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Crystal Structure of Rac1 in Complex with the Guanine Nucleotide Exchange Region of Tiam1

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Beamline(s): X4A

Introduction: The Rho family of small monomeric GTPases are involved in the regulation of a variety of important cellular processes including: cell cycle progression, gene transcription, cell adhesion, and cell growth and differentiation^{1,2}. Like all G-proteins, Rho GTPases are specifically activated upon exchange of bound GDP for GTP facilitated by guanine nucleotide exchange factors (GEFs). Cellular GEFs specific for Rho GTPases are in general, large (>100kD) proteins possessing diverse functional domains. However, all contain a ~200 residue Dbl homology (DH) domain immediately preceding a ~100 residue pleckstrin homology (PH) domain. Studies have shown that nucleotide exchange activity resides within the DH domain, while the exact function of the adjacent PH domain is unclear and may include a role in regulation of the activity of the DH³. We report the structure of the DH/PH element of the T-lymphoma invasion and metastasis factor 1 (Tiam1) protein in complex with its cognate Rho-family G-protein Rac1⁴.

Methods and Materials: Tiam1(DH/PH) and Rac1 were each expressed in bacteria and purified to homogeneity using standard chromatographic techniques. The proteins were incubated together in the presence of the magnesium chelator EDTA prior to loading onto a gel exclusion column. Fractions containing the GEF/G-protein complex were pooled, concentrated, and crystallized by vapor diffusion. Phases were acquired utilizing selenomethionine incorporated Tiam1(DH/PH) and the technique of multi-wavelength anomalous dispersion. The final model contains 16,031 protein atoms and 114 solvent atoms with $R_{\text{cryst}} = 26.2\%$ ($R_{\text{free}} = 29.3\%$) using data $|F| > 0$ to 2.8Å.

Results: Over 3,000Å² of solvent accessible surface area is buried at the GEF/G-protein interface. No contact is seen between the PH domain and Rac1 or between the helical insertion of Rac1 (characteristic of Rho-family GTPases) and Tiam1. In addition, the PH domain of Tiam1 is oriented with respect to the DH domain in a manner that is radically altered from that seen in structure of the DH/PH element from Sos1⁵. While no Tiam1 residues are directly inserted into the GTPase active site, interaction with Tiam1 has displaced and remodeled both switch regions of Rac1 in a way that blocks magnesium binding and reduces favorable interaction between Rac1 and a bound nucleotide. A comparison of amino acid conservation and residues of the GEF and G-protein that form the interface has delineated regions likely to be important for dictating proper Rho-family GEF: Rho-family GTPase pairing.

Conclusions: This structure provides insight into the details of nucleotide exchange of Rho-family G-proteins by their cellular GEFs. The structure will be of great utility in understanding a large body of existing mutagenesis data, and will allow for the design of GEF and G-protein mutants that will aid in the understanding of signal transduction pathways involving Rho-family GTPases.

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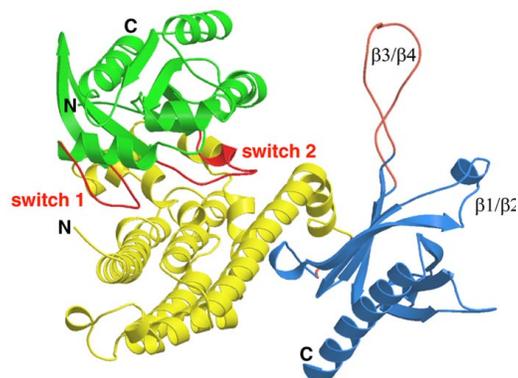


Figure 1. The Tiam1(DH/PH)•Rac1 complex. Rac1 is shown in green with switch regions in red. The DH and PH domains of Tiam1 are in yellow and blue respectively. The disordered β_3/β_4 loop of the PH domain is in orange.