www.elsevier.com/locate/developmentalbiology

An Optogenetic approach to control protein localization during embryogenesis of the sea urchin

Alicia Uchida, Mamiko Yajima

PII: S0012-1606(17)30738-8
DOI: https://doi.org/10.1016/j.ydbio.2018.06.015
Reference: YDBIO7797

To appear in: *Developmental Biology*

Cite this article as: Alicia Uchida and Mamiko Yajima, An Optogenetic approach to control protein localization during embryogenesis of the sea urchin, *Developmental Biology,* https://doi.org/10.1016/j.ydbio.2018.06.015

An Optogenetic approach to control protein localization during embryogenesis of the sea urchin

Alicia Uchida and Mamiko Yajima*

MCB Department, Brown University, 185 Meeting Street, BOX-GL173, Providence, RI 02912, USA

***Correspondence to:** mamiko_yajima@brown.edu

Summary

Light inducible protein–protein interactions have been used to manipulate protein localization and function in the cell with utmost spatial and temporal precision. In this technical report, we use a recently developed optogenetic approach to manipulate protein localization in the developing sea urchin embryo. A photosensitive LOV domain from *Avena sativa* phototropin1 cages a small peptide that binds the engineered PDZ domain (ePDZ) upon blue light irradiation. Using this system, mCherry tagged proteins fused with the LOV domain were recruited to ectopic sub-cellular regions such as the membrane, microtubules, or actin by GFP tagged proteins fused with the ePDZ domain upon blue light irradiation within 1~3 minutes in the sea urchin embryo. The efficiency and speed of recruitment of each protein to its respective subcellular region appeared to be dependent on the power and duration of laser irradiation, as well as the respective level of affinity to the tagged location. Controlled laser irradiation allowed partial recruitment of the spindle to the membrane, and resulted in cell blebbing. Vasa, a cell cycle and germline factor that localizes on the spindle and enriches in the micromeres at 8-16 cell stage was recruited to ectopic sites, preventing normal enrichment. Continuous blue light activation with a regular blue aquarium light over two days of culture successfully induced LOV-ePDZ binding in the developing embryos, resulting in continued ectopic recruitment of Vasa and failure in gastrulation at Day 2. Although some cytotoxicity was observed with prolonged blue light irradiation, this optogenetic system provides a promising approach to test the sub-cellular activities of developmental factors, as well as to alter protein localization and development during embryogenesis.

Keywords:

Optogenetics, Live imaging, Embryo, Sea urchin

Introduction

Embryonic cells display dynamic protein expression and localization in each blastomere, contributing to differential cell fate determination in the embryo during development. In the sea urchin embryo, cell division occurs every 30~40-minutes (i.e. 20 minutes of M-phase and 20 minutes of S-phase and no G-phase). Several proteins exhibit differential expression and/or localization in each blastomere. Traditionally, a general gene knockdown or overexpression has been used to identify the functional contributions of specific proteins during embryogenesis, but