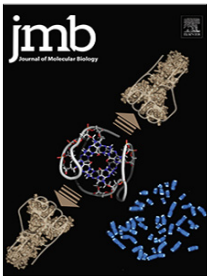

OptoLRP6 Illuminates Wnt Signaling in Early Embryo Development

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Cell fate determination and tissue organization during early development requires precise spatiotemporal coordination of cell-to-cell signaling. In metazoans, both adult stem cell niches and early embryos require continuous cell–cell communication using precise gradients of signaling molecules throughout the tissue, conveying positional and cell fate information through complex, multicomponent signal relay pathways.[1] Such information is transmitted across large distances via extracellular ligands, such as Wnt, to stimulate receptor activation and promote downstream gene expression programs.

Wnt signaling is an ancient and conserved signal transduction pathway with a number of central roles in early embryonic development.[2] In the blastula stage of amphibian embryos, Wnt signaling through Frizzled (Frz) and the LRP5/6 co-receptors is responsible for stabilizing β-catenin in dorsal cells and for establishing the Spemann Organizer, thereby promoting the formation of the dorsoventral axis.[3] Further studies have shown that canonical Wnt signaling mechanisms promote the formation of similar dorsal organizer in other phyla, suggesting conservation in most, if not all, vertebrate species.[4] Later, during gastrulation, Wnt signaling contributes to posterior cell fates. Activation of the canonical Wnt pathway or stabilization of β-catenin in inappropriate tissues, such as in ventral cells, is sufficient to form a secondary axis in amphibian embryos.[5–8]

At the molecular level, the flux through the canonical Wnt pathway is determined by modulation of β-catenin degradation. The core destruction complex, consisting of Axin, GSK-3 and APC, promote phosphorylation which ultimately leads to degradation of β-catenin.[9] When cells are stimulated by the Wnt ligand binding to Frz and LRP6, components of the destruction complex translocate to the membrane and are downregulated, thus stabilizing β-catenin. In support of this model, Wnt pathway induction can also be achieved by inhibiting destruction complex function with small molecules (e.g. LiCl inhibition of GSK3), or by increasing the expression of Wnt pathway components.[3] However, it is far more challenging to precisely control the timing of gene expression using siRNA or morpholino knockdown approaches in large, complex tissues. Further, it is difficult to spatially re-pattern signaling pathways chemogenically.

The advent of optogenetic tools has facilitated careful dissection of signals that control tissue organization through spatiotemporal illumination and activation of specific pathways in individual cells. Light-induced optical switches, based on dimerization of cryptochromes or LOV domains have revolutionized cell and developmental biology.[10] These light-responsive molecular switches come in a number of formats, including allosteric regulation of protein conformation and enzymatic activity, clustering of a pathway components, or subcellular translocation of a receptor or ligand to an activation site.[11] One of the major engineering challenges of these platforms is to suppress activation in the dark state. For membrane associated receptors or co-receptors, a hurdle in converting them to optogenetic switches is high basal dimerization in the unilluminated state.[12,13] The authors overcome this by localizing the co-receptor to the cytosol, away from plasma membrane in the off state.

In this issue of the *Journal of Molecular Biology*, Krishnamurthy and colleagues report the development of a generalizable strategy to

optically translocate co-receptors to the plasma membrane to promote downstream activation and demonstrate its efficacy in promoting Wnt signaling in a model vertebrate embryo.[14] The authors engineered the cytosolic domain of the Wnt co-receptor, LRP6, into a blue light responsive switch, OptoLRP6opt. They show that photo-induced localization of the LRP6 cytosolic domain to a membrane associated anchor promotes downstream canonical Wnt signaling, activation of target genes, and sculpts the morphology of early embryos.

Engineering an optically responsive LRP6 required splitting the co-receptor into two functional components and fusing them to light-induced dimerization domains. LRP6 is composed of an extracellular, transmembrane, and cytosolic domain. Previous studies demonstrated that the cytosolic domain of LRP6 was capable of promoting constitutive signaling, but only with very high levels of expression. However, the addition of the transmembrane domain fused to the cytosolic domain was sufficient to activate constitutive signaling at native expression levels.[15] Further, clustering of LRP6 into cytosolic signalosomes is sufficient to promote signaling.[15,16] Guided by these results, the authors constructed split components into a membrane associated CaaX domain and the C-terminal cytosolic domain of LRP6, and tagged them with cognate light-dimerized CIB and Cry2 domains. Because the cytoplasmic domain of LRP6 freely diffuses in the cytosol, this strategy ensures low basal activity in the absence of illumination. Only when cells are illuminated, does the cytosolic domain of LRP6 translocate to the membrane and initiate canonical Wnt signaling.

The signaling response of OptoLRP6opt is specific and robust and works in both cell culture and in large vertebrate embryos. The authors used several rounds of structural optimization of the membrane associated anchor fused to CIB and the cytosolic LRP6 fused to Cry2. Importantly, multivalency of CIB81 domain and close proximity of LRP6 to membrane, by removal of a fluorophore, were key to a high ratio of activation in the light versus dark state. To measure Wnt activation in transfected human cell lines, the authors used a TopFlash reporter assay. In the final optimized construct, OptoLRP6opt, they found light-induced translocation of the LRP6 cytosolic domain to the transmembrane domain occurs in a matter of minutes, and the system achieves a nearly 50-fold range of activation between on and off states. Importantly, the authors also demonstrate their system achieves higher levels of induction in response to light compared to a previous OptoWnt system.[16]

To test the efficacy of this new optogenetic Wnt signaling platform in a complex tissue, they interrogated axis formation in embryos from the model vertebrate, *Xenopus laevis*. This tissue environment represents a very challenging test case because the embryos are millimeter size and contain pigment granules and yolk that may attenuate light penetrance. Rather spectacularly, they demonstrate near perfect light-dependent axis duplication upon injection of ventral blastomeres with OptoLRP6opt and illumination during the blastula stage of development. With an optimized injected dose, 25 pg RNA, they show no axis duplication in the dark and 100% in the light. Optical induction promotes formation of an ectopic Spemann-Mangold organizer and later full morphological axis duplication.

This study elegantly demonstrates power of optogenetics for control of cell-to-cell signaling. The authors implemented a simple, modular membrane proximity switch to photoregulate co-receptor function and signal initiation. Further, they advanced beyond cell lines, developing a new tool to supplement the limited optogenetic toolbox that functions in large vertebrate embryos. The tools presented are likely generalizable for other signaling pathways that rely on membrane bound receptors. Notably, OptoLRP6opt outperforms previous Cry2-based Wnt activation systems and expands optical control of this pathway to a complex tissue. Additionally, this study provokes new questions about the temporal window required for Wnt signaling and the minimum spatial domain of cells sufficient to induce axis specification. β-catenin signaling controls many distinct features of embryogenesis across wide ranging periods of development and tissue organization. To the extent that blue light can penetrate embryonic tissues of instance, such as those near the surface of the embryo for non-placental models, this platform should provide a new strategy to interrogate the temporal and spatial impacts of Wnt activation.

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