Supporting information Notes

This file contains the supporting information Figures, supporting information Methods, supporting information Tables and supporting information References.

Supplemental data

Fig. 10. Complex formation and structures of ARA7/VPS9a. **a, b** and **c**, Cartoon models of ARA7-$GDPNH_2$/VPS9a and ARA7-GDP/VPS9a(D185N), respectively, are colored based on the secondary structures labeled as in SI Figs 10a and b. $GDPNH_2$ is drawn as stick model with small balls of carbon (grey), oxygen (red), and phosphorus (magenta) and nitrogen (blue) atoms. Switch I of ARA7 (residues 32-44) in ARA7-GDP/VPS9a(D185N) is disordered and indicated as transparent green dots.

Fig. 11. Amino acid sequences of Rab5 and Vps9 proteins. **a**, VPS9a, b and human Rabex-5 are aligned based on the crystal structures. Secondary structures of VPS9a and human Rabex-5 are divided into three domains according to Rabex-5 (ref. 5); N-terminal helical domain, Vps9 domain, and C-terminal helix are colored with green, cyan, and orange, respectively. Residues corresponding to the aspartate finger are highlighted with bold letter. **b**, Amino acid sequences of Rab5 proteins including an *Arabidopsis* ARA7 homologue, RHA1, and human Rab5A-B are aligned. Secondary structures of the ARA7 moiety of ARA7-GDP/VPS9a, Rab5A-GppNHp-Mg^{2+}as a GTP form (1R2Q) and Rab5A-GDP-Co^{2+} as a GDP form (1TU4) are indicated as E (β-strand, magenta), H (α-helix, green), and G (3_{10}-helix, cyan) with segment names. Loops are indicated as small circles. Identical residues are colored with red. Amino acid identities with ARA7 are shown at the end of the alignment.

Fig. 12. Interactions between ARA7 and VPS9a. **a-d**, Hydrogen bonds (blue), electrostatic interactions (red) and van der Waals contacts (orange) between residues of ARA7 (magenta) and VPS9a (cyan) are shown.

Fig. 13. Interactions between ARA7/VPS9a and nucleotides. **a-d**, Polar interactions surrounding nucleotides are shown as SI Fig. 12. In **b**, position of a missing nucleotide is indicated as a grey GDP. The star mark shows the short distance between same charges.

Fig. 14. Converting GppNHp to $GDPNH_2$. **a,** Various nucleotides are separated by an anionic exchanger for controls of the following experiment. GppNHp contains $GDPNH_2$ from the beginning. **b,** Boiling of GppNHp solution leads to a complete conversion to $GDPNH_2$.

Fig. 15. Temperature factors of ARA7/VPS9a. **a-d**, Residual temperature factors for the proteins and atomic temperature factors for the nucleotides are mapped on the respective models of Figs 1b, c and Supplementary Figs 5a, b using a blue-to-red color gradient between overall mean *B* value ± 10 $Å^2$.

Fig. 16. Schematics of GEF reactions in the Rab/Vps9 domain. **a**, Inactive Rab binds to GDP through the interactions with a conserved p-loop Lys and a Mg^{2+} ion. The Mg^{2+} ion should have a coordination of six oxygen atoms as usual. **b**, A stable intermediate of Rab-GDP bound to Vps9 domain, which has a shorter and unzipped β2/β3 sheet of Rab. Asp finger in Vps9 domain makes interactions with β-phosphate of GDP and the p-loop Lys. **c**, The p-loop Lys shifts up and makes a new interaction with Asp residue in switch II of Rab to release GDP, which also makes the switch I of ARA7 flexible. Asp finger would support the movement of the p-loop Lys residue. **d**, In the binary complex between Rab21 and Vps9a domain, the conserved p-loop Lys and Asp finger in Vps9 domain of Rabex-5 also play a important role for GDP release. The Lys residue shifts further away form nucleotide binding site, which produce the enough space to put GTP nucleotide (**e**). f, After GTP coming into the binary complex of Rab/Vps9 domain, the conformational change is occurred in switch I and II of Rab and Rab is re-orientated to the compacted form.

Fig. 17. Difference between Rab21/Rabex-5 and ARA7/VPS9a. **a,** Superposition of nucleotide-free Rab21/Rabex-5 and ARA7-GDP/VPS9a at the GEF moiety. The GTPase moiety shows 18º rotation with 0.8 Å shift along the rotation axis calculated by DynDom (http://www.sys.uea.ac.uk/dyndom/). **b**, The rotation of the GTPase moiety makes a room for the γ-phosphate of GTP in Rab21/Rabex-5. The GTP-bound model in Rab21/Rabex-5 predicts hydrogen bonds between the the γ-phosphate of GTP and the aspartate finger as shown in Fig. 6.

Fig. 18. Electron densities around nucleotides. Electron densities of nucleotides and the surrounding residues are contoured as indicated.

Fig. 19. Omit map of the nucleotide binding region in the ternary complex structures.

Table. 1. MAD data of Se-Met labeled nucleotide-free ARA7/VPS9a.

Table. 2 Crystallographic data and refinement statistics of ARA7/VPS9a.

Supporting information Methods

Preparation of $GDPNH_2$-GppNHp was dissolved in water as 400 mM solution, and boiled for 10 minutes at 95ºC. Purity of $GDPNH_2$ and GppNHp were checked by anionic exchange chromatography.

Crystallography-The diffraction data were processed with HKL2000 (14). For the nucleotide-free ARA7/VPS9a crystal data, the initial phase set was calculated using SOLVE/RESOLVE (15) followed by iterative auto and manual model refinement by REFMAC5 (16) in the CCP4 suit (17) and COOT (18), or O (19), respectively. For the other crystal data, molecular replacement by MOLREP (20) in the CCP4 suit gave an initial model using the coordinate of nucleotide free Se-Met ARA7/VPS9a. The model was refined same as above, with the help of wARP (21). Structural figures were prepared by RASMOL (22), MOLSCRIPT (23), and RASTER3D (24). Stereochemical quality of protein structures was checked by PROCHECK (25), and no main-chain torsion angels were located in disallowed regions of the Ramachandran plot.

Supplementary References

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