Supplementary information

Methods

Plasmid construction and production of SidMcd–Rab1(1-175;N121I)

Genomic DNA of *Legionella pneumophila* (ATCC 33152) was the source for polymerase chain reaction amplification of the SidM/DrrA gene. DNA fragments encoding full-length or truncated SidM/DrrA and human Rab1A (denoted as Rab1 throughout the text) were subcloned into the pPROEx HTa plasmid (Invitrogen) and the pGEX-4T-3 plasmid (GE Healthcare), respectively. The SidMcd–Rab1(1-175;N121I) complex was produced in the *E. coli* BL21 (DE3) RIG strain (Novagen) at 18 ^{o}C. Bacterial lysates were prepared by sonication in buffer A consisting of 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. The protein complex bound to a column packed with Ni-NTA resin (QIAGEN) was eluted with buffer A containing 200 mM imidazole. The eluted fraction was reacted with TEV protease to cleave the N-terminal $(His)_6$-tag and loaded onto a HiTrapQ HP column (GE Healthcare). The bound proteins were eluted with a linear gradient of 0.05-0.5 M NaCl. Further protein purification was performed with a HiLoad 26/60 Superdex 75 gel-filtration column (GE Healthcare) equilibrated with 20 mM Tris-HCl (pH 7.5). The selenomethionine-substituted complex was produced in the *E. coli* B834 (DE3) methionine auxotroph (Novagen), and purified as described above. For crystallization, the complex was concentrated to 20 mg/mL in a buffer solution containing 20 mM Tris-HCl (pH 7.5), 100 mM NaCl and 1 mM dithiothreitol (DTT).

Preparation of full-length SidM/DrrA and SidMcd

Full-length SidM/DrrA and SidMcd were expressed from the pPROExHTa plasmid in the *E. coli* BL21 (DE3) RIG strain at 18 °C. Cell lysates were prepared by sonication in buffer A. The two proteins were purified by using a column packed with Ni-NTA resin and a HiTrap Q HP column. The N-terminal $(His)_6$-tag was cleaved with TEV protease.

Preparation of full-length Rab1 and Rab1(1-175)

Full-length Rab1 and Rab1(1-175) were expressed from pGEX-4T-3 plasmid (GE Healthcare) in the *E. coli* BL21 (DE3) RIG strain at 18 °C. Bacterial cell lysates were prepared by sonication in buffer A containing 1 mM DTT additionally. The proteins were bound to a column packed with GST bind resin (Novagen) and eluted with a buffer solution consisting of 100 mM Tris-HCl (pH 8.0), 100 mM NaCl , 10 mM reduced glutathione and 1 mM DTT. The eluted fraction was reacted with TEV protease to cleave the N-terminal GST. The two proteins were further purified by using a HiTrap Q HP column (GE Healthcare) and subsequently a HiLoad 26/60 Superdex 75 gel-filtration column (GE Healthcare).

Preparation of GDI1

Human GDI1 (Accession number: NM_001493) was expressed as a full-length protein from the pPROEx HTa plasmid in the *E. coli* BL21 (DE3) RIG strain at 18 °C. The cell lysate was prepared by sonication in buffer A containing 2 mM β-mercaptoethanol additionally. GDI1 was purified by using a column packed with Ni-NTA resin followed by the cleavage of N-terminal $(His)_6$-tag by TEV protease. Further purification was performed with HiTrap Q HP and HiLoad 26/60 Superdex 75 gel-filtration columns.

Preparation of the GDI1–p-Rab1:GDP complex

GDI1–p-Rab1:GDP was produced by coexpression of the two full-length proteins in the Sf9 insect cells based on the Baculovirus expression vector system employing the pFastBac Dual plasmid (Invitrogen). p-Rab1 was expressed as a fusion protein with a $(His)_6$-tag at its N-terminus, while GDI1 was expressed as an N-terminal GST-tagged fusion protein. The expression level of GDI1 was much lower than that of Rab1. Cell lysates were prepared by sonication in buffer A containing 0.1% CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate), 1 mM $MgCl_2$ and 0.1 mM GDP and incubated with additional GDI1 produced from *E. coli* to increase the yield of the complex. The purification of the complex involved a column packed with Ni-NTA resin, the cleavage of the $(His)_6$-tag and the GST tag with TEV protease, a HiTrap Q column, a Superdex 200 column and a Mono Q HP column (GE Healthcare). The final protein sample was concentrated in a buffer solution containing 20 mM Tris-HCl (pH 7.5), 220 mM NaCl, 1 mM $MgCl_2$ and 1 mM DTT.

Preparation of phosphatidylcholine liposomes

Phosphatidylcholine (PC) lipids (Avanti Polar Lipids, Inc.) stored in chloroform at -80 °C were dried under an air stream and desiccated for 3 hr. The lipids were resuspended in a buffer solution containing 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. Ten cycles of freeze-and-thaw were carried out and 2 mM PC lipids were used for experiments.

Charging Rab1 proteins with mant-GDP

According to the reported procedure(Murata et al, 2006), purified GDP-bound Rab1(1-175) was incubated with the 20-fold molar excess of 1'(3)-bis-O-(N-methylanthraniloyl) GDP (mant-GDP; Invitrogen) for 30 min at 37 °C in the loading buffer containing 30 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA and 1 mM dithiothreitol. The charging reaction was completed by addition of 10 mM $MgCl_2$, and the excess mant-GDP was removed using HiTrap Desalting column (GE Healthcare) equilibrated with a buffer containing 30 mM Tris-HCl (pH 8.0), 150 mM NaCl and 0.5 mM $MgCl_2$.

References

Murata T, Delprato A, Ingmundson A, Toomre DK, Lambright DG, Roy CR (2006) The Legionella pneumophila effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol* **8**(9)**:** 971-977