Shining Light on Calcium-Mediated Morphogenesis : Forward Engineering Organ Development with Optogenetics

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Cells communicate to coordinate cellular processes across tissues, and Ca^{2+}-ions are second messengers facilitating such multiscale coordination. However, current understanding of the biological mechanisms precludes direct control of Ca^{2+}-mediated processes. Here, we have adopted a forward engineering approach to understand the role of Ca^{2+}-signaling in morphogenesis using optogenetics. This is a novel method of regulating Ca^{2+}-signaling in epithelial tissues. The output of Ca^{2+}-signaling in terms of tissue morphogenesis can thus be controlled by adjusting width-half-max of optogenetic activation. Broader implications of this work include organism and organ-based drug screening assays and disease treatments connected to dysregulation of Ca^{2+}-signaling dynamics.

I. Purpose

Calcium (Ca^{2+})-ions are second messengers facilitating multiscale coordination for cellular processes across tissues [1]. Ca^{2+}-selective cation channels mediate Ca^{2+} influx from the extracellular environment into the cell cytosol upon activation. However, the biological mechanisms involved in Ca^{2+}-regulated processes remain elusive as there is no direct way to control Ca^{2+}-dynamics. In this work, an optogenetic channel, CsChrimson is investigated to define how Ca^{2+}-signaling dynamics impact epithelial growth and morphogenesis. Toward this end, we outline the phenotypic results of controlled stimulation of CsChrimson [2]. *Drosophila melanogaster* was used as an in vivo system, whose wing is acutely sensitive to changes in developmental pathways [3]. We hypothesize that by controlling Ca^{2+}, growth of cells and tissues can be directly controlled and the optimum level of cytosolic Ca^{2+}-signaling to promote growth can be established. Additionally, while optogenetic channels have been expressed in neurons [4], the consequence of their manipulation directly in epithelial tissues to perceive morphological outcomes remains unexplored.

II. Results

We employed optogenetics to forward engineer growth control as a bottom-up approach toward tissue engineering. Temporal programming of light was utilized to stimulate Ca^{2+}-influx and control cellular processes in an in vivo organ model of epithelial morphogenesis.

A. Stimulation of Ca^{2+} spiking with light activation

We have shown a significant level of increase in Ca^{2+} spiking with light activation when CsChrimson is expressed in epithelial cells of the wing imaginal disc of *Drosophila*. This definitively proves that CsChrimson can be used control the concentration of Ca^{2+}-ions in tissue.

B. Biphasic control of organ size

Genetic overexpression of CsChrimson in the wing disc, and subsequent light activation cause high flux of Ca^{2+} and progressively stronger phenotypes in adult wings. Preliminary results suggest that the size of organ expressing CsChrimson increases with lower intensity of red light (~5 lux), compared to dark: 0 lux. However, size reduces with higher intensity of light and more Ca^{2+} in cytosol. Hence, an optimum Ca^{2+}-level is achievable to program tissue shape.

C. Light-induced apoptosis

Cell death significantly increases while cell proliferation decreases in optogenetically activated regions of wing discs on longer periods of exposure to high intensity light. This is congruous with the phenotypes observed in adult wings. This demonstrates that CsChrimson regulating cytosolic Ca^{2+}-dynamics impacts downstream proteins and growth-related biological pathways.

III. Conclusion

Growth control of epithelial organs is possible by controlling Ca^{2+} flux and spatiotemporally controlled apoptosis can occur via optogenetics. A “Goldilocks' Zone” can be identified for the optimum level of Ca^{2+} that promotes growth by controlling the width-half-max of optogenetic activation and, in turn, program tissue and organ shapes by regulating cytosolic Ca^{2+}-dynamics. We also aim to translate the work in human endothelial cells and optochemical tools to map Ca^{2+}-signaling in terms of membrane potential, $V_{mem.}$ The end goal of this work is to establish optogenetics as a sensitizer for drug screening applications.

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