Reversible Optogenetic Control of Growth Factor Signaling During Cell Differentiation and Vertebrate Embryonic Development

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Abstract: To decipher the kinetic regulation of growth factor signaling outcomes, I will introduce our recently developed non-neuronal optogenetic strategies that enable reversible control of growth factor signaling during cell differentiation and embryonic development.
OCIS codes: (180.2520) Fluorescence microscopy; (170.1530) Cell analysis

1. Introduction

Growth factor signaling pathway regulates cell proliferation, differentiation, survival, migration, and apoptosis. Of significant importance is the nerve growth factor (NGF), which controls proper development of the mammalian peripheral nervous system. NGF interacts with its high-affinity receptor, TrkA, and mediates an intracellular signaling network that includes the extracellular-signal-regulated kinase (ERK), phosphoinositide 3-kinase (PI3K)/Akt, and phospholipase-γ (PLCγ) pathways. These signaling subcircuits work synergistically to determine cellular outcomes. Delineation of signaling contributions from individual subcircuits remains challenging, which is further complicated by the identification of a low-affinity receptor of NGF, p75NTR. Here, we utilize optogenetics to decouple signaling outcomes at the level of receptor and intracellular kinases. Optogenetics offers precise spatiotemporal control of growth factor signaling during cell differentiation and embryonic development.

2. Methods

The rat PC12 cells, an *in vitro* model to study sympathetic neuron development, were cultured in F12K medium supplemented with 15% horse serum and 2.5% FBS. All cell cultures were maintained in a standard incubator at 37 ºC with 5% CO_2. *Xenopus* embryos were obtained and injected as described [1]. The optogenetic TrkA (optoTrkA) was constructed by a fusion of the blue-light-inducible, homo-associating light-oxygen-voltage domain of aureochrome1 from *Vaucheria frigida* (AuLOV) to the C-terminus of the rat TrkA intracellular domain (ICD) [2]. The optoRaf system was constructed with an optimized bicistronic optogenetic system using *Arabidopsis thaliana* cryptochrome 2 (CRY2) protein and the N-terminal domain of cryptochrome-interacting basic-helix-loop-helix (CIBN) (**Fig. 1c**) [3]. Light stimulation was performed on a home-built LED lightbox [4].

3. Results

Decoupling receptor signaling outcomes by optoTrkA. Upon treatment with NGF, PC12 cells adopt a differentiated state, characterized by halted proliferation and the outgrowth of neurites several times longer than the cell body. This response has been attributed to the signaling mediated by TrkA, which is expressed abundantly in PC12 cells. Biochemical and genetic analysis has identified two key tyrosines within the TrkA intracellular domain, Y490 and Y785, which serve as signal-initiating residues for the ERK and PLCγ pathways, respectively. Delineation of the role of each tyrosine is complicated due to the potential signaling mediated by the low-affinity NGF receptor, p75NTR.

The optoTrkA uses light to activate TrkA in the absence of NGF, therefore, decouples the signaling outcomes of TrkA from p75NTR. Activation of TrkA is based on light-induced homo-association of AuLOV, which brings TrkA kinase domains within proximity to initiate the cross- and autophosphorylation of specific tyrosines (**Fig. 1a**). To accurately assign the phenotypic roles of Y490 and Y785 in PC12 cell differentiation, tyrosine-to-phenylalanine mutants (Y490F, Y785F, and Y490/785F). In combination with pathway-specific pharmacological inhibition, we demonstrated that Y490 and Y785 each contributes to PC12 cell differentiation through the ERK pathway in an additive manner (**Fig. 1b**). In line with previous findings, we found that Y490 regulated ERK signaling, while Y785 primarily regulated PLCγ signaling. Y785 also promoted ERK signaling in a PLCγ-PKC-dependent manner [2].

OptoRaf delineates the Raf/MEK/ERK signaling cascades from other kinase pathways downstream of TrkA. To further delineate the signaling outcome of downstream signaling pathways, we designed optoRaf1, an optogenetic system

that interrogates the Raf/MEK/ERK signaling subcircuits. Unlike optoTrkA, optoRaf1 uses light-mediated membrane translocation of the Raf1 to activate the ERK activity in a ligand-free manner (**Fig. 1c**). Thus, optoRaf1 bypasses the receptor and solely acts on the Raf/MEK/ERK signaling cascades. Western blot analysis reveals that optoRaf1 enables reversible phosphorylation of ERK at the time scale of minutes. A 24-h blue light stimulation (0.2 mW/cm^2) in PC12 cells transfected with optoRaf1 caused significant neurite outgrowth.

ERK signaling plays multifaceted roles during vertebrate embryonic development. During gastrulation, ERK signaling is essential for mesoderm induction. After gastrulation, high levels of ERK activity can be detected in the tailbud and is thought to control the formation of tail structures. It was unclear, however, whether the functions of ERK in mesoderm induction and tail formation could be uncoupled. Taking advantage of optoRaf, which allows us to activate ERK signaling at any desired time, we addressed this important question using *Xenopus* embryos. We injected mRNAs encoding optoRaf1 into embryos at the 8-cell stage and modulated the temporal profile of blue light stimulation. As expected, activation of optoRaf1 during gastrulation induced ectopic mesoderm and formation of somite-like structures. When activated after gastrulation, optoRaf1 stimulated the growth of mesenchyme, leading to ectopic tail-like structures (**Fig. 1d-f**) [3]. This result thus demonstrates that ERK signaling can promote tail formation independent of its function during germ layer specification.

4. Conclusion

Besides temporal regulation of the ERK pathway, optogenetics also allows spatial regulation of the ERK signaling in other model systems such as *Drosophila* [5]. Accumulating work has demonstrated that optogenetics can complement with current cell biological and biochemical approaches. Continuous advances in biophotonics promise to enhance the spatial and temporal resolution of optical control of intracellular signaling pathway in live cells.

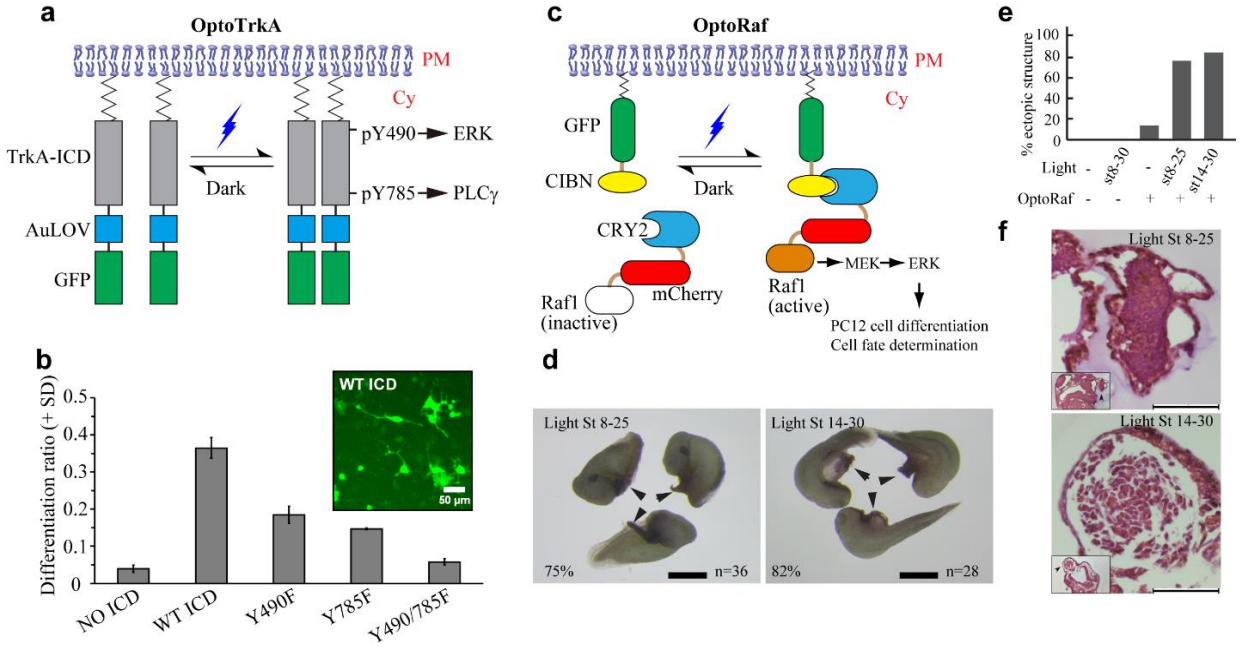

Fig. 1. (a) Scheme of the optoTrkA system. (b) PC12 cell differentiation ratio induced by optogenetic activation of the optoTrkA mutants. (c) Scheme of the optoRaf system. (d) Ectopic tail-like structure (arrows) induced by optoRaf during (stage 8-25) and after (stage 14-30) gastrulation. Scale bars: 1mm. (e) Quantification of the percentage of ectopic structure under different conditions. (f) Histological analysis of tissues in the ectopic structures in (d). Scale bars: 100 μm.

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