氏　　名	千 原 崇 裕
学位（専攻分野）	博士(理学)
学 位 記 番 号	総研大甲第533号
学位授与の日付	平成１３年３月２３日
学位授与の要件	生命科学研究科　遺伝学専攻 学位規則第４条第１項該当
学 位 論 文 題 目	Role of Wingless signaling during Drosophila tracheal development
論 文 審 査 委 員	主　査　教授　廣海　健 教授　武田　洋幸 教授　桂　勲 教授　上村　匡（京都大学） 助教授　平田　たつみ（国立遺伝学研究所）

（博士論文の要旨）

Cell differentiation and cell migration are key processes in the development of multicellular organisms. In the nervous system, for instance, the pattern of cell differentiation and cell migration is tightly regulated in order to form an elaborate neural network. For studies of regulatory mechanisms of cell differentiation and cell migration, the Drosophila tracheal system has become an appropriate model system. The tubular epithelium of the Drosophila tracheal system forms a network with a stereotyped pattern consisting of cells and branches with distinct identity. The tracheal primordium undergoes primary branching due to an induction by the FGF homolog Branchless (Bnl), differentiates into cells with specialized function such as fusion cells that perform target recognition and adhesion during branch fusion, and extends branches toward specific targets. Specification of an unique identity for each primary branch is essential for directed migration, as a defect in either the EGF receptor (EGFR) or Decapentaplegic (Dpp) signalings leads to a loss of branch identity and the misguidance of tracheal cell migration.

To elaborate the stereotyped pattern of tracheal network, cell differentiation and cell migration must be tightly regulated during embryogenesis. Following cell differentiation of primary branches, direction of tracheal cell migration must be also regulated so that tracheal branches reach specific targets precisely. However, little of known about what kind of signal in addition to EGFR and Dpp induces differentiation of primary branches and how migration of tracheal cells is guided.

Wingless (Wg) is known as a secreted signaling protein essential for segmentation events. The Drosophila -catenin homolog, Armadillo (Arm), is a downstream component of Wg signaling. Previously it was reported that wg and arm mutant embryos exhibit the severe tracheal defects with loss of specific branch (dorsal trunk) and all tracheal fusion events. Although these phenotypes indicate that Wg signaling is essential for specification of cell and branch identity in the tracheal system, little is known about how Wg signaling acts in the tracheal cells.

To investigate the role of Wg signaling in the specification of cell and branch identity in the tracheal system, I first observed expression pattern of Wg during tracheal development, and found that Wg is expressed in stripes of ectodermal cells located on the anterior and posterior side of each tracheal primordium. The tracheal phenotypes in wg temperature-sensitive mutants and zygotic arm mutants were categorized into the following three phenotypes. First one is the invagination defect. In wild-type embryos, tracheal placodes invaginate into the inside of the embryo. But in wg temperature-sensitive mutants invagination failed, and descendants of tracheal placode were retained in the ectodermal layer. Second, dorsal trunk (DT) was lost. DT normally expresses Spalt (Sal) which is essential for the DT identity, but in arm zygotic mutants Sal expression was lost. Finally, tracheal fusion was defective and Escargot (Esg) expression was lost at all fusion points. Esg is a zinc-finger type DNA binding protein, and its expression is required for fusion cell identity. The latter two phenotypes were further investigated, and I demonstrate that Wg signaling is required within tracheal cells for expression of Sal and Esg in tracheal cells. From these results, I show that Wg and its intracellular signal transducer, Arm, have multiple functions, one specifying dorsal trunk through activation of Sal expression and the other inducing differentiation of fusion cells in all fusion branches.

Moreover, I demonstrate that Wg signaling regulates not only Esg expression itself, but also singling-out of Esg expressing cell at the tip of migrating branches by regulating Notch activity. Notch is a transmembrane receptor stimulated by a ligand Delta. A single fusion cell expressing Esg always locates at the tip of each migrating branch. High level of Delta expression by Bnl signaling is limited to fusion cell at the tip of tracheal branches. The Delta expression in fusion cell activates Notch signaling in nearby cells and that activated Notch signaling represses the fate of fusion cell. As the results, fusion cell is singled out at the tip of tracheal branches by Notch-dependent lateral inhibition. I here show that expression of Delta is also up-regulated by Wg signaling at the transcriptional level, and the high accumulation of Delta permits Esg expression

only in fusion cell. Because Notch activity does not affect Sal expression, expression of Sal is permitted in all DT cells.

From these results, I propose that Wg signaling controls the formation of DT by regulating three genes sal, esg, and Delta in distinct ways. Wg signaling activates Sal expression in tracheal cells, and the expression of Sal is required to render DT an identity to become thick tubule and to migrate in anteroposterior direction. Wg signaling also activates Esg in all DT cells. In addition, Wg and Bnl signals are combined to activate Delta expression at the tip of DT. Elevated Delta activates Notch in nearby cells, leading to repression of Esg in the stalk of tracheal branches. Stimulation of both positive and negative regulation of Esg by Wg signaling comprises a self-limiting assignment of single fusion cells that mark the end of tracheal tubule. In combination with the specification of thick tubules through regulation of Sal, Wg signaling determines the shape of the tracheal tubule.

In addition to the studies described above, I investigated the guidance mechanism of tracheal cell migration. I focused on well-studied dorsal branch (DB), which migrates dorsally and fuses at the dorsal midline with DB from the other side of the same segment during embryogenesis. Previous studies reported that several signaling molecules (Bnl, Dpp and Notch) affect DB formation by inducing cell differentiation, but little is known about the guidance mechanism of DB migration. I found that DB fusion points are precisely located posterior to Wg stripes, suggesting that the direction of DB migration must be also tightly regulated.

To obtain insight into the guidance mechanism of DB migration, I first observed migration pattern of fusion and terminal cells, a pair of cells that occupy the tip of DB. Fusion cells were identified by expression of Esg and were initially located anterior to terminal cells labeled with SRF expression. After the initial expression of these genes, their relative positions switched. In this process, Esg expressing cells continue to contact Engrailed expressing ectodermal cells. I also analyzed the distribution of actin cytoskeleton in migrating DB, and found that fusion and terminal cells have filopodia and lamellipodia similar to those found in neuronal growth cone.

Based on these observations, I next investigated which signaling guides DB migration. I show that planar cell polarity (PCP) signaling has a role in the guidance of DB migration. It is known that PCP signaling regulates small GTPases Rho/Rac1 activity, and controls cytoskeletal rearrangements within the plane orthogonal to their apical-basal axis. I also show that a component of PCP signaling, Drac1 has an essential role on the guidance of DB migration, but not motility of tracheal cells. As it has been reported that axon guidance is regulated by Drac1 activity, these results imply that there is a common guidance mechanism in tracheal cell migration and axonal outgrowth.

In summary, I demonstrate that Wg signaling controls the shape of tracheal tubule in concert with Notch signaling. In addition, I show the guidance mechanism of tracheal cell migration is similar to that of axon guidance, and demonstrate that PCP signaling has effects on guidance of tracheal cell migration. From these results, I propose that Wg acts on both cell differentiation and the guidance of cell migration via distinct signaling pathway to elaborate Drosophila tracheal network.

論文の審査結果の要旨

肺や血管のような複雑な管状網目構造をとる器官が形作られる過程には，細胞特異化，細胞分化，細胞運動などの様々なプロセスが関与している．千原君はショウジョウバエ胚の気管を用い，気管として運命決定を受けた細胞群がいかにして管状網目構造を形成するか，という問題に取り組み，遺伝的，細胞生物学的，分子生物学的手法を巧みに組み合わせた研究を行った．

ショウジョウバエの気管ネットワークは，予定気管細胞群が５つの方向に伸長して５つの側管（気管支）を作り，伸長した気管支のいくつかが体節間で融合することによって形成される．これまでに，予定気管細胞がFGF様因子Branchlesssによって誘導された後，EGF及びDPPというふたつの分泌性因子によって背腹軸に沿って３群の細胞に区画化されることがわかっていた．千原君は第３の分泌因子Winglessが予定気管細胞群の周囲で限局したパターンで発現していることに注目し，Winglessの気管形成における機能を解析した．

まず，wingless遺伝子の高温感受性変異を使うことにより，Winglessが気管発生過程で，（１）予定気管細胞の体内への陥入，（２）５つの気管支の一つであるDorsal trunkの形成，（３）気管支の融合，の３つの過程で必要とされていることを見いだした．Winglessシグナル伝達経路でシグナルを核に伝える役割を担っているArmadillo蛋白質の機能を気管細胞内で阻害すると（２）と（３）の過程が阻害されることを示すことにより，Winglessを受容するのは気管細胞そのものであることを証明した．そして，Winglessシグナルによって（２）の過程では転写因子Spaltの転写が，（３）の過程では転写因子Escargotの転写がそれぞれ活性化されること，さらに，Winglessシグナルは気管支の先端領域でNotchのリガンドであるDeltaの発現を誘導することも示した．Deltaが隣接細胞のNotchを活性化する結果，Wingless発現領域から遠い細胞ではNotchシグナルによりEscargotの発現が抑制され，Escargotが気管支先端の細胞にのみ発現誘導されると推論した．SpaltはDorsal trunk細胞の特異化に必要であり，Escargotは気管支先端細胞の融合に必要であることが知られているので，Winglessは細胞群（Dorsal trunk）特異化と細胞種（先端細胞）特異化を連続して行うことにより気管ネットワーク形成を制御していることが明らかになった．

次に，千原君は気管支伸展における細胞移動機構について解析した．Dorsal branchと呼ばれる気管支は背側に枝を伸ばし，背側正中線で反対側からの気管支と融合した後，方向を変えて腹側に伸展する．千原君はこの過程での気管支形態を詳しく解析し，その先端細胞が神経軸索先端と同じ様にフィロポディアやラメリポディアを有することを発見した．さらに，Dorsal branchの道筋付近でもWinglessが発現しており，Winglessを異所的に発現すると気管支走行が乱れることを見いだした．Armadilloの機能を気管細胞内で阻害する実験から，この現象においてもWinglessを受容しているのは気管細胞そのものであることがわかった．しかし，標準的なWinglessシグナル経路でArmadilloの下流で働くDTcfや，先端細胞での転写因子Escargotの発現は細胞移動には関与していないことが判明した．従って，Armadilloの下流に細胞移動を制御する新しいシグナル伝達経路が存在する可能性がある．一方，細胞極性シグナル経路はWinglessシグナル伝達経路と構成要素の一部を共有しており，その下流には細胞骨格の制御因子である低分子GTPアーゼDrac1があることが知られている．千原君は，Drac1の機能を阻害すると気管支伸長そのものは起こるが，正常な方向性を失うことを見いだした．Drac1は神経軸索誘導にも関与しているため，この結果は気管と神経細胞に共通なガイダンスの分子機構の存在を示唆するものとして重要である．

千原君の仕事は，気管の細胞集団が空間的に限局した分泌因子の情報を利用して複雑な気管ネットワークを形成するメカニズムを初めて明らかにしたものとして世界的にも高く評価されている．細胞移動機構についても，Drac1とその上流のシグナルの関係を調べることによって研究がさらに発展すると期待される．以上の理由で，この論文は博士（理学）の学位論文として充分な内容を持つ，と審査員全員の意見が一致した。