Transcriptional profiling in zebrafish using optimized photoswitchable MEK

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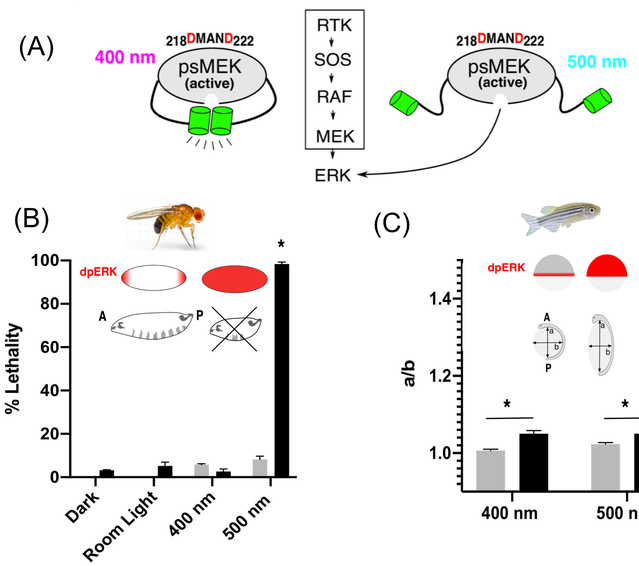
Abstract

Optogenetic tools enable fine control of signaling pathway activation in developing embryos. A photoswitchable version of the MEK1 enzyme (psMEK) directly triggers the Extracellular Signal-Regulated Kinase (ERK) pathway by short-circuiting signal transduction at the most proximal step of effector kinase activation. This optogenetic tool, however, is only weakly activating *in vivo* because it uses suboptimal phosphorylation-mimicking substitutions in the activation loop of MEK. We optimize psMEK by adding gain-of-function, missense mutations associated with human diseases into the kinase domain. Simply adding mutations, chosen for their activating potential, tunes and enhances photoswitchable MEK activity. We use optimized psMEK, which is the first tool for optogenetic ERK activation in zebrafish embryos, to study the effects of ERK signaling on gene expression in the first 6 hours of development, when key cell fate patterning events take place. Preliminary data show that ERK activation significantly changes expression levels of many genes that are spatially variable along the dorsal-ventral axis. In the future, we plan to generate short, targeted optogenetic pulses of ERK activation to establish the long-term transcriptional effects of ERK signals restricted to very specific, early time windows.

Phenotypic effects of photoswitchable MEK (psMEK)

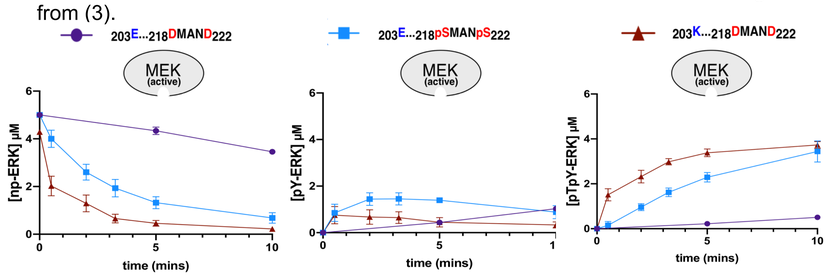
A photoswitchable version of the penultimate kinase in the ERK signaling cascade, MEK, directly controls activity of the pathway's effector molecule. Photoswitchable MEK (psMEK) is maintained in the kinase's active conformation by phosphorylation-mimicking serine to aspartic acid mutations in the activation loop (S>D at residues 218 and 222) (1). psMEK-induced constitutive pathway activity, indicated by dually phosphorylated ERK (dpERK) patterns induce strong developmental defects in Drosophila, but not in zebrafish.



(A) 500 and 400 nm light controls photoswitchable MEK function by reversibly caging the active site of constitutively active MEK. (B) In *Drosophila,* lethal defects occur in the larval cuticle when there is psMEK-induced ERK activation in the middle of the 3-hr old embryo. (C) Activating psMEK cannot induce a characteristic elongation of the zebrafish embryo at 11 hours post fertilization (2), a consequence of ERK activation outside of the margin of the embryo. Figures from (3).

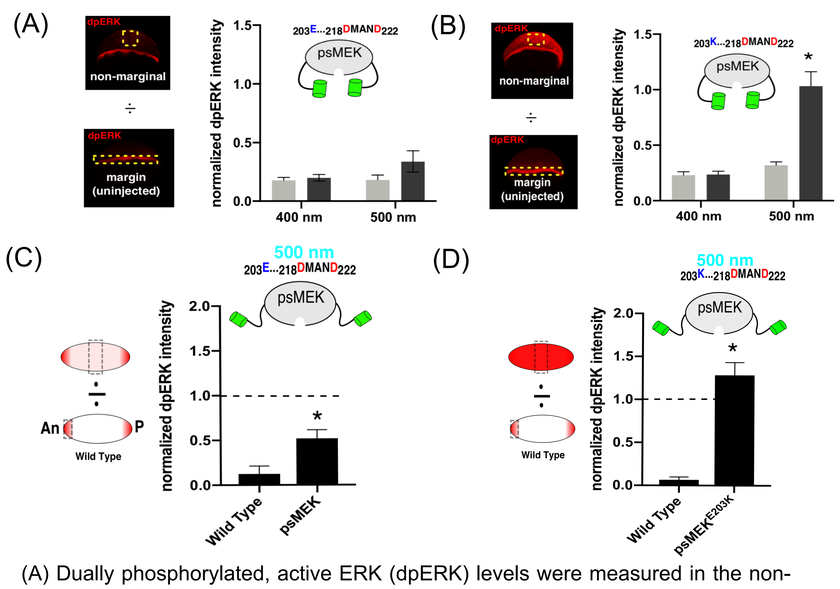
Destabilizing mutations increase phosphomimetic MEK activity

Phosphomimetic mutations in the activation loop are suboptimal substitutions for constitutively activating MEK. Adding mutations that destabilize the inactive conformation of MEK further favors the active conformation, and dramatically increases phosphomimetic MEK kinase activity. The curves below reflect the two-step phosphorylation of ERK by MEK that has been phosphorylated, contains phosphomimetic mutations, or contains both phosphomimetic mutations and an additional E203K mutation known to destabilize the inactive conformation. Figures from (3).



E203K substitution optimizes psMEK activity *in vivo*

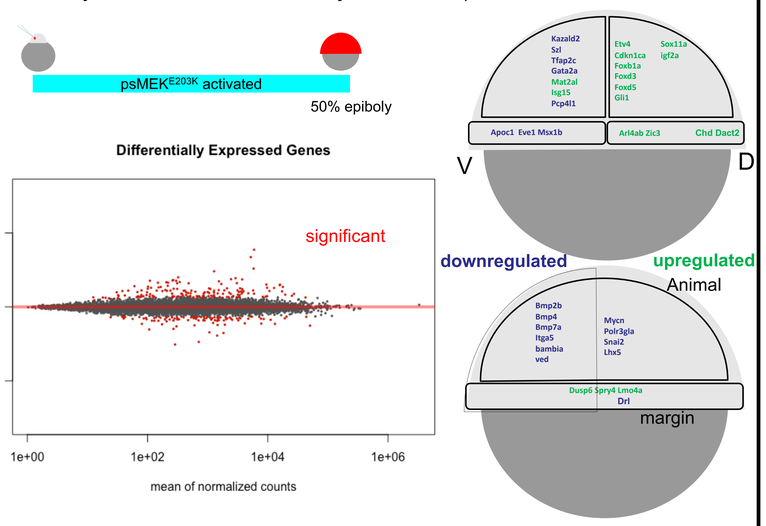
We hypothesized that an additional E203K mutation in psMEK will improve its function as an optogenetic source of ERK activation. Indeed, psMEK with E203K restores maximal levels of ectopic ERK signaling.



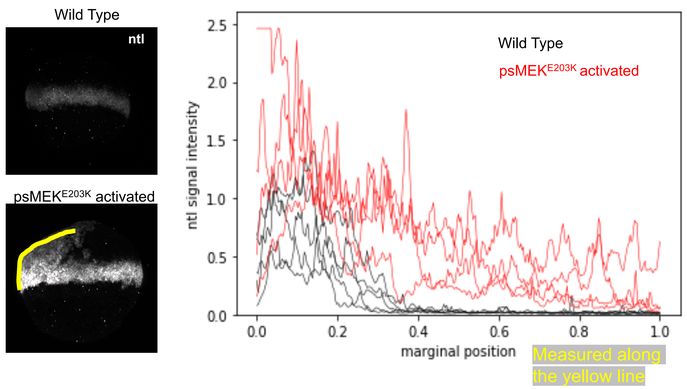
(A) Dually phosphorylated, active ERK (dpERK) levels were measured in the non-marginal cells of zebrafish embryos expressing psMEK, and normalized to the levels in the margins of non-psMEK expressing embryos. There was no significant increase in dpERK levels, reflecting the non-elongated embryo. (B) Simply adding the E203K mutation to psMEK renders it able to induce ectopic dpERK levels that are closer to the endogenous levels. (C-D) The same constructs expressed in *Drosophila* embryos also demonstrate that the E203K mutation enhances psMEK activity. Figures from (3).

Probing gene expression changes with light

We injected and activated optimized psMEK until 50% epiboly, and then assayed for gene expression changes using bulk RNAseq from single embryos (N=3 for psMEK^{E203K}-injected and N=3 for WT uninjected control).



Genes that were significantly upregulated (green) or downregulated (purple) and spatially variable are grouped by their WT spatial patterns in the 50% epiboly embryo as identified by scRNAseq and SEURAT described in (4). Interestingly, many dorsally expressed genes are upregulated, and ventrally expressed genes are downregulated.



Some target genes like notail (ntl) in the margin may not have been identified by bulk RNAseq because of small fold changes. HCR in situs for ntl, however, show that activating ERK does extend the expression domain, but maintains the graded expression along the margin-animal pole axis, likely reflecting the joint requirement for Nodal signals in regulating ntl expression in the margin. *These preliminary results provide a framework for future studies using sequencing and imaging assays in combination with temporally restricted ERK pathway perturbations provided by optimized psMEK.*

References:

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4. Satija et. al. Spatial reconstruction of single-cell gene expression. *Nature* (2015)

Acknowledgments:

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