

Supplementary Information

Targeting small GTPases and their prenylation in diabetes mellitus

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A

	10	20	30	40	50	60	70	80	90	100				
Rab1a	MSSMNPEY	DYLFKLLLI	G DSGVGKS	CLL	LRFADDTY- T	ESYIST	IGVD	
Rab35	MARDY	DHLFKLLII	G DSGVGKS	SLL	LRFADNTF- S	GSYITT	IGVD	
Rab13	MAKAY	DHLFKLLLI	G DSGVGKT	CLI	IRFAEDNF- N	NTYIST	IGID	
Rab3a	MASATDSRY	GQKESSDQNF	DYMFKILII	G NSSVGKT	SFL	FRYADDSF- T	PAFVST	TVGID
Rab3b	MASVTDGKT	GVKDASDQNF	DYMFKLLII	G NSSVGKT	SFL	FRYADDTF- T	PAFVST	TVGID
Rab26	MSRKKTPKSK	GASTPAASTL	PTANGARPAR	SGTALSGPDA	PPNGPLQPGR	PSLGGGVDFY	DVAFKVMLV	G DSGVGKT	CLL	VRFKDGAFLA	GTFIST	TVGID		
Rab37	M	WLMSEAHGAE	PVLLREAARP	FTQTLRLCVP	SGNSKVMLL	G DTGVGKT	CFL	IQPKDGAFLS	GTFIAT	TVGID	
Rab8a	MAKTY	DYLFKLLLI	G DSGVGKT	CVL	FRFSEDAF- N	STFIST	IGID	
Rab10	MAKTY	DLFLKLLLI	G DSGVGKT	CVL	FRFSDDAF- N	TFFIST	IGID	
Rab24	MS- GQ	RVDVKVVMLG	KEYVGKT	SLV	ERYVHDFLVL	GPYQNT	IGAA	
Rab7b	MNPRK	KVDLKLII	V G AIGVGKT	SLL	HQYVHKTF- Y	EEYQTL	LGAS	
Rab18	MDEDV	LTTLKILII	G ESGVGKS	SLL	LRFTDDTF- D	PELAAT	IGVD	
Rab27a	MSDGDY	DYLIKFLAL	G DSGVGKT	SVL	YQYTDGKF- N	SKFIT	TVGID	
Rab7a	MTSRK	KVLLKVIIL	G DSGVGKT	SLM	NQYKAT	IGAD		
Rab2a	MAY	AYLFKYIII	G DTGVGKS	CLL	LQFTDKRF- Q	PVHDLT	IGVE	
Rab4a	MSQTAMSETY	DFLFKFLVI	G NAGTGKS	CLL	HQFIEKKF- K	DDSNHT	IGVE	
Rab11a	MGTR- DDEY	DYLFKVVLI	G DSGVGKS	NLL	SRFTRNEF- N	LESKST	IGVE	
Rab11b	MGTR- DDEY	DYLFKVVLI	G DSGVGKS	NLL	SRFTRNEF- N	LESKST	IGVE	
Rab14	MATA- PNYN	SYIFKYIII	G DMGVGKS	CLL	HQFTEKKF- M	ADCPHT	IGVE	
Rab4b	M- AETY	DFLFKFLVI	G SAGTGKS	CLL	HQFIEKKF- K	QDSNHT	IGVE	
Rab28	MSDS- EESQ	DRQLKIVVL	G DGASGKT	SLT	TCFAQETF- G	KQYKQT	IGLD	

P loop

Switch I

G1

G2

Switch II

G3

	110	120	130	140	150	160	170	180	190	200		
Rab1a	FKIRTIELD-	G	K- TIKLQIW-	DT	AGQ	ERFRITIT	SSYYRGAHGI	IVVYDVTDQE	SFNNVKQWL-	
Rab35	FKIRTVEIN-	G	E- KVKLQIW-	DT	AGQ	ERFRITIT	STYYRGTHGV	IVVYDVTSAE	SFVNVKRWL-	
Rab13	FKIRTVDIE-	G	K- KIKLQVW-	DT	AGQ	ERFKTIT	TAYYRGAMGI	ILVYDITDEK	SFENIQNWM-	
Rab3a	FKVKTIIYRN-	D	K- RIKLQIW-	DT	AGQ	ERYRTIT	TAYYRGAMGF	ILMYDITNEE	SFNAVQDWS-	
Rab3b	FKVKTIVYRH-	E	K- RVKLQIW-	DT	AGQ	ERYRTIT	TAYYRGAMGF	ILMYDITNEE	SFNAVQDWA-	
Rab26	FRNKVLDVD-	G	V- KVKLQMW-	DT	AGQ	ERFRSVT	HAYYRDAHAL	LLLYDVTNKA	SFDNIQAWL-	
Rab37	FRNKVVTVD-	G	V- RVKLQIW-	DT	AGQ	ERFRSVT	HAYYRDAQAL	LLLYDITNKS	SFDNIRAWL-	
Rab8a	FKIRTIELD-	G	K- RIKLQIW-	DT	AGQ	ERFRITIT	TAYYRGAMGI	MLVYDITNEK	SFDNIRNWI-	
Rab10	FKIKTVELQ-	G	K- KIKLQIW-	DT	AGQ	ERFHTIT	TSYYRGAMGI	MLVYDITNGK	SFENISKWL-	
Rab24	FVAKVMSVG-	D	R- TVTLGIW-	DT	AGS	ERYEAMS	RIYYRGAKAA	IVCYDLTDSS	SFERAKFWV-	
Rab7b	ILSKIIILG-	D	T- TLKLQIW-	DT	GGQ	ERFRSMV	STFYKGS DGC	ILAFDVTDL	SFEALDIWR-	
Rab18	FKVKTISVD-	G	N- KAKLAIWV	TLHQQTANFF	LKSQIGNSPI	LKWAMWQY	DT	AGQ	ERFRSLT	PSYYRGAQGV	ILVYDVTRRD	TFVKLDNWL-
Rab27a	FREKRVVYRA	SGPDGATGRG	Q- RIHLQLW-	DT	AGQ	ERFRSLT	TAFFRDAMGF	LLFLDLTNEQ	SFLNVRNWI-	
Rab7a	FLTKEVMVD-	D	R- LVTMQIW-	DT	AGQ	ERFQSLG	VAFYRGADCC	VLVFDVTPAN	TFKTLDSWR-	
Rab2a	FGARMITID-	G	K- QIKLQIW-	DT	AGQ	ESFRSIT	RSYYRGAAGA	LLVYDITRRD	TFNHLTTWL-	
Rab4a	FGSKIINVG-	G	K- YVKLQIW-	DT	AGQ	ERFRSVT	RSYYRGAAGA	LLVYDITSRE	TYNALTNWL-	
Rab11a	FATRSIQVD-	G	K- TIKAQIW-	DT	AGQ	ERYRAIT	SAYYRGAVGA	LLVYDIAKHL	TYENVERWL-	
Rab11b	FATRSIQVD-	G	K- TIKAQIW-	DT	AGQ	ERYRAIT	SAYYRGAVGA	LLVYDIAKHL	TYENVERWL-	
Rab14	FGTRIIIEVS-	G	Q- KIKLQIW-	DT	AGQ	ERFRAVT	RSYYRGAAGA	LMVYDITRRS	TYNHLSSWL-	
Rab4b	FGSRVVNVG-	G	K- TVKLQIW-	DT	AGQ	ERFRSVT	RSYYRGAAGA	LLVYDITSRE	TYNSLAAWL-	
Rab28	FFLRRITLP-	G	NLNVTLQIW-	DI	GGQ	TIGGKML	DKYIYGAQGV	LLVYDITNYQ	SFENLEDWYT	

Supplementary Figure 1 corresponding to Figure 1. Amino acid sequence alignment of human GTPases involved in diabetes and insulin resistance

Protein sequences were downloaded from String: functional proteins association network. Alignment of Rab (A), Rho (B) and Ras (C) proteins was performed using Praline (developed in the Centre for Integrative Bioinformatics Vrije Universiteit Amsterdam). Important regions are indicated: the P loop (orange), switch I (green) and switch II (magenta), the nucleotide-binding G-motifs (yellow, G1-G5) and the C-terminal cysteine important for prenylation (cyan). Numbers at the top indicate the residue number.

Supplementary Table 1 corresponding to Figure 1. List of crystal structures of small GTPases playing a role in diabetes.

GTPase	GDP-bound	GTP&- bound	In complex with effector	In complex with GEF	In complex with GAD
Rab GTPases					
Rab1a	2FOL	ND	ND [^]	ND [^]	ND [^]
Rab2a	ND	ND	ND	ND	ND
Rab3a	ND	3RAB	ND	ND	ND
Rab3b	3DZ8	ND	ND	ND	ND
Rab4a	2BMD	1YU9 2BME	ND	ND	ND
Rab4b	2O52	ND	ND	ND	ND
Rab5a	1TU4 2BMD	1N6H	ND [^]	ND [^]	ND
Rab7	1VG1	1T91 1VG8	1YHN (RILP)	ND	ND
Rab8a	4LHV 6STF	4LHW 6STG	ND [^]	ND [^]	ND [^]
Rab10	ND	ND	ND [^]	ND	ND
Rab11a	1OIV 1OIX (and Pi) 6IY1	1OIW 1YZK	ND [^]	ND [^]	ND

Rab11b	2F9L	2F9M	ND^	ND	ND
Rab13	ND	ND	ND	ND	ND
Rab14	1Z0F 4DRZ	ND	ND^	ND	ND
Rab18	ND	1X3S	ND	ND	ND
Rab24	ND	ND	ND	ND	ND
Rab26	ND	2G6B	ND	ND	ND
Rab27a	ND	HUF6	ND^	ND	ND
Rab28	2HXS	3E5H	ND	ND	ND
Rab35	ND#	ND	ND^	ND^	ND
Rab37	ND	ND	ND	ND	ND
Rho GTPases					
RhoA	1FTN 5E26 6KX2	1A2B 3TVD	ND^	ND^	ND
Cdc42	ND#	2QR2	1CEE (WASP)	ND^	ND^
Rac1	5N6O 6AGP	1MH1 3TH5	ND^	1FOE (TIAM1) 4YON (P-Rex1) 5O33 (Kalirin DH1)	ND^

TC10	ND	2ATX	2KE4 (CDc42-interacting domain of CIP4)	ND	ND
RND3/ RhoE	ND	1GWN (only core domain) 1M7B	ND^	ND	ND
Ras GTPases					
RalA	1U90 1U8Z 6P0O 6P0J	ND	1UAD (Sec5) 1ZC3 (Exo84) 1ZC4 (Exo84)	ND	ND
(R)Rad	2DPX 2GJS	3Q72 (G domain) 3Q7P (G domain)	ND	ND	ND
Rap1	ND	ND	ND^	ND	ND
H-Ras	<u>1CRR</u> <u>1CRQ</u> <u>1CRP</u> 2CLD 4Q21	121P 1CTQ 1PLK 1QRA 3TGP 4RSG 4EFL 5P21	ND^	1NVX (SOS) 1NVW (SOS) 1NVU (SOS) 1BKD(SOS-1) 4URY(SOS)	1WQ1 (RasGAP)
N-Ras	ND	5UHV	ND	ND	ND

K-Ras	4OBE 5VQ8 5W22 6MBT 6MBU	5VQ2 5VQ6 6GOD 6VC8	ND [^]	ND	ND
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Structures identification codes (PDB ID codes) were assessed from the Protein Data Bank, <http://www.pdb.org>. As source organism taxonomy was selected *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*. X-ray and NMR structures are included. NMR structures are underlined. Only the structures of GTPases with a single Mg²⁺ were included. Structures in the complex with the effectors unrelated to diabetes were omitted. ND - not determined for GTPases; ND[^] - there exist crystal structures of GTPase-effector/GEF/GAP complexes, but unrelated to diabetes, insulin resistance or insulin-dependent tissue; ND[#] - crystal structure of GDP-bound form exists only in the in complex with GEF; >P or its non-hydrolyzable analog, including GppNHp, GTPgammaS, GMP-PCP; PDB ID codes in bold represent structures used for the generation of Figure 1.

Supplementary Table 2 corresponding to Figure 3. List of crystal structures of the enzymes of mevalonate pathway playing a role in diabetes mellitus

Enzyme	Only enzyme	With substrate/product	With inhibitor
HMG-CoA reductase	ND [^]	1DQ8 (HMG and CoA) 1DQ9 (HMG-CoA) 1DQA (HMG, CoA and NADP ⁺)	1HW9 (Simvastatin) 1HWI (Fluvastatin) 1HWK (Artovastatin)
Farnesyl pyrophosphate synthase (FPPS)/ farnesyl diphosphate synthase (FDPS)	1ZW5 2F7M 4XQR 4XQS (Mg ²⁺) 4XQT (3Mg ²⁺)	5JA0 (FPP)	2F89 (pamidronate) 2F8C (zoledronate) 2F92 (alendronate)
Geranylgeranyl diphosphate synthase (GGPPS)	ND [^]	2Q80 (GGPP)	ND [^]
Farnesyltransferase (FTase)	1FT1	1FPP (FPP) 1FT2 (FPP, Zn ²⁺) 1JC6 (FPP, TKCVFM peptide substrate) 1KZP (farnesylated K-Ras4b) 1TN8 (FPP, H-Ras) 2H6F (farnesylated DDPTASACVLS peptide)	1SA4 (R115777) 1SA5 (BMS-214662)

Geranylgeranyltransferase-I (GGTase-I)	1DCE	<p>1N4P (GRG)</p> <p>1N4Q (GGPP*, geranylgeranylated KKKSKTKCVIL peptide)</p> <p>1N4R (geranylgeranylated KKKSKTKCVIL peptide)</p> <p>1N4S (GGPP, geranylgeranylated KKKSKTKCVIL peptide)</p> <p>1TNO (GGPP*, geranylgeranylated KKKSKTKCVIM peptide from K-Ras4b)</p> <p>1TNZ (GGPP*, geranylgeranylated RRCVLL peptide from Cdc42)</p>	ND^
Geranylgeranyltransferase type-2 (GGTase-2)	3DSS	<p>3DST (GRG)</p> <p>3DSU (FPP, Ca²⁺Zn²⁺)</p> <p>3DSV (monoprenylated SCSC peptide from Rab7)</p> <p>3DSW (monoprenylated SCSC peptide from Rab7)</p> <p>3DSX (diprenylated SCSC peptide from Rab7)</p>	ND^
Geranylgeranyltransferase type-III (GGTase-III)	6J6X &	ND^	ND^

Supplementary Table 2 corresponding to Figure 3. List of crystal structures of the enzymes of mevalonate pathway playing a role in diabetes mellitus.

Structures identification codes (PDB ID codes) were assessed from the Protein Data Bank, <http://www.pdb.org>. As source organism taxonomy was selected *Homo sapiens*, *Mus musculus*, and *Rattus norvegicus*. Structures in the complex with the inhibitors unrelated to diabetes were omitted. ND - not determined for the enzymes, ND^ - there exist crystal structures of enzyme/substrate/product or

enzyme-inhibitor complexes, but unrelated to diabetes, insulin resistance or insulin-dependent tissue, *analog, & deposited, but unpublished. PDB ID codes in bold represent structures used for the generation of Figure 3.