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Title	Development of FRET-based indicators for visualizing homophilic trans interaction of a clustered protocadherin
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様式３

論　文　内　容　の　要　旨

氏　名　（　星野　七海　）	
論文題名	Development of FRET-based indicators for visualizing homophilic *trans* interaction of a clustered protocadherin（クラスター型プロトカドヘリンのホモフィリックなトランス相互作用の可視化のためのFRETを用いたインジケーターの開発）

論文内容の要旨

Clustered protocadherins (Pcdhs), which are cell adhesion molecules, play a fundamental role in self-recognition and non-self-discrimination by conferring diversity on the cell surface. Although systematic cell-based aggregation assays provide information regarding the binding properties of Pcdhs, direct visualization of Pcdh trans interactions across cells remains challenging. Here, I present Förster resonance energy transfer (FRET)-based indicators for directly visualizing Pcdh trans interactions. I developed the indicators by individually inserting FRET donor and acceptor fluorescent proteins (FPs) into the ectodomain of Pcdh molecules. They enabled successful visualization of specific trans interactions of Pcdh and revealed that the Pcdh trans interaction is highly sensitive to changes in extracellular Ca2+ levels.

The FRET indicators above, did not show any FRET signals at contact sites when the acceptor and the donor indicators were co-expressed in cells. To enable FRET-based visualization of the Pcdh homophilic trans interaction in self-neurites is essential for asking the spatio-temporal information of the interaction during self-recognition. To achieve the goal, I fused two FPs into a single Pcdh molecule, which means the two indicators are united into a single indicator which donor and acceptor proteins are combinatorially fused. This “combined” FRET indicator successfully showed FRET signals at cell-cell contact sites but no FRET signals in intracellular space.

Although two FPs are fused in extracellular (EC) domains of a Pcdh, cell aggregation assay and immunoprecipitation revealed that the FP-fused Pcdh showed normal binding affinity to other proteins which are reported to interact with EC domains of Pcdhs.

Furthermore, I constructed a transgenic mouse which expresses the combined FRET indicator in a Cre-dependent manner. With this mouse, I revealed that a single isoform of Pcdhγ can rescue the self-crossing phenotype in dendrites of Pcdhγ-deletion Purkinje cell.

I expect that FRET-based indicators for visualizing Pcdh trans interactions will provide a new approach for investigating the roles of Pcdh in self-recognition and non-self-discrimination processes including regulation of synaptic specificity.

In this thesis, I present introduction of why I worked on the development of the indicator of the homophilic trans interaction of Pcdh as chapter 1. Then, I present the results of separated FRET indicators and the ones of a combined FRET indicator. As a co-first author, I reported the former results in (Kanadome et al., 2021). Lastly, I discuss the usage of these indicators including the pros and cons of the two concepts.

様式 7

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

星野七海さんは、Förster共鳴エネルギー移動（FRET）技術を用いて、クラスター型プロトカドヘリン(Pcdh)のPcdhγB2のtrans相互作用を可視化することに成功した。最初は、CFPとYFPの蛍光タンパク質を別々のPcdhγB2タンパク質のEC1とEC5領域に融合させたタンパク質させることでFRETの観察に成功した。また、このFRETシグナルがCa2+依存的であるということを示した。更に、このCFPとYFPの2種類のプローブを同時にPcdhγB2タンパク質に融合させた結合型PcdhγB2-FRETタンパク質での、ホモフィリック相互作用のFRETによる可視化に成功し、海馬神経細胞でのPcdhγB2のホモフィリック相互作用を初めて可視化することに成功した。さらに、Cre依存的に結合型PcdhγB2-FRETタンパク質を発現するトランスジェニックマウスの作製に成功し、小脳プルキンエ細胞の樹状突起形成の自己忌避活性を示すことに成功している。これまでにカドヘリンを含めホモフィリック相互作用をin vivoで可視化した例はなく、極めて独創性の高い研究であり、博士号取得に値すると考える。