Dissecting kinase-mediated downstream pathways with temporal and spatial control of its activation

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Protein kinases control cell behavior by regulating signaling pathways through posttranslational modification of proteins in a cell. Complex interdependence of the downstream components in the signaling network makes understanding of the specific roles of kinases a difficult challenge. In traditional genetic approaches a downregulation of a kinase expression or overexpression of its mutants does not provide temporal control of kinase activity and allows the cell to compensate before it is assayed. Here we report a novel method of temporally controlled activation of specific kineses[1] while simultaneously restricting their interactions to individual downstream targets[2]. To study immediate effects of kinase activation, we first insert a modified FK506-binding protein (iFKBP) into a highly conserved portion of the kinase catalytic domain, which inactivates the kinase. Then, addition of rapamycin leads to heterodimerization of the inserted iFKBP with a coexpressed FKBP12-rapamycin binding domain (FRB), causing kinase reactivation. Furthermore, fusing a modified FRB to a specific kinase target, we restrict activation to that kinase-target interaction alone. We used this new technique, dubbed RapR-TAP (rapamycin-regulated targeted activation of pathways) to dissect the role of Src kinase interactions with specific effectors (FAK and p130Cas) in cell motility and morphodynamics. To this end, we created chimeras of FAK and p130Cas wherein the binding site for the Src SH2

domain was replaced by the FRB domain. Expression of the mutated RapR Src with either p130Cas-FRB or FAK-FRB produced different subsets of the behaviors induced simply by Src activation. Src complexed with p130Cas, but not with FAK, generated filopodia and increased their length. The Src-p130Cas complex was also much more effective at increasing adhesion number than was the Src-FAK complex. In contrast, Src-p130Cas had little effect on adhesion morphology, while Src-FAK produced an exaggerated version of the adhesion elongation that had been produced by Src alone. To provide systematic and unbiased quantification of these observations we developed a computational framework that allows accurate automated identification and tracking of all cellular protrusions with arbitrarily complex shapes[3]. Together these tools allowed us to differentiate the roles of specific Src-effector interactions from the overall Src response and reveal transient changes in cell morphology synergistically generated through different Src-mediated pathways. Importantly, structural analysis suggests that RapR-TAP method can be broadly applied to many kinase-target systems.

1. A.V. Karginov et al. (2010) Engineered allosteric activation of kinases in living cells. *Nature Biotechnology*, **28**: 743-747.

2. A.V. Karginov et al. (2013) Control of kinase activation and interaction in vivo: dissection of Src signaling pathways. *Science* (under review)

3. D. Tsygankov et al. (2013) A computational method for tracking cell protrusions of arbitrary complex geometries. *Nature Methods* (submitted)