Fluorescent probes illuminate role of Rac1A in regulation of the actin cytoskeleton in *D. discoideum*

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Rac proteins are members of a broad family of monomeric Rho GTPases that act as key regulators of the actin cytoskeleton. We are using genetically tractable and highly motile *Dictyostelium discoideum* cells to study localization, dynamics and activity of Rac1A in processes that involve actin remodeling. In their active state, Rac1 GTPases interact with downstream effectors and participate in signal transduction from the cell surface to the actin cytoskeleton. Previously it was shown that Rac1A binds to the IQGAP-related protein DGAP1, which forms a cortical complex with cortexillin heterodimer [1]. This complex localizes to the rear end of moving cells and to the cleavage furrow of dividing cells, and appears to be important for efficient cytokinesis in *D. discoideum*. To gain insight into the role of Rac1A in *D. discoideum,* we set out to create a fluorescent probe that would specifically bind its active form.

Since *D. discoideum* Rac GTPases are strongly related to each other, we employed yeast two hybrid (Y2H) assay to test the interaction between 4 potential binding partners and 11 Rac proteins. In this way, we could pinpoint an interaction partner specific for the active form of Rac1A. In Y2H assay, we tested the full-length DGAP1 and its truncated form harboring a GAP-related domain (GRD). We also tested regulatory domains of two other downstream effectors of Rac1 GTPases that contain CRIB (*Cdc42/Rac interactive binding*) motifs: PBD (*p21-binding domain*) from *D. discoideum* PAKa kinase, and GBD (GTPase-binding domain) from rat PAK1 kinase. Only GBD from rat PAK1 interacted under stringent conditions in Y2H assay with Rac1A. Moreover, it was the only partner that did not interact with the constitutively inactive form of Rac1A. This interaction was confirmed by a GST pull-down assay. Based on these results, PAK1_GBD was fused N-terminally to YFP and expressed in wild-type *D. discoideum* cells. In non-motile cells, our probe was strongly enriched throughout the cortex, while in motile cells it always localized to the leading edge. In phagocytosis and macropinocytosis, it localized to the endocytotic cup. During cytokinesis and chemotaxis the probe didn't show any prominent localization.

In order to demonstrate an interaction between PAK1_GBD and GTP-Rac1A in living cells, we decided to employ fluorescence resonance energy transfer (FRET) approach. We constructed a unimolecular probe consisting of PAK1_GBD and Rac1A sandwiched between florescent proteins YFP and mRFP. This FRET probe has a prominent cortical localization and to a certain extent localizes also to endomembranes of *D. discoideum* cells. Initial results obtained by sensitized emission of the acceptor indicate that PAK1_GBD and GTP-Rac1A interact *in vivo*.

Localization of PAK1_GBD-YFP in actin-dependent processes did not correspond to localization of GFP-DGAP1 and GFP-cortexillin, the other components of the

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aforementioned cortical complex that contains Rac1A. In addition, its localization was not altered in mutant cells deficient in DGAP1. We hypothesize that GTP-Rac1A trapped in the tetrameric complex is not accessible to our probe for steric reasons. On the other hand, population of GTP-Rac1A which participates in signaling at the cell front, possibly as a component of a hypothetical frontal complex, undergoes fast recycling, rendering it accessible to our probe. Based on these results, we propose that Rac1A has a dual role in regulation of the actin cytoskeleton in *D. discoideum*. In addition to its established role in recruitment of the DGAP1-cortexillin complex to the rear parts of a polarized cell, it also participates in signaling pathways that control *de novo* actin polymerization at the protruding regions of the cell.

1. J. Faix et al., EMBO J. **20** (2001) p3705.
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