Supplementary information

Production of Rab8 and MSS4

Purification of MSS4

Recombinant MSS4 protein was produced in *Escherichia coli* BL21(DE3) strain. Cells were grown in LB-medium containing 125µg/ml ampicillin at 37°C, and induced with 1mM IPTG at OD_{600nm} 0.8. The cells grew for 15h at 20°C and were harvested by centrifugation. After washing with PBS, the resuspended cells (25mM NaPi pH 7.5, 500mM NaCl, 1mM PMSF, 1mM β-mercaptoethanol) were disrupted using a microfluidizer, 1% (v/v) Triton X-100 was added, cells were centrifuged at 100000g for 40min, and the supernatant was applied to a nickel loaded Pharmacia-HiTrap Chelating HP column. Protein was eluted with a linear gradient of imidazol and the fractions containing MSS4 were pooled. The His-tag was removed using His_6-tagged TEV-protease in 25mM NaPi pH 7.5, 500mM NaCl, 2mM β-mercaptoethanol, 10µM $ZnCl_2$ buffer at 4°C over night. TEV-protease and uncleaved protein were removed by passing the solution over a Nickel-NTA-column. The concentrated protein solution was subjected to size exclusion chromatography on Superdex75 media (buffer: 10mM HEPES pH 7.5, 100mM NaCl, 5mM DTT, 5% glycerole, 100µM $ZnCl_2$). Samples containing the protein complex were pooled, concentrated to 7mg/ml, snap-frozen in liquid nitrogen and stored at -80°C.

Purification and refolding of Rab8

Since all approaches to produce Rab8 as a soluble protein failed, the protein was produced as insoluble inclusion bodies in *Escherichia coli*, denatured with guanidinium hydrochloride and refolded by rapid dilution in the presence of GDP. The recombinant Rab8 protein was produced in BL21(DE3) CodonPlus RIL cells, which were grown at 37°C in LB-medium containing 34µg/ml chloramphenicol, 125µg/ml ampicillin until reaching an OD_{600nm} of 0.8, induced with 1mM IPTG, and harvested 5 hours later. The cells were lysed with a microfluidizer (lysis buffer: 100mM TrisHCl pH 7.5, 5mM EDTA, 1mM PMSF, 5mM DTE), stirred for 20min in the presence of 2% (v/v) TritonX-100, and inclusion bodies were sedimented by centrifugation at 22000g for 30min. The sediment bodies was washed in 100mM TrisHCl pH 7.5, 5mM EDTA, 5mM DTE, 8M Urea, 2% (v/v) TritonX-100, and TritonX-100 was removed by washing in lysis buffer. Inclusion bodies were denaturated in 6M guanidinium hydrochloride, 50mM Tris pH 7.5, 5mM DTE, 5mM EDTA to a protein concentration of 9mg/ml.

200mg of the denatured protein was very slowly dropped (over 45min) into 2L of renaturation buffer (50mM HEPES pH 7.5, 400mM arginine HCl, 400mM sucrose, 330mM guanidinium HCL, 1mM EDTA, 5mM DTE, 50µM GDP, 2mM $MgCl_2$, 100mM $(NH_4)_2SO_4$ under intensive stirring at 16°C. The protein was precipitated with $(NH_4)_2SO_4$ (75% saturation) at 4°C over night. Protein was collected using a paper filter and dissolved in 50mM HEPES pH7.5, 150mM NaCl, 5mM DTE, 10% glycerole, 10µM GDP, 1mM $MgCl_2$. The protein was further purified on a gel sizing Superdex75 column in the resuspension buffer. Samples containing Rab8 were pooled, concentrated to 10.6mg/ml, snap frozen in liquid nitrogen, and stored at -80°C.
Nucleotide-free Rab8 was prepared by removal of the tightly bound GDP as described previously for Rab5 and Rab7 (Simon et al, 1996).

Figure S1.
Comparison of different Rabs sequence alignment with sequence alignment of exocytosis Rabs. Amino acid residues homologous among all Rabs (Rab8, rab3a, rab1a, ypt1, rab10, sec4, rab5a, rab6, rab11, rab4, rab7, rab9a) a highlighted in grey, homologous only in exocytic Rabs (Rab8, rab3a, rab1a, ypt1, rab10, sec4) – in yellow. Consensus line cording: 1:DN; 2:EQ, 3:ST; 4:KR; 5:FYW; 6:LIV 7:ED; 8:LIVMFCAYW.

MOVIE. Morphing movie of structural rearrangements of Rab8 when binding to MSS4. The movie shows the structural changes in the nucleotide binding pocket of Rab8 when the GTPase binds to MSS4. It starts with Rab8 in the conformation bound to a guanosine nucleotide and then sequentially transforms into Rab8 in its nucleotide-free conformation bound to MSS4. Rab8 is drawn in cartoon representation (grey) with the P-loop in yellow, α_1-helix in orange, Switch I in red, and Switch II in purple. MSS4 is shown as a blue surface; the structure of MSS4 is taken from the MSS4:Rab8-complex structure. The movie demonstrates the steric clash of the α_1-helix of Rab8 (bound to nucleotide) with the surface of MSS4. The steric hindrance is released by displacement of the α_1-helix, concomitant with a structural rearrangement of the nucleotide-binding pocket. The structure of Rab8 bound to nucleotide is not known and has been modeled by sequence homology using Ypt1-Rab and Sec4-Rab structures as models (SWISS-MODEL (Automated Protein Modelling Server, http://swissmodel.expasy.org/SWISS-MODEL.html, (Guex & Peitsch, 1997; Schwede et al, 2003; Peitsch 1995)). Additionally, the conformation of residues Gly18

to Asp31 of Rab8 in the MSS4-bound structure is unknown because of lack of electron density. These loops have been modeled using Swiss PdbViewer (version 3.7, http://www.expasy.org/spdbv/, (Guex & Peitsch, 1997)). Morphing of structures between Rab8 bound to nucleotide and Rab8 bound to MSS4 was performed using the program lsqman (Jones et al, 1991; Kleywegt, 1996). Movie frames were prepared using the program pymol (DeLano 2002) and subsequently converted into a Quicktime-movie using Adobe ImageReady CS.

REFERENCES

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Guex N and Peitsch MC (1997) SWISS-MODEL and the Swiss-PdbViewer: an environment for comparative protein modeling. *Electrophoresis* **18:** 2714-2723

Jones TA, Zou JY, Cowan SW, and Kjeldgaard (1991) Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr A* **47 (Pt 2):** 110-119

Kleywegt GJ (1996) Use of non-crystallographic symmetry in protein structure refinement. *Acta Crystallogr D Biol Crystallogr* **52:** 842-857

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Schwede T, Kopp J, Guex N, and Peitsch MC (2003) SWISS-MODEL: An automated protein homology-modeling server. *Nucleic Acids Res* **31:** 3381-3385

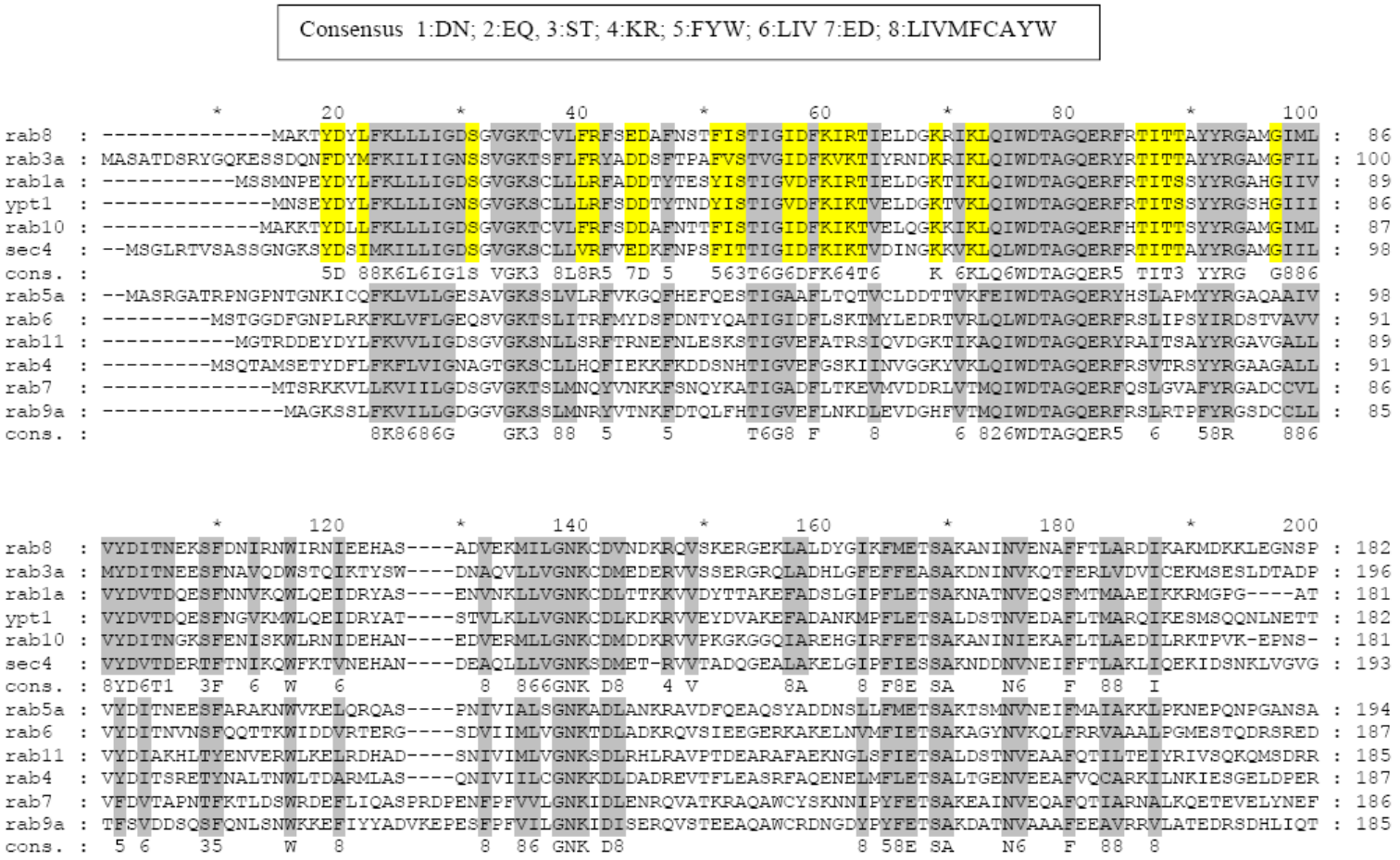
Consensus 1:DN; 2:EQ, 3:ST; 4:KR; 5:FYW; 6:LIV 7:ED; 8:LIVMFCAYW
rab8 : ----------MAKTYDYLFKLLLIGDSGVGKTCVLFRFSEDAFNSTFISTIGIDFKIRTIELDGKRIKLQIWDTAGQERFRTITTAYYRGAMGIML : 86
rab3a : MASATDSRYGQKESSDQNFDYMFKILIIGNSSVGKTSFLFRYADDSFTPAFVSTVGIDFKVKTIYRNDKRIKLQIWDTAGQERYRTITTAYYRGAMGFIL : 100
rab1a : ----------MSSMNPEYDYLFKLLLIGDSGVGKSCLLLRFADDTYTESYISTIGVDFKIRTIELDGKTIKLQIWDTAGQERFRTITSSYYRGAHGIIV : 89
ypt1 : ----------MNSEYDYLFKLLLIGNSGVGKSCLLLRFSDDTYTNDYISTIGVDFKIKTVELDGKTVKLQIWDTAGQERFRTITSSYYRGSHGIII : 86
rab10 : ----------MAKKTYDLLFKLLLIGDSGVGKTCVLFRFSDDAFNTTFISTIGIDFKIKTVELQGKKIKLQIWDTAGQERFHTITTSYYRGAMGIML : 87
sec4 : --MSGLRTVSASSGNGKSYDSIMKILLIGDSGVGKSCLLVRFVEDKFNPSFITTIGIDFKIKTVDINGKKVKLQLWDTAGQERFRTITTAYYRGAMGIIL : 98
cons. : 5D 88K6L6IG1S VGK3 8L8R5 7D 5 563T6G6DFK64T6 K 6KLQ6WDTAGQER5 TIT3 YYRG G886
rab5a : --MASRGATRPNGPNTGNKICQFKLVLLGESAVGKSSLVLRFVKGQFHEFQESTIGAAFLTQTVCLDDTTVKFEIWDTAGQERYHSLAPMYYRGAQAAIV : 98
rab6 : ---------MSTGGDFGNPLRKFKLVFLGEQSVGKTSLITRFMYDSFDNTYQATIGIDFLSKTMYLEDRTVRLQLWDTAGQERFRSLIPSYIRDSTVAVV : 91
rab11 : ----------MGTRDDEYDYLFKVVLIGDSGVGKSNLLSRFTRNEFNLESKSTIGVEFATRSIQVDGKTIKAQIWDTAGQERYRAITSAYYRGAVGALL : 89
rab4 : ---------MSQTAMSETYDFLFKFLVIGNAGTGKSCLLHQFIEKKFKDDSNHTIGVEFGSKIINVGGKYVKLQIWDTAGQERFRSVTRSYYRGAAGALL : 91
rab7 : ----------MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMVDDRLVTMQIWDTAGQERFQSLGVAFYRGADCCVL : 86
rab9a : ----------MAGKSSLFKVILLGDGGVGKSSLMNRYVTNKFDTQLFHTIGVEFLNKDLEVDGHFVTMQIWDTAGQERFRSLRTPFYRGSDCCLL : 85
cons. : 8K8686G GK3 88 5 5 T6G8 F 8 6 826WDTAGQER5 6 58R 886
rab8 : VYDITNEKSFDNIRNWIRNIEEHAS----ADVEKMILGNKCDVNDKRQVSKERGEKLALDYGIKFMETSAKANINVENAFFTLARDIKAKMDKKLEGNSP : 182
rab3a : MYDITNEESFNAVQDWSTQIKTYSW----DNAQVLLVGNKCDMEDERVVSSERGRQLADHLGFEFFEASAKDNINVKQTFERLVDVICEKMSESLDTADP : 196
rab1a : VYDVTDQESFNNVKQWLQEIDRYAS----ENVNKLLVGNKCDLTTKKVVDYTTAKEFADSLGIPFLETSAKNATNVEQSFMTMAAEIKKRMGPG----AT : 181
ypt1 : VYDVTDQESFNGVKMWLQEIDRYAT----STVLKLLVGNKCDLKDKRVVEYDVAKEFADANKMPFLETSALDSTNVEDAFLTMARQIKESMSQQNLNETT : 182
rab10 : VYDITNGKSFENISKWLRNIDEHAN----EDVERMLLGNKCDMDDKRVVPKGKGGQIAREHGIRFFETSAKANINIEKAFLTLAEDILRKTPVK-EPNS- : 181
sec4 : VYDVTDERTFTNIKQWFKTVNEHAN----DEAQLLLVGNKSDMET-RVVTADQGEALAKELGIPFIESSAKNDDNVNEIFFTLAKLIQEKIDSNKLVGVG : 193
cons. : 8YD6T1 3F 6 W 6 8 866GNK D8 4 V 8A 8 F8E SA N6 F 88 I
rab5a : VYDITNEESFARAKNWVKELQRQAS----PNIVIALSGNKADLANKRAVDFQEAQSYADDNSLLFMETSAKTSMNVNEIFMAIAKKLPKNEPQNPGANSA : 194
rab6 : VYDITNVNSFQQTTKWIDDVRTERG----SDVIIMLVGNKTDLADKRQVSIEEGERKAKELNVMFIETSAKAGYNVKQLFRRVAAALPGMESTQDRSRED : 187
rab11 : VYDIAKHLTYENVERWLKELRDHAD----SNIVIMLVGNKSDLRHLRAVPTDEARAFAEKNGLSFIETSALDSTNVEAAFQTILTEIYRIVSQKQMSDRR : 185
rab4 : VYDITSRETYNALTNWLTDARMLAS----QNIVIILCGNKKDLDADREVTFLEASRFAQENELMFLETSALTGENVEEAFVQCARKILNKIESGELDPER : 187
rab7 : VFDVTAPNTFKTLDSWRDEFLIQASPRDPENFPFVVLGNKIDLENRQVATKRAQAWCYSKNNIPYFETSAKEAINVEQAFQTIARNALKQETEVELYNEF : 186
rab9a : TFSVDDSQSFQNLSNWKKEFIYYADVKEPESFPFVILGNKIDISERQVSTEEAQAWCRDNGDYPYFETSAKDATNVAAAFEEAVRRVLATEDRSDHLIQT : 185
cons. : 5 6 35 W 8 8 86 GNK D8 8 58E SA N6 F 88 8

Figure S1