氏　　名	加藤　輝
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学位論文題目	Analysis of dynamic tissue organization using Drosophila tracheogenesis as a model
論文審査員	主　査　教授　桂　勲
	教授　上田　龍
	助教授　鈴木　えみ子
	助教授　平田　たつみ
	助教授　伊藤　啓（東京大学）

論文内容の要旨

Movement and polarized growth of cells are important processes of tissue morphogenesis. The tracheal system of *Drosophila* is a respiratory organ consisting of a stereotyped network of tubular epithelia, which ramifies and delivers air to target organs. The tracheal network is formed by a series of branching, migration and fusion of the tracheal primordia. While the tracheal branches are extended, rearrangement and shape changes of the cells in the tracheal promordia takes place without cell division. Those properties of tracheogenesis make it a highly suitable for studies of rearrangement and guided migration of epithelial cells in tissue organization. To further analyze the highly dynamic events of tracheogenesis, novel approaches for tracing the behavior of the individual tracheal cells with high temporal and spatial resolutions are needed. In order to visualize the tracheogenesis in living animal, time-lapse observation system based on the confocal laser microscope was constructed. Green fluorescent protein fused to the actin-binding domain of moesin was expressed exclusively in tracheal cells. GFP-moesin illuminated the fine details of tracheal cell shape, and the time course of tracheal cell shape change was successfully traced over most of the period of tracheal network formation. By applying this system, I analyzed two fundamental mechanisms required for tubulogenesis, organized cell rearrangement (Part 1) and guidance mechanism for polarized cell growth (Part 2).

In Part 1, I described the outline of time-lapse recording system and its application for analysis of the function of the small GTPase Rac. Cell rearrangement, accompanied by the rapid assembly and disassembly of cadherin-mediated cell adhesions, plays essential roles in epithelial morphogenesis. Various in vitro and cell culture studies on the small GTPase Rac have suggested it to be a key regulator of cell adhesion, but this notion needs to be verified in the context of embryonic development.

To investigate the function of Rac in the epithelial cell rearrangement, I used the tracheal system. Rac activities were altered by over-expressing constitutively active or dominant negative form of Rac1 transgenes in tracheal primordia, and tracheal phenotypes were examined by time-lapse observations. I found that a reduced Rac activity inhibited the outgrowth and dynamic cell rearrangement of the tracheal branches. On the other hand, hyperactivation of Rac caused loss of tracheal cell adhesion, resulting in cell detachment from the epithelia. Together with its role in cell motility, Rac regulates plasticity of cell adhesion and thus ensures smooth remodeling of epithelial sheets into tubules.

In Part 2, I demonstrated that the two morphogens, Hedgehog and Decapentaplegic instruct the polarized growth of tracheal cells. The migration of cellular extensions is guided by signals from tissues with which they contact. Many axon guidance molecules regulate growth cone migration by directly regulating actin cytoskeletal dynamics. Secreted morphogens control global patterns of cell fate decisions during organogenesis through transcriptional regulation, and constitute another class of guidance molecules. Terminal branch is a single cellular extension of the terminal cell located at the terminus of tracheal dorsal branch. The terminal cells adhere to the

internal surface of the epidermis whereas other cells such as stalk cells of dorsal branch do not make contact with epidermis, and elongates terminal branch in ventral direction.

In this study, I focused on the guidance mechanism of cellular extension of the terminal branch. Terminal cells extend numerous filopodia in every directions during the elongation of terminal branch. These filopodial movements exhibit no strong directional bias and that filopodial extensions may be selectively stabilized in ventral direction. Terminal branch is extended over the Posterior compartment of the epidermis, which expresses Hedgehog. Even when the number of terminal cells was increased, most of them were localized to posterior compartment as their substrate for elongation. In addition, terminal cells were located immediately adjacent to the Decapentaplegic expressing epidermal cells. These observations suggest a model that morphogens Hedgehog and Decapentaplegic guide the directed outgrowth of cytoplasmic extension of the terminal branch. To test this idea, I manipulated input levels of Hedgehog and Decapentaplegic to the tracheal cells. Alteration of Hedgehog expression pattern by overexpressing Hedgehog in the dorsal epidermis induced misrouted terminal branches. Over-expression of the Hedgehog signal transducer transgenes in tracheal cells also produced misguided terminal branch phenotype. Thus, I concluded that Hedgehog promotes terminal branch extension over the posterior compartment of the epidermis. Altered levels of Decapentaplegic signaling activity in the trachea caused defect of polarized extension of the terminal cells. It was concluded that Decapentaplegic, expressed at the onset of terminal branching, restricts dorsal extension of terminal branch and ensures its monopolar growth. From these findings, I concluded that orthogonal expression of Hedgehog and Decapentaplegic in the epidermis instructs monopolar extension of the terminal branch along the posterior compartment, thereby matching the pattern of airway growth with that of the epidermis.

In summary, I showed that mechanisms of cell rearrangement and guided cellular extension in tracheaogenesis can be revealed by applying in vivo time-lapse analysis. This temporally high-resolution assay system may be a valuable tool for unveiling the fundamental processes of dynamic tissue morphogenesis.

論文の審査結果の要旨

この博士論文は、ショウジョウバエの気管形成において、低分子量G蛋白質Racが細胞の再配置に果たす役割と、シグナル分子Hedgehog(Hh)およびDecapentaplegic(Dpp)が細胞の特定方向への伸長に果たす役割を解明したものである。

ショウジョウバエの気管形成は細胞分裂を伴わず、細胞運動や細胞形態の変化によって達成されるため、種々の遺伝子がこれらの細胞機能をどのように制御して器官形成を行うかを解析する実験系として優れている。しかし、この解析には優れた観察法が必要となる。

論文の第1部で、加藤君は、まずショウジョウバエ胚での気管形成を高度の時間・空間分解能で追跡する方法を開発した。それは、気管細胞特異的なプロモーターの制御下にGFP-モエシン融合蛋白質を発現するショウジョウバエの系統を作り、これを共焦点顕微鏡をとおして動画像として記録する方法である。次に彼は、培養細胞の細胞接着を制御するRacに注目し、気管細胞のRac活性を変化させると気管形成がどうなるかを上記の方法で観察し、気管形成においてRacが気管細胞の細胞接着や再配列を制御することを示した。

論文の第2部で、加藤君は、上記の観察法を用い、気管背側分枝の先端細胞の突起伸長においてHhおよびDppの信号伝達系が果たす役割を調べた。先端細胞はfilopodiaを多方向に伸ばし上皮の内側表面に沿って突起を伸ばすが、正常発生では腹側に伸びた突起が安定化されていた。また、この突起は、Hhを発現する各体節のP区画の上皮細胞に接して伸びていた。そこで、Hhの発現パターンを変えたり、Hh信号伝達系下流の転写因子Ciの活性を先端細胞内で強制的に増減させると、先端細胞の突起伸長の方向が大きく乱れたので、突起の正しい伸長にはCiを含むHh信号伝達系が適切な強度に活性化されることが必要とわかった。もう1つの信号分子Dppは先端細胞のすぐ背側で発現し、先端細胞の突起がこの方向にはあまり伸びないことがわかった。そこで、気管細胞でDppを過剰発現すると、突起の伸長が停止し、時には短縮した。また、Dpp信号系の阻害因子Dadを過剰発現させると、突起先端がしばしば前後軸に沿って異常に伸長した。これらの結果から、Dpp信号系が先端細胞の突起伸長を阻害することが示された。

以上のように、申請者は優れた時間・空間分解能の記録法を開発し、ショウジョウバエの気管形成におけるRac、Hh、Dppの役割を解明した。これらの結果は、ショウジョウバエの気管形成だけでなく、動物の器官形成全般における細胞の移動・突起伸長・再配置の制御機構への示唆を含み、審査委員会は、全員一致で、この論文が博士論文として十分であるとの結論に達した。

博士論文審査会では、論文の内容およびその基礎となる分野について、様々な質問が出たが、加藤君は適切な回答をした。この質疑応答、および公開発表と博士論文の内容から判断して、加藤君は専門分野および基礎分野で、博士号を得るのに十分な知識と理解力を持つと結論した。また、博士論文が英語で書かれていたので、これを読んで、英語能力も十分と判断した。