論文の内容の要旨
Dissertation Abstract

論文題目

Bioluminescence Analysis and Optical Control of Phosphatidylinositol (3,4,5)- triphosphate on the Cell Membrane
（細胞膜上ホスファチジルイノシトール 3, 4, 5−三リン酸の生物発光分析と光制御法）

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Phosphatidylinositol (3,4,5)- trisphosphate (PIP3) is a lipid second messenger that mediates central cellular events such as growth, motility and development by recruiting and activating downstream proteins. The PIP3 level in an un-stimulated cell is balanced between its synthesis by the phosphatidylinositol 3-kinase (PI3K) upon receptor stimulation and its degradation by lipid phosphatases. Loss of such equilibrium results in an elevation of PIP3 amount which was found in tumorigenic

cells or a suppressed PIP3 level which retards the growth of nerve cells. Although PIP3 abnormality is related to fatal diseases, the various roles of PIP3 have not been resolved thoroughly because of limitations of PIP3 analytical methods. Previous methods have used PIP3 antibodies, instrumental separation, or fluorescence imaging to analyze the PIP3. However, the in vitro immunoblotting and instrumental analysis involve cell demolishing sample pretreatment that may decompose the PIP3; fluorescence imaging enables the visualization of PIP3 in living cells, however the observation is confined by a microscope and plausible quantification is caused by the instability of a fluorescent protein. The spatiotemporal and quantitative analysis of PIP3, which is of critical importance to unveil the precise regulative roles of PIP3, remains difficult.

In this study, I focused on the development of bioluminescent probes to analyze the relative amount of PIP3 using split luciferase complementation, and furthermore, the establishment of an optical system to produce PIP3 on cellular compartments using light sensitive protein-protein interactions.

In chapter 1, the background and basic concepts for this study such as PIP3, bioluminescence analysis and light inducible protein-protein interactions are introduced.

In chapter 2, a pair of split luciferase probes was developed to quantify the production of PIP3 on the plasma membrane. A full-length luciferase was dissected into N- and C- terminal luciferase fragments and their activities are completely lost. When the two fragments approach each other with a close proximity, they would reconstitute to recover the luciferase activity and produce the bioluminescence signal. The PIP3 probes using split luciferase fragments took advantage of a Pleckstrin Homology domain that specifically binds with PIP3. Tandem PH domains were fused with C-terminal luciferase fragment, expressed in the cytosol, whereas the N-terminal

luciferase fragment is predominantly localized on the plasma membrane. In response to PIP3 production, PH domain would be brought to the plasma membrane by the PHD-PIP3 interactions and promotes the co-localization of two luciferase fragments. The co-localized fragments spontaneously reconstitute to form an active luciferase producing bioluminescence recovery. (Figure 1) The bioluminescence signals are proportional to the amount of PIP3 produced on the plasma membrane. The bioluminescence probe was also used to bioluminescence imaging of PIP3 production and also to establish a High Throughput Screening (HTS) system for PIP3 related compounds. The PIP3 probes provide important features including temporal recording of PIP3 production by bioluminescence and analysis of relative PIP3 amount on the plasma membrane with minimal cell toxicity. The study also used a novel self-associative luciferase fragment complementation that enables the further analysis of a lipid messenger with bioluminescence signals with high specificity.

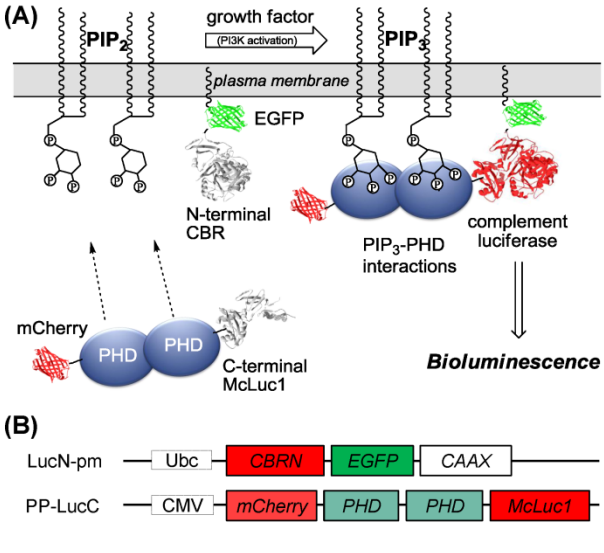

Figure 1. (A), Schematics for the detection of membrane PIP3. (B), Domain structures of PIP3 probes.

In chapter 3, optical control of PIP3 production is performed. PIP3 is a pivotal lipid messenger that plays various roles. Diverse functions are likely to be resulted from spatiotemporal effects of PIP3 naturally produced in specific organelles as a signal network. Therefore, we seek to establish a method to control the production of PIP3 on specific cellular compartments. Previously PIP3 was generated by ligand stimulation with low organelle specificity. In substitution of the chemical stimulation,

optogenetic system that uses light sensitive protein-protein interactions is utilized to perturb the PIP3 production spatiotemporally. Arabidopsis cryptochrome 2 (Cry2) interact with CIBN protein with sub second timescale when irradiated with blue light and dissociates with CIBN when kept in the dark or irradiated by red light. In our method to control the PIP3 production, A CIBN is anchored on a subcellular organelle membrane, whereas a Cry2 is fused with a constitutively active PI 3-kinase (acPI3K), which synthesizes PIP3, expressed in the cytosol. acPI3K would be recruited to the membrane after light stimulation by the CIBN. As PIP3 is synthesized on the membrane, its production is visualized by the translocation of the PH domain, which specifically binds PIP3. (Figure 2) Fluorescent proteins are fused with each protein to monitor the dynamics. The PIP3 was reversibly produced by blue light and some cellular effects such as growth, movements were observed after the irradiation. This method clarifies a path for more extensive study of PIP3 functions.

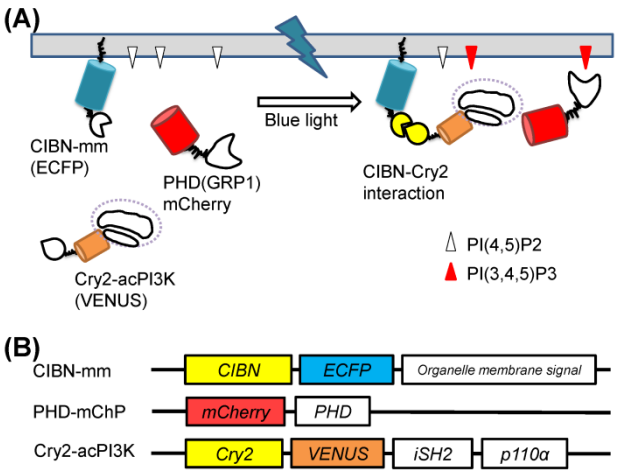

Figure 2. (A), Schematics for the optical control of PIP3 production on organelle membrane. (B), Domain structures of fusion proteins.

In chapter 4, the concluding remarks are given.