A dual role for Rac1 GTPases in the regulation of cell motility in *Dictyostelium*

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Introduction

Rac proteins are the only canonical Rho family GTPases in *Dictyostelium*, where they act as key regulators of the actin cytoskeleton. In order to monitor the dynamics of activated Rac1 in *Dictyostelium* cells, a fluorescent probe was developed that specifically binds to GTP-bound form of Rac1. The probe is based on the GTPase-binding domain (GBD) from PAK1 kinase, and was selected on the basis of yeast two-hybrid and GST pull-down screens (Figure 1). An interaction between PAK1_GBD and activated Rac1 was corroborated in living cells by fluorescence resonance energy transfer (FRET) (Figure 2).

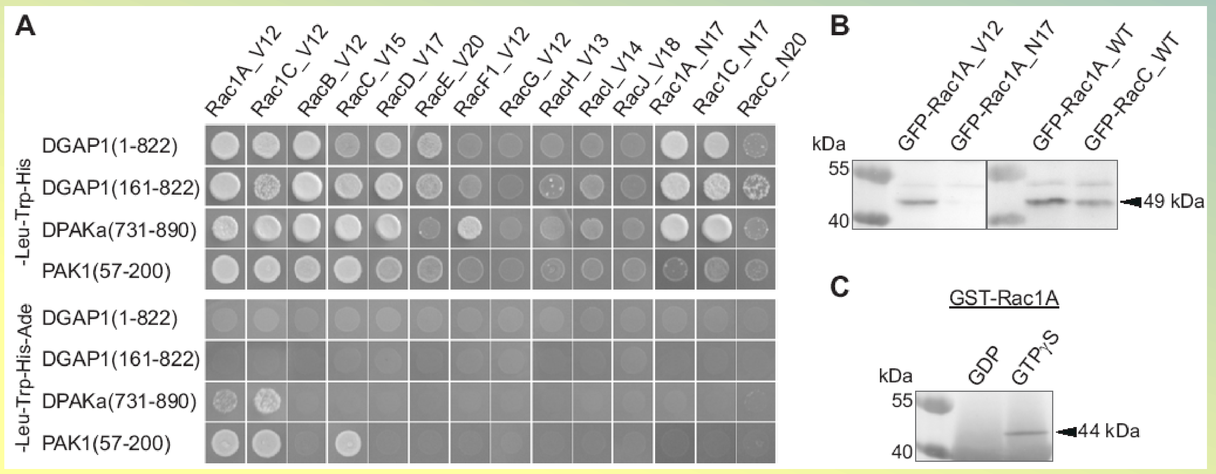

Figure 1. *Regulatory domain of rat PAK1 kinase specifically interacts with active forms of Dictyostelium Rac1 and RacC GTPases. (A) Yeast two-hybrid assay was used to test interactions between full-length and truncated variants of Dictyostelium DGAP1, the GBD of Dictyostelium DPAKa kinase, and the regulatory domain of rat PAK1 kinase as baits, with constitutively active forms of 11 Dictyostelium Rac proteins and constitutively inactive forms of 3 of these proteins as preys. Only PAK1_GBD (aa 57-200) interacts with active forms of Rac1A, Rac1C and RacC, but not with their inactive forms, under both low stringency (upper panel) and high stringency (lower panel) conditions. (B) GST-binding assay shows interactions of PAK1_GBD with wild-type forms of Rac1A and RacC, and with constitutively active form of Rac1A, but not with constitutively inactive Rac1A. GST-(PAK1_GBD)2 recombinant protein was bound to glutathione-sepharose and used to affinity-purify GFP-fusion proteins from whole cell lysates, as indicated. After elution proteins were immunoprecipitated with anti-GFP antibody. (C) GST-Rac1A was bound to glutathione-sepharose and loaded with either GDP or GTPγS as indicated. Using the whole cell lysate, PAK1_GBD-DYFP was immunoprecipitated with anti-PAK1_GBD antibody only from the column containing GTPγS-loaded GST-Rac1A.*

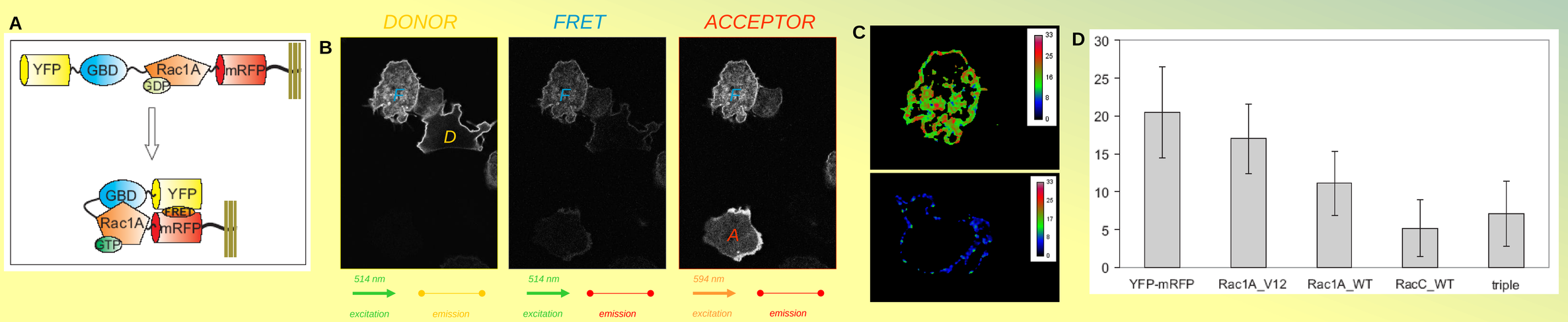

Figure 2. (A) Intramolecular construct used for measurements of FRET efficiency. Quadruple construct $Rac1A_{FRET}$ reports FRET upon interaction between Rac1A-GTP and PAK1_GBD. (B) An example of raw-data images where three cells, expressing the $Rac1A\_V12_{FRET}$ construct (F), a pure donor species (D), and a pure acceptor species (A) were sequentially recorded in donor, fret and acceptor channels. (C) Colour-coded maps of the FRET efficiency as determined for a cell expressing $Rac1A\_V12_{FRET}$ (left) and a cell expressing DYFP-PAK1_GBD-mRFPmars triple construct (right). (D) Apparent FRET efficiencies measured for the five designated constructs (means standard deviations).

Results

In moving *Dictyostelium* cells, PAK1_GBD is strongly enriched at the leading edge where it co-localizes with F-actin, and it also localizes to endocytotic cups during phagocytosis and macropinocytosis (Figure 3). As in vertebrates, activated Rac1 therefore appears to participate in signalling pathways that control de novo actin polymerization at protruding regions of the cell. Additionally, the IQGAP-related protein DGAP1 sequesters active Rac1 into a quaternary complex with the actin-binding proteins cortexillin I and II and, notably, this complex localizes to the trailing, retracting regions of migrating cells (Figure 4). As assessed by latrunculin B treatment, cortical localization of PAK1_GBD strictly depends on the integrity of the actin cytoskeleton, whereas cortical localization of DGAP1 does not (Figure 5).

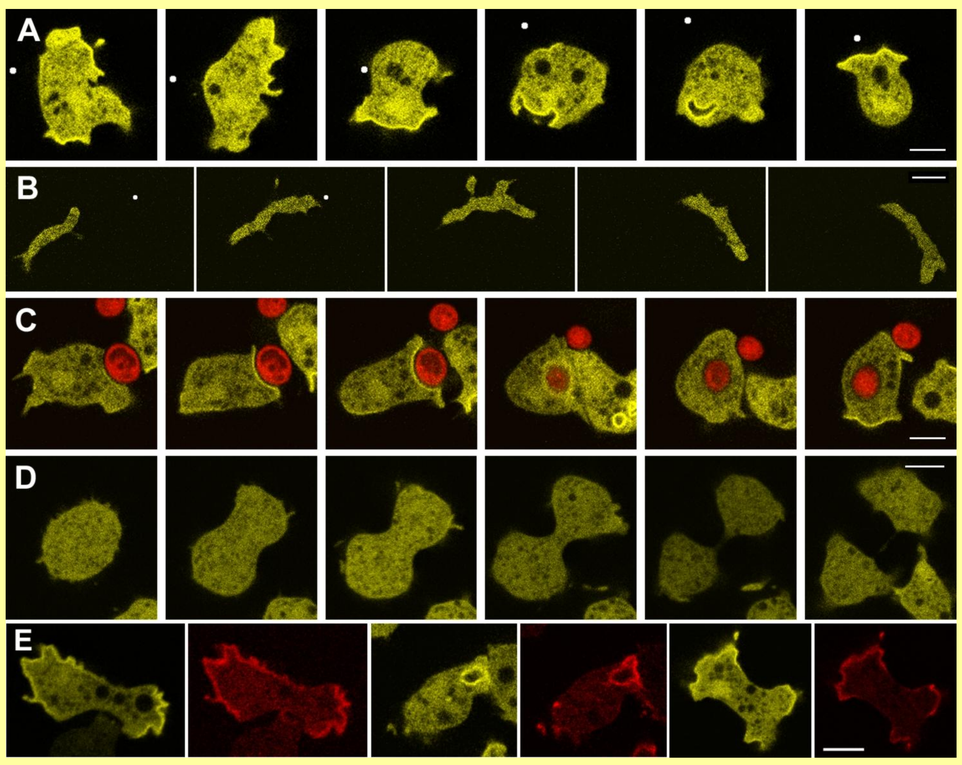

Figure 3. Localization of PAK1_GBD-DYFP fusion protein in wild-type *Dictyostelium* cells during random movement (A); directed movement of chemotactically competent cells (B); phagocytosis (C); and cytokinesis (D). (A) White dots designate a fixed point on the substratum. Note that the cell moved significantly downward between the third and the fourth frame. (B) White dot marks the position of the micropipette tip, i.e. the source of chemoattractant, in the first two frames. Afterwards, the micropipette was relocated 20 μm underneath the lower left corner of the displayed frame. (C) Phagocytosis was induced using fluorescent, TRITC-labelled yeast particles. (E) Co-localization of co-expressed PAK1_GBD-DYFP (yellow) and mRFPmars-LimEΔcoil (red), a marker of F-actin. Bars: 5 μm (A, C, D); 10 μm (B, E). Time spans: 325 s (A); 120 s (B); 210 s (C); 200 s (D).

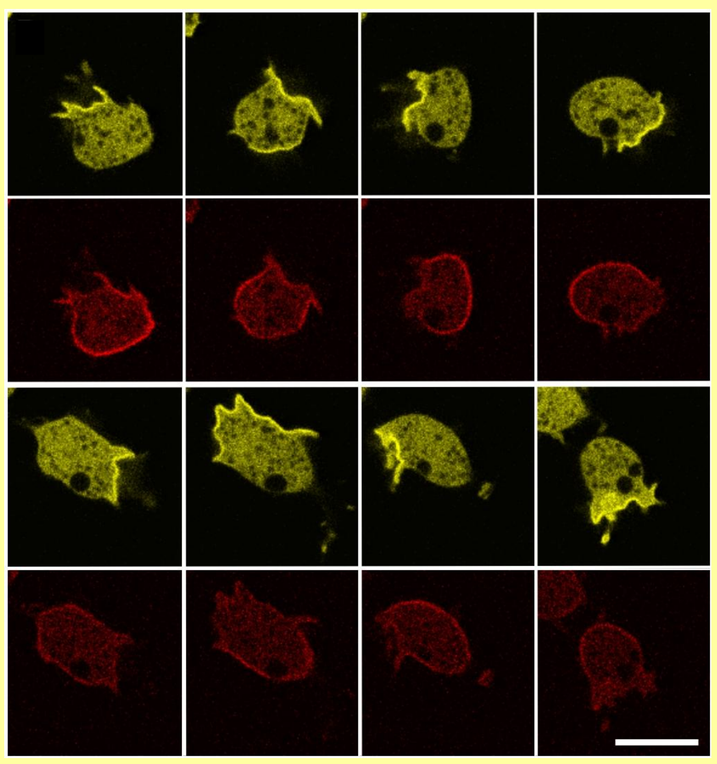

Figure 4. An opposite localization of the PAK1_GBD-DYFP probe (yellow) and the mRFPmars-DGAP1 fusion (red) in polarized *Dictyostelium* cells that express both fusion proteins. Bar: 10 μm. Time span: 250 s.

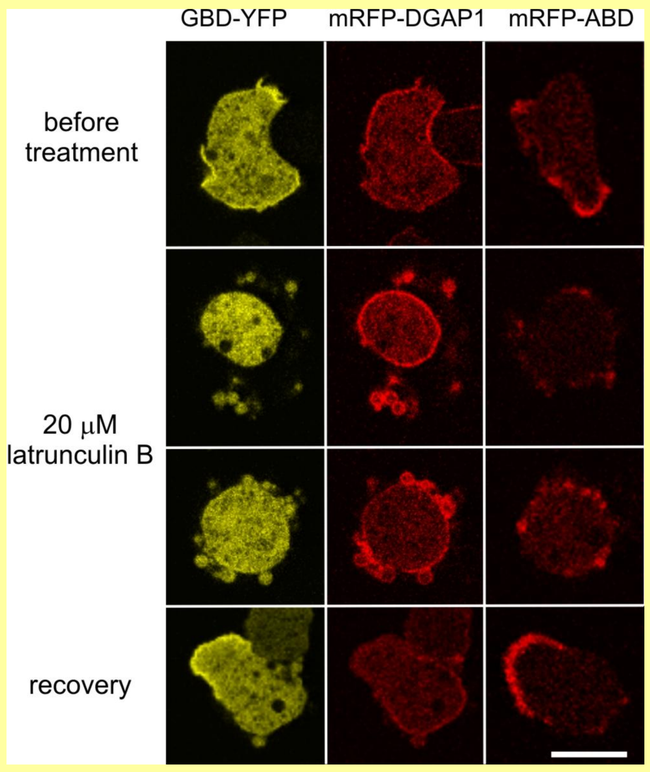

Figure 5. Cells that express PAK1_GBD-DYFP (yellow, left column) and mRFPmars-DGAP1 (red, middle column) before treatment with latrunculin B (upper row), 20 minutes after addition of 20 μM latrunculin B (two middle rows), and 30 minutes after the washout (last row). The rightmost column shows cells that express an F-actin marker, the actin-binding domain ABD from ABP-120 kDa protein fused with mRFPmars. Bar: 10 μm.

Conclusions

Presented results imply that Rac1 GTPases play a dual role, both at the front and in the back, in migrating *Dictyostelium* cells. In our view, DGAP1-containing posterior complex act as a kinetic trap for Rac1-GTP, whereas at the front it is constantly recycled at a high rate and therefore being repeatedly detected by the PAK1_GBD sensor.