

A high throughput optogenetic system to interrogate MAPK signaling network dynamics at the single cell level

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Short Abstract — We have coupled the FGF/MAPK pathway to an optogenetic FGF receptor and an ERK activity reporter. This system allows us to activate the pathway with light in a highly automated and reproducible way and to measure ERK signaling output in hundreds of single cells with high temporal resolution. By combining dynamic INPUT stimulation patterns together with drug and siRNA perturbations and by recording ERK activity OUTPUTs, we aim to identify molecular players and feedback wiring involved in the regulation of the MAPK network.

Keywords — MAPK signaling, ERK, optogenetic, single-cell measurements, drug perturbations, siRNA screen.

I. BACKGROUND

RECEPTOR tyrosine kinases (RTK) convert extracellular inputs such as a growth factor binding into specific cellular outputs through the activation of signaling networks [1]. Despite numerous studies of the MAPK pathway, we still miss crucial information about how network components are wired, and how various dynamic responses of the network orchestrate the cell fate choice.

Due to this complexity, we need to combine live ERK activity measurement with network perturbations to elucidate network wiring and how network components affect dynamic responses.

This approach has already allowed to discover new pathway connections as recently demonstrated by Pertz et al. [2]. Mathematical models of different pathway topologies could be discriminated by measuring dynamic single-cell ERK activity responses to growth factors delivered in a pulsatile manner using microfluidic devices.

II. RESULTS

To further elucidate the MAPK pathway topology, we have built a synthetic system where the MAPK pathway can be activated with light of different intensity, duration, or stimulation pattern (single- or multi-pulse) using an optogenetic FGF receptor (optoFGFR1). The use of dynamic light input provides high temporal and spatial resolution and enables to fully automatize the experiments. We then measure ERK signaling output in hundreds of single cells at the same time with an ERK activity reporter (ERK-KTR).

The optoFGFR1 receptor activates specifically and reversibly the ERK, AKT and PLC γ MAPK pathways with

488nm light pulses. The receptor activation is based on the self-dimerization of CRY2PHR, a photosensitive domain fused to the cytosolic part of the receptor [3]. ERK-KTR is a substrate of ERK that gets exported from the nucleus to the cytosol upon its phosphorylation by activated ERK [4]. This translocation event is used as a proxy to measure ERK activity without modifying ERK protein itself.

We generated a stable NIH3T3 cell line expressing the optoFGFR1 and the ERK-KTR and characterized ERK activity in response to light stimulations. To increase the throughput of our experiments, we established a pipeline to highly automatize the image acquisition and the stimulations, as well as the data processing. Using this pipeline, we could observe that light triggers specific and reproducible ERK responses depending on the pulses intensity and frequency.

As a proof of concept, this system was used to study the effect of two well-known MEK inhibitors, UO126 and PD0325901, on the activation of the pathway. Using time series classification and clustering we could identify subpopulations of cells with distinct dynamic patterns despite high variability in ERK activation levels in response to the same treatment conditions.

III. CONCLUSION

Our system is a tool to study the effects of targeted system perturbations. It enables triggering of homogenous and reproducible ERK activity dynamics in hundreds of single cells with a high temporal resolution. Such measurements are essential for building and calibrating predictive mathematical models of the MAPK pathway.

Combined with a highly-automatized image analysis workflow, this system provides the required throughput to screen for proteins involved in the network regulation using drugs and siRNAs targeting selected molecular players [5].

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