Analysis of the Akt isoform-specific signaling pathway regulation using optogenetics and mathematical modeling

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A Ser/Thr kinase, Akt, plays a pivotal role in various protein signal transductions in live cells. There are three Akt isoforms, Akt1, Akt2, and Akt3, of which functional differences in signaling pathways have been investigated. However, the temporal activation patterns of the Akt isoforms is unclear. Additionally, the relationship between the isoforms' temporal activation patterns and their regulation mechanisms of downstream signal transduction remains elusive. The purpose of this research is to examine the differences in the temporal aspects of the Akt isoforms' signal transduction. Previously, we developed an optogenetic method named photoactivatable Akt (PA-Akt) system for controlling the activity of Akt1[1]. The PA-Akt system enables us to artificially activate Akt by utilizing a light-induced dimerization of a photoreceptor CRY2 with CIBN (Fig. 1a). In this research, we newly applied this system to Akt2 and Akt3 (Fig. 1b) . By using the PA-Akt systems for the three Akt isoforms, we enable individual manipulation of the activation pattern of each Akt isoform with high temporal resolution. We have confirmed that all the PA-Akt systems activated CRY2-Akt upon light stimulation by observing CRY2-Akt translocation to the plasma membrane and detecting the phosphorylated CRY2-Akt.

To quantitatively analyze the temporal activation patterns of the Akt isoforms, the combination of the PA-Akt system and a mathematical model is effective. For the construction of a mathematical model, we quantified the CRY2-Akt phosphorylation levels using western blotting. By constructing the mathematical model, the factors contributing to the differences among the isoforms' temporal phosphorylation level changes would be revealed. We aim to individually manipulate the Akt isoforms' activity and investigate the isoforms' selectivity to the downstream signaling.

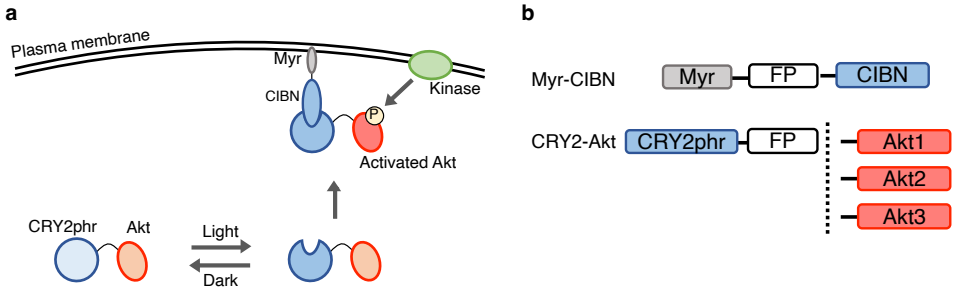

Fig 1. PA-Akt system. (Created based on [1])

1) Y. Katsura, H. Kubota, K. Kunida, A. Kanno, S. Kuroda, T. Ozawa. *Sci. Rep.* **2015**, *5*, 1–10.