々な現象を仲介するシグナル分子である．たとえば，ショウジョウバエの原腸陥入過程では，上皮細胞の細胞変形・移動にはＧα12が必要であることが知られている．しかし，Ｇタンパク質の下流の細胞内構造やその調節機構はわかっていなかった．兼崎君はＧタンパク質のターゲットの一つは上皮細胞の細胞膜を裏打ちしているアクチン細胞骨格であることを見いだした．

兼崎君はまず，Ｇα12やＧαi の変異では細胞表層のアクチン骨格形成が破壊され，アクチンが細胞質に散っていることを見いだした．この現象は原腸陥入を行う細胞だけでなくすべての上皮細胞で見られ，原腸陥入においてＧα12の上流で働くリガンドであると考えられているFOGは不要であった．Ｇタンパク質シグナルをさらに包括的に解析する手段として，兼崎君はＧタンパクを細胞膜に局在させるのに必要だとされているRic-8の変異系統を用いた.Ric-8変異では４種のＧαタンパク質の細胞膜への局在が失われており，原腸陥入を行っている領域で細胞膜が不安定になってブレブ形成を起こしていた．これらの結果を基に兼崎君は，Ｇタンパク質によるアクチン骨格制御は，細胞変形によって生じるストレスに細胞が拮抗するために必要である，と提唱している．

アクチン細胞骨格はダイナミックに変化する．たとえば，ショウジョウバエの多核性胞胚期には，核分裂の度にアクチン骨格が核の上のキャップからネットワーク構造へと変化する．兼崎君はこのアクチン構造のダイナミクスにも３量体Ｇタンパク質が関与していることを示した．Ｇタンパク質はこの過程でずっとアクチン骨格に局在していた．アクチン骨格との共局在はRic-8変異では失われており,Ric-8変異ではアクチン骨格の再編成が大幅に遅れていた．この結果は，３量体Ｇタンパク質が伝えるシグナルが細胞分裂の際のアクチン骨格のダイナミックな変化を制御していることを示唆している．

アクチン骨格は細胞の運動や形態形成に直結する働きをする細胞内構造だが，その制御機構としては低分子量ＧタンパクであるRhoが知られているだけであった．兼崎君はアクチン骨格制御因子として新たに３量体Ｇタンパク質を見いだした．これは新規制御因子の発見としてだけではなく，その上流のシグナル解析の手がかりとなる重要な発見である．以上の理由で兼崎琢磨君の論文は博士号授与の要件を満たすと審査員全員一致で判断した．