FAST RAC1 ACTIVATION CYCLES AT THE LAMELLIPODIUM TIP CONTROL WAVE COMPLEX FUNCTION

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The first step of cell migration is the formation of the lamellipodium (LM), which is a thin sheet of membrane-enclosed actin filaments (F-actin) networks propelled by actin polymerization. The spatiotemporal coordination of actin regulators in the LM determines the dynamics and architecture of branched F-actin networks during cell migration. The WAVE complex, effector of Rac1 during cell protrusion, is concentrated at the LM tip. Yet, correlation of Rho GTPases activation with cycles of membrane protrusions, suggested that Rac1 activation is not synchronized with membrane protrusion and occurs behind the LM tip. However, RhoA activation is maximal at the cell edge and synchronized with edge progression. Combining single protein tracking (SPT) and super-resolution imaging with loss- or gain-of-function of Rho GTPases mutants, we demonstrated that Rac1 immobilizations at the LM tip were correlated with Rac1 activation, contrary to RhoA. We showed that Rac1 effector WAVE and regulator IRSp53 accumulated at the LM tip by membrane free-diffusion and trapping. Nevertheless, wild-type Rac1, which directly interacts with WAVE and IRSp53, only displayed slower diffusion at the LM tip, suggesting fast local activation/inactivation cycles. Coupling tracking with optogenetic activation of Rac1, triggered by Tiam1 membrane recruitment, demonstrated that Rac1-WT diffusive properties were unchanged despite enhanced LM protrusion. Taken together, our results support a model where Rac1 is rapidly switching between activation and inhibition at the LM tip, ensuring a local and fast control of Rac1 actions on its targets.