Multiscale analysis of spatio-temporal Rho GTPase signaling networks controlling neuronal outgrowth

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Abstract

Precise spatio-temporal Rho GTPase signaling mechanisms are thought to fine tune cytoskeletal dynamics allowing neurite outgrowth. This is not compatible with the current view that Rac1 and Cdc42 activity control neurite outgrowth, whereas RhoA controls neurite retraction. Using 2nd generation, improved FRET-based biosensors, we uncover a much more complex picture. In an advancing growth cone, RhoA, Cdc42 and Rac1 are activated in discrete subcellular domains, most probably to position different effectors at different locations to fine tune cytoskeletal dynamics. During growth cone collapse, RhoA and Cdc42 are globally activated in the growth cone, while Rac1 switches off. We hypothesize that each of these distinct spatio-temporal Rho GTPase pools are controlled by the action of distinct GEFs and GAPs, to specifically activate different effectors. The challenge is now to map relevant "spatio-temporal GEF/GAP – Rho GTPase – effector modules". For that purpose, we performed a siRNA screen targeting a candidate 220 proteins Rho GTPase signaling network (identified using a proteomics approach) with the 20 hour-long dynamic neurite outgrowth process as readout. We devised a computer vision pipeline to automatically track and segment neurons in 8000 movies. Statistical analysis then extracted morphological and morphodynamic signatures relevant to each perturbation. Our approach pinpoints Rho GTPase signaling networks important for neurite initiation, extension, retraction, branching

and for neuronal migration. However, the limited phenotypic space we observe precludes identification of relevant Rho GTPase signaling modules. We observe that morphodynamic phenotypes observed at the 20 hour timescale can be explained by a wide variety of defects, only evident when growth cone dynamics are examinated at much shorter timescales. We report on a new computer vision pipeline to segment and track growth cone morphodynamics. Understanding spatio-temporal Rho GTPase signaling complexity therefore requires to sample FRET measurements and the effect of perturbations at multiple time/length scales.