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学 位 論 文 題 目	Dual function of Src tyrosine kinase in epithelial morphogenesis in *Drosophila*
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論文内容の要旨

Cell migration and cell determination play important roles during establishment of multicellular organs. Although cell determination and cell migration take place simultaneously in many cases of organogenesis, the mechanism which coordinates those processes is not well understood. In this study, I identified the Drosophila homolog of c-Src, src42A as a candidate for a gene that controls both cell migration and cell determination during tracheal formation.

The Drosophila tracheal system is a network of tubules with a stereotyped pattern formed by a series of branching, migration, and fusion of tubular ectodermal epithelia and provides an ideal system to study the processes of organogenesis. In this work, I performed gain-of-function screening using the Gal4-UAS system to search for novel genes functioning in tracheal cell morphogenesis in Drosophila. The phenotypes of tracheal defects were classified into 3 classes; the branching, migration or fusion defect class, the cell attachment defect class, and the cell volume defect class. By mapping the GS vector insertion sites on the Drosophila genome map, I identified candidate genes whose expression might be forced in the GS lines causing the tracheal defects.

In this study, I focused on the roles of the Src family protein in Drosophila tracheal development. Src family tyrosine kinases are first identified as transforming proteins encoded by oncogenic retroviruses. Numerous studies suggest that Src function to regulate cell-cell adhesion and the actin cytoskeleton. However, in vivo roles of this family proteins is unclear.

There are 2 c-src homologues in *Drosophila* genome; src42A and src64B, which are shown to play functionally redundant roles in the closure of dorsal epidermis. Overexpression of src42A, as well as src64B, caused mesenchyme-like transformation of the tracheal epithelium and abnormal F-actin accumulation. These results suggest that both src42A and src64B have important roles in tracheal development and in epithelial morphogenesis in general.

During tracheal development, activated Src was localized to the apical cell-cell junction site and activation of Src is strictly regulated spatially and temporally when cell rearrangement activity is high. In src42A mutant embryos, F-actin organization at adherens junction where E-cadherin/β-catenin/α-catenin complex is localized was abnormal. Such a phenotype might reflect cell-cell contact defects in src42A mutants.

By using a functional DE-cadherin-GFP fusion protein to distinguish newly synthesized E-cadherin from pre-existing one of maternal origin, I found that Src42A activity regulates DE-cadherin turnover rate. Overexpression of dominant negative form of src42A, src42ADN caused pre-existing E-cadherin signal to predominantly occupy the apical cell-cell junction. On the other hands, overexpression of constitutively active form of src42A, src42AACT reduced the amount of pre-exinting E-cadherin signals, allowing newly synthesized E-cadherin to occupy adherens junction of trachea.

Consistent with this observation, Src was shown to post-transcriptionally reduce the level of E-cadherin. Furthermore I identified the Drosophila homolog of Hakai, DHakai, which ubiquitinates E-cadherin in a src-dependent manner. The reduced level of DE-cadherin caused by hyperactivation of src was restored by inactivation of DHakai by RNA interference. These results

suggest that Src down-regulates DE-cadherin by ubiquitination and degradation mediated by DHakai.

In addition, src42A mutants lost the expression of a target gene of Wg signaling Escargot (Esg), which is usually expressed only in the fusion cells of the trachea. On the other hand, hyperactivation of Src caused increase of the number of Esg expressing cells. Increase in the number of Esg expressing cells upon hyperactivation of src was suppressed by coexpression of the dominant negative form of Tcf, TcfΔN. Furthermore hyperactivation of Src42A caused increase of Arm, the key transducer of Wg signaling, in tracheal cytoplasm. These results suggest that Src activity is involved in the control of tracheal formation by Wg signaling. The increase of Esg expressing cells caused by hyperactivation of Src was enhanced by coexpression of DAxin, which forms a complex with GSK-3 and APC, and promotes degradation of Arm. This result suggests that Src might work to inactivate the degradation machinery of Arm by regulating the Axin function.
From these analyses, I concluded that Src42A coordinates cell adhesion and cell differentiation by regulating E-cadherin turnover rate and Arm stabilization. Furthermore, activation of Src42A in fusion cells at later tracheal development amplifies Wg signaling in tracheal cells.

論文の審査結果の要旨

発生における器官形成では、細胞の運動と分化が秩序だって進行するが、それらを統合する機構には不明な点が多い。ショウジョウバエの呼吸器官である気管の形成では、上皮細胞が陥入して細胞分化を伴って様々に形態変化し、複雑な三次元構造を構築する。新道さんは、細胞の運動と分化を統合する機構を明らかにする目的で、気管の形成について遺伝学的解析を行った。新道さんは、まず強制発現したときに気管の形成に異状を生じる遺伝子をスクリーニングし、それらの中で気管上皮の間充織様組織への変形と F-アクチンの異常な蓄積を示した *src* に注目した。気管形成において Src は、細胞運動や分化が盛んに行われる時期に部位特異的に活性化され、活性化型 Src は細胞接着部位に局在していた。Src の活性を実験的に上昇させると、E-カドヘリンのターンオーバーが亢進されたことから、Src は細胞接着能を低下させることで細胞運動を促進していると考えられる。

一方、Src の活性化は、Wg シグナル伝達系の標的遺伝子の発現を誘導し、Wg シグナル伝達系の下流因子 Arm を安定化した。従って、Src は Wg シグナル伝達系を通して細胞分化を誘導すると考えられる。これらの結果は、気管形成において細胞の運動と分化が Src の時期特異的活性化により秩序だって進行することを示している。

以上のように、この論文の内容は器官形成における細胞の運動と分化の秩序だった進行の機構を解明したもので、遺伝学専攻の博士論文としての条件を満たすことを審査員全員が認めた。

博士論文審査に関わる公開発表会の後に、論文審査員と新道さんの間で質疑応答がなされた。この結果、新道さんは博士論文に関わる研究分野および、関連する研究分野について充分な知識をもっており、その知識に基づいて考察する能力をそなえていることが解った。また本論文は英語で書かれており、学位にふさわしい英語の能力を持つと判断した。