Supplementary information to

Signaling Gateway Molecule Pages – a data model perspective

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MOLECULE PAGE DETAILS:

Though molecule pages can be assigned to sequences of any species, only those with mouse sequences can have author annotation. This limitation is due to the origin of the SGMP as part of the Alliance for Cellular Signaling (Gilman et al, 2002) – which used the mouse cell as a basis for the experiments. In practice, many of the molecule pages contain information derived from other species than the mouse, so viewing the SGMP as hybrid model is more accurate. Removing the mouse sequence limitation, and presenting the data based on homologous groups, is a future goal of the SGMP.

Automated data for each molecule page gets updated at least once a month. As of now, all published molecule pages only have one version. Extending our system to allow for updated versions is a feature we plan on adding in 2011.

Table S1: **Molecule page categories**. Presently there are 4017 molecule pages (from 3992 genes) and 655 published as of March 4, 2011, all assigned to a specific in-house category. **Note:** The sequence section of a molecule page and the BioPAX export file have complete Gene Ontology (GO) annotation details, which gives complete details on a gene function, process and compartment.

S.No	Molecule Page Category	Total Molecule Pages	Published
1	Actin binding protein	28	4
2	Activator protein	29	5
3	Adaptor/scaffold	196	40
4	Adenylyl cyclase	10	5
5	Adhesin/agglutinin	96	15
6	Apoptosis	12	3
7	Calcium-binding protein	52	17
8	Cell surface, *misc*.	129	18
9	Channel, calcium	44	7
10	Channel, cation	28	8
11	Channel, chloride	4	0
12	Channel, *misc*.	12	0
13	Channel, potassium	70	1
14	Channel, sodium	21	2
15	Chaperone	17	8
16	Chemokine	32	1
17	Contractile protein	14	0
18	Cytokine	36	0
19	Cytoskeletal protein	106	8
20	Cytosolic, *misc*.	213	43
21	DNA binding protein	8	0
22	Dystrophin complex	17	0
23	Enzyme, *misc*.	35	5
24	Extracellular matrix	17	1
25	G protein, heterotrimeric	34	12
26	G protein, monomeric (non-Rab)	69	24
27	G protein, Rab	55	12
28	GTPase activating protein, ARF	28	7

29	GTPase activating protein, misc.	9	1
30	GTPase activating protein, Rac/Rho	18	4
31	GTPase activating protein, Ras	9	0
32	GTPase activating protein, RGS	23	12
33	Guanine nucleotide exchange factor, ARF	9	0
34	Guanine nucleotide exchange factor, *misc*.	14	3
35	Guanine nucleotide exchange factor, Rab	5	1
36	Guanine nucleotide exchange factor, Rac/Rho	40	10
37	Guanine nucleotide exchange factor, Ras	9	3
38	Guanylyl cyclase	4	1
39	Hydrolase, esterase	12	0
40	Hydrolase, non-esterase	9	5
41	Immunoglobulin superfamily	32	0
42	Inhibitor protein	68	11
43	Isomerase	5	2
44	Kinase (non-protein)	27	3
45	Kinase, lipid	46	7
46	Ligand, GPCR	7	1
47	Ligand, receptor tyrosine kinase	43	3
48	Lipid binding protein	34	2
49	Lipid modification, protein	15	7
50	Lyase	2	0
51	Motor protein	16	1
52	Myosin binding protein	20	0
53	Nuclear, *misc*.	59	5
54	Oxidoreductase	33	9
55	Phosphatase	3	1
56	Phosphatase (non-protein)	52	4
57	Phosphatase, lipid	6	0
58	Phosphodiesterase	23	12
59	Phospholipase	35	4
60	Phosphorylase	3	0
61	Protease (non-proteasomal)	62	1
62	Protease (proteasomal subunit)	41	7
63	Protein kinase, dual-specificity	28	15
64	Protein kinase, Ser/Thr	59	10
65	Protein kinase, Ser/Thr (non-receptor)	273	67
66	Protein kinase, tyrosine (non-receptor)	39	11
67	Protein phosphatase, dual-specificity	42	7
68	Protein phosphatase, Ser/Thr	1	0
69	Protein phosphatase, Ser/Thr (non-receptor)	60	2
70	Protein phosphatase, tyrosine (non-receptor)	24	2
71	Receptor, cytokine	63	3
72	Receptor, GPCR	219	47
73	Receptor, guanylyl cyclase	6	2
74	Receptor, ligand-gated channel	65	2
75	Receptor, *misc*.	90	33
76	Receptor, nuclear (transcription factor)	26	1
77	Receptor, protein phosphatase, tyrosine	19	1
78	Receptor, protein translocating	2	0
79	Receptor, Ser/Thr kinase	13	3
80	Receptor, tyrosine kinase	56	1

81	RNA binding protein	29	1
82	Secreted protein	123	10
83	Transcription factor	131	17
84	Transcription; coactivator/corepressor	46	7
85	Transferase	15	5
86	Translation, protein	9	2
87	Transporter, ABC	52	5
88	Transporter, active	34	4
89	Transporter, facilitator	188	17
90	Ubiquitin conjugating system	23	3
91	Vesicle protein	77	11

Table S2**: SGMP proteins present in signaling pathways from the KEGG database**.
Kegg_total: KEGG total genes (includes orthologs, predicted, pseudo and hypothetical), Kegg_mouse: KEGG total genes (excludes orthologs, predicted, pseudo and hypothetical), Sgmp_total: SGMP total genes and Sgmp_published: SGMP published pages (as of March 4, 2011).

S.No	KEGG Pathway ID	KEGG Pathway Name	Kegg_total	Kegg_mouse	Sgmp_total	Sgmp_published
1	path:mmu04010	MAPK signaling pathway	269	264	247	54
2	path:mmu04020	Calcium signaling pathway	178	177	164	37
3	path:mmu04062	Chemokine signaling pathway	188	177	166	31
4	path:mmu04310	Wnt signaling pathway	154	150	127	25
5	path:mmu04722	Neurotrophin signaling pathway	132	129	117	25
6	path:mmu04912	GnRH signaling pathway	99	98	90	22
7	path:mmu04660	T cell receptor signaling pathway	112	110	101	20
8	path:mmu04662	B cell receptor signaling pathway	76	74	73	17
9	path:mmu04910	Insulin signaling pathway	137	132	117	17
10	path:mmu04370	VEGF signaling pathway	76	74	71	14
11	path:mmu04012	ErbB signaling pathway	87	86	81	16
12	path:mmu04664	Fc epsilon RI signaling pathway	80	77	73	18
13	path:mmu04350	TGF-beta signaling pathway	85	84	59	13
14	path:mmu04620	Toll-like receptor signaling pathway	101	100	87	11
15	path:mmu04070	Phosphatidylinositol signaling system	78	77	70	8
16	path:mmu04150	mTOR signaling pathway	53	48	45	8
17	path:mmu04340	Hedgehog signaling pathway	54	54	43	7
18	path:mmu04115	p53 signaling pathway	70	68	41	6
19	path:mmu04621	NOD-like receptor signaling pathway	62	60	37	7
20	path:mmu04630	Jak-STAT signaling pathway	153	148	119	4
21	path:mmu04920	Adipocytokine signaling pathway	68	68	54	5
22	path:mmu04622	RIG-I-like receptor signaling pathway	69	68	42	6
23	path:mmu03320	PPAR signaling pathway	81	81	32	1
24	path:mmu04330	Notch signaling pathway	51	51	21	1
25	path:mmu02010	ABC transporters	45	45	45	5
26	path:mmu04512	ECM-receptor interaction	86	84	56	8
27	path:mmu04742	Taste transduction	60	58	23	4
28	path:mmu04110	Cell cycle	128	125	74	12
29	path:mmu05014	Amyotrophic lateral sclerosis (ALS)	56	55	41	5

STATES:

State Naming

The following rules are used to create a state name in order to preserve continuity across all published molecule pages. For example: "ProA/ProB-P2 (nuc)" represents Protein A bound to Protein B (phosphorylated twice with the state existing in the nucleus).

(1) The first letter of the protein is capitalized, with subsequent letters in lowercase.
(2) If the protein name contains a number, then the space or dash that normally separates the number from the name is removed.
(3) Post-translational modifications are represented as a short code (see Table S5). The modification code follows the protein name and is separated by a dash ('-') as in ProB-P.
(4) Multiple modifications are separated by dashes and appear after the protein name.
(5) If the protein contains two or more of the same modifications, then the modification code is followed by the number of modifications.
(6) Ligands are listed in the same way as post-translational modifications. All common abbreviations for the ligand name are used (e.g. GTP, PIP3).
(7) Short code that corresponds to cellular location appears in parentheses after the state name (see Table S6). Cellular localization is not listed in all state names. However, if a state shuttles between two or more locations, it helps to clarify the state transition network map.
(8) A backslash is used to separate proteins in the state name.
(9) If a protein exists as a dimer or multimer, then the state name is enclosed in parentheses, and the number of monomers is added before the first parenthesis.
(10) If a state name exceeds the character limit (30), then cellular location or post-translational modifications are removed from the name or protein names are shortened.
(11) Greek letters are acceptable in protein names.

Table S3. **State names with description.** States capture information about protein-protein and protein-small molecule interactions, post-translational modifications and changes in cellular location.

Sr.No	State Name	Description
1	NTPDase8-G4	NTPDase8 that has been glycosylated 4 times
2	2(TgfβRI-P)	phosphorylated TGF beta receptor type I dimer
3	Rsk1-P4/Erk1-P2/Pdk1-P	Rsk1 that has been phosphorylated 4 times, bound to diphosphorylated Erk1 and monophosphorylated Pdk1
4	Kras-GG-proteo-Me-GTP	GTP-bound K-Ras that is bound to a geranylgeranyl isoprenoid and has been proteolytically cleaved and methylated

Some key features of a molecule page state are:

1. State is either a Protein or a Complex molecule.
2. If Protein, it may associate with one or more modifications and/or cellular location.
3. If Complex, it may be associated with one or more proteins, small molecules, complexes and/or class molecules, and may also associate with one or more modifications and/or cellular location.
4. Stoichiometry is included in a state when it is used as a catalyst or in other functions, since the state is created with stoichiometric details.

Table S4**: A molecule state may associate with one or more of the following five types of functions**. Nuclear receptors may function as receptors and transcription factors and receptor tyrosine kinases may function both as an enzyme and as a receptor.

S.No	State Name	Function	SGMP_ID	STATE_ID
1	4(Trpc7)[2]	Channel	A002418	20261
2	(Lcmt1)	Enzyme	A003167	18449
3	(Aplnr 2G)	Receptor	A000304	15611
4	(Pknox1) (Hoxb1) (Pbx[1-4])	Transcription Factor	A004115	25250
5	(Abca1-2ATP)	Transporter	A002593	10440

Table S5: **Some covalent modifications and corresponding codes used in state names**.

S.No	Covalent Modification	Code
1	Phosphorylation	P
2	Glycosylation	G
3	O-linked Glycosylation	O(G)
4	Methylation	Me
5	Myristoylation	M
6	Palmitoylation	PA

7	Farnesylation	F
8	Geranylgeranylation	GG
9	Phosphatidic acid	PhtAc
10	Ubiquitination	Ub
11	Sumoylation	Sumo
12	Proteolysis	Proteo

Table S6**: Some of the cellular locations and corresponding codes used in state names**. Cellular locations are mapped to their respective GO term.

S.No	Cellular Location	Code
1	Nucleus	(nuc)
2	Cytoplasm	(cyto)
3	Endoplasmic reticulum	(ER)
4	Golgi	(Golgi)
5	Plasma membrane	(PM)
6	Secreted	(sec)

STATE TRANSITIONS:

Based on the components, modifications and localization differences between the two states, state transitions are classified under the following categories and appropriate kinetic parameters (see STATE FUNCTIONS) are provided. The categories are

1. Ligand association/dissociation
2. Protein association/dissociation
3. Intrinsic enzyme activity
4. Addition/removal of covalent modification
5. Change of cellular localization and
6. Multiple modification

Further, state transition differences (computed differences) are calculated by comparing the starting and ending state signatures. To calculate computed differences, each state is considered as a *complex* with *complex components* like proteins and/or class molecules with associated modifications, small molecule binding and cellular location.

STATE FUNCTIONS:

A state may associate with one or more of the following functions. Not all protein states are associated/defined with functions in molecule pages; for example, *scaffolds/adaptor* proteins, *binding* proteins, *RGS* proteins and *small GTPases*. These proteins regulate signal transduction by localizing pathway components or mediating specific protein-protein interactions (Pawson and Scott, 1997).

Channel Function

Channels are trans-membrane protein complexes (states) found in the lipid bilayer membranes (Brown, 1993). These channels allow permeation of specific ions/molecules and the majority of channels are defined with respect to how and/or what opens and closes the channels. Thermodynamic data as shown in Table S7 is provided for ion channels. *Voltage-gated* ion channels, like cation channels, open or close depending on the voltage gradient (potential change) across the membrane. *Ligand-gated* ion channels open or close depending on binding of ligands to the channel. Examples of ligand gated channels are nicotinic acetylcholine receptor, the γ-aminobutyric acid receptors, glycine receptor *etc*.

Table S7. **The channel function is associated with the following thermodynamic data.** Ion channels exist in different conformational states, including an open state, which permits ions through the pore, or a closed state. The transition between these states is often called ion channel gating, and it is influenced by a number of different factors.

Sr.No	Parameter	Units	Description
1	*Tau open*	*milli sec*	mean channel open time
2	*Tau close*	*milli sec*	mean channel close time
3	Activation gating	*milli volts*	
4	Inactivation gating	*milli volts*	
5	Inactive gate time const	*milli sec*	
6	I.V curve	-	current-voltage properties
7	*Channel Ion conductance*	-	small molecule
		pS	pico-siemens, measure of current (in amps)

			divided by voltage (in volts)
	γ value	*pS*	average conductance
	γ max value	*pS*	maximum average conductance
8	Ionic selectivity	-	list of *Channel ion* with selectivity order
9	Blocker	-	protein or small molecule (ionic or other)

The channel *ionic selectivity* is defined by a list of channel ions with selectivity order. A channel ion is a *small molecule* with *conductance* details. A *channel blocker* is categorized into a protein or a *small molecule* that interferes with the entry of a channel ion into the cell through the membrane. Some ligand-gated channels are associated with receptor activity, for example *Inositol 1,4,5-triphosphate ($InsP_3$)* receptor is associated with Ca^{+2} channel on the endoplasmic reticular membrane.

Enzyme Function

A state's enzymatic/catalytic activity is represented as a reaction with substrate kinetic parameters. Each reaction has reaction components (reactants and products) which are either proteins (states) and/or small molecules with stoichiometry. Two types of kinetic models, *Michaelis-Menten* and *Co-operative,* are available as in Table S8. Examples of enzymes include kinases, phosphatases, phospholipases *etc*.

Table S8. **Enzyme kinetic parameters with units and description**. Enzyme kinetics gives information on how enzymes bind substrates and turn them into products.

Sr.No	Parameter	Units	Description
1	Kinetic Pattern	-	Michaelis-Menten / Positive Cooperativity
2	K_m	*µmol*	Michaelis-Menten Constant
3	*Kprime*	*µmol*	analogous to K_m in cooperative binding
4	*Hill coefficient*	-	cooperativity factor
5	V_{max}	*µmol/min/mg*	maximum enzyme velocity
6	k_{cat}	*per sec*	maximum number of enzymatic reactions catalyzed per second
7	Observed rate (V_o)	*µmol/min/mg*	
8	Substrate *conc*. *([S])µmol*		

A catalytic state may associate with one or more reactions or a particular reaction might be carried out by more than one catalyst (second one is called reference enzyme), quantitative data is displayed where available in peer reviewed literature. In the latter case the activity of the function is defined as a percentage of the activity of the old function (*relative activity*). These values can be positive or negative. If the relative activity is greater than 1 then the new function activity is represented as '*increase (relative activity-1.00) fold*'. If it is less than 1 then the new function activity is '*decrease (1.00 – relative activity)*100 percent'*. Receptor tyrosine kinases may function both as an enzyme and as a receptor. All intrinsic enzymatic reactions of a state are described in process data of state transitions section.

Receptor Function

A receptor may be embedded in the membrane in an insoluble form or in a soluble state when localized in the cytoplasm/nucleus of a cell (Gutkind, 1998; Gutkind, 1998). The receptor will be activated upon binding of a specific kind of signaling molecule called a *ligand*. This ligand is a protein in a specified state or a small molecule which may bind to the extracellular domain of a transmembrane receptor or enter into the cell and bind to intracellularly localized receptors. Examples of receptors include transmembrane (G-protein coupled) receptors, cell surface receptors, receptor tyrosine kinases and nuclear receptors. Receptor-ligand binding efficiency is described by estimating the rate constants and/or dissociation constants from experimental kinetic or equilibrium data (see Table S9).

Table S9. **Receptor-Ligand kinetic parameters with units and description**. Some states are associated with receptor activity. Ligand may be either a state or a small molecule. The life span of the receptor-ligand complex is controlled primarily by the dissociation rate of the bound ligand.

Sr.No	Parameter	Units	Description
1	Ligand	-	either a state or a small molecule
2	*Stoichiometry*	-	number of binding sites
3	*Dissociation constant (K_d)*	*µmol*	affinity between a ligand and receptor
4	*Hill coefficient*	-	cooperativity factor in a binding process

Not every ligand that binds to a receptor activates the receptor. Based on the ligand's role on receptor activation, ligands are classified. AGONISTS are able to activate the receptor and result in a maximal biological response. Most natural ligands are full agonists. PARTIAL AGONISTS do not activate receptors fully, causing responses which are partial compared to those of full agonists. ANTAGONISTS bind to receptors but do not activate them, this result in receptor blockage, inhibiting the binding of other agonists. INVERSE AGONISTS reduce the activity of receptors by inhibiting their constitutive activity. The receptor tyrosine kinases may function as both an enzyme and as a receptor. Nuclear receptors may function as receptors and transcription factors (ligand-controlled transcription factors).

Transcription Factor Function

The primary controlling factors of the gene transcriptional activity are called transcription factors (TFs), which bind to *cis-acting* DNA sequences and thereby influence the transcription process (Brivanlou and Darnell, 2002; Mitchell and Tjian, 1989). Each TF function is associated with a list of regulated *target genes* and information on the bound DNA sequence. *Bound DNA sequence* is represented by using IUPAC nucleotide ambiguity codes (Cornish-Bowden, 1985). Each TF modulates transcription of *one or more* target genes, and if a TF's target modulated gene list contains *two or more* same family genes with the same recognition sequence then a *class molecule* is assigned as a target. The TFs can exert a negative or positive influence on gene transcription upon binding to their DNA sequence and this effect is represented in molecule pages as *regulation type* which is either UP, DOWN or NO CHANGE. In order to attain full stimulatory activity sometimes the TFs themselves require the aid of further protein factors, known as *effectors* - which are either activators or repressors (Thomas and Chiang, 2006; Xu, et al., 1999). This class of proteins is represented as transcription factor, coactivators/corepressors.

Transporter Function

A transporter is a transmembrane protein complex that helps a certain substances (proteins or small molecules) to cross the membrane by facilitated diffusion or active transport (Pajor, 1999; Palacin, et al., 1998). Examples of transporters include glucose transporters and sodium/potassium ATPases. If two states have similar transporter function and kinetic parameter data (Table S10) then the new function activity is expressed as a percentage of the activity of the old function (similar to reference enzyme activity mentioned in Enzyme function section).

Table S10. **Transporter function with substrate kinetic parameters.** The transporter state may assist in the movement of substances by facilitated diffusion or active transport across the membrane.

Sr.No	Parameter	Units	Description
1	*Stoichiometry*	-	number of cycles/times that substrate molecules, passing through the transporter
2	*Turnover number*	*cycles/min*	
3	K_{m}*_inside*	*µmol*	
4	K_{m}*_outside*	*µmol*	
5	*Common direction*	-	in/out
6	*Electrogenic*	-	yes/no
7	*ATPage*	-	yes/no
8	K_{m}*_atp*	*µmol*	

CLASS MOLECULE:

A *class molecule* (also called Protein Class) is a set of two or more protein molecules with sequence and/or functional homology that behave similarly in the context of states, transitions and functions. To create a class molecule, a protein should interact with two or more member proteins of the family. A class molecule does not need to include all *homologs* of a protein family if the molecule has not been demonstrated to interact with a given protein (see Table S11). The use of a class molecule permits the creation of interactions between a protein molecule and each member of the protein class simultaneously by creating a single state that represents the prototypical complex. Therefore, class molecules are not the same thing as protein complexes. The class molecule is represented with a *'Stem Grouping'* method. This tries to group proteins based on a common symbol stem and then collapses the suffixes, producing something much shorter than simply enumerating the components. For example (Gnb1 | Gnb2 | Gnb3 | Gnb4) class molecule is represented by Gnb[1-4] and (Sfn | Ywhaz | Ywhab | Ywhae | Ywhah | Ywhag | Ywhaq) is represented by Sfn | Ywha[b,e,g-h,q,z].

Table S11**: Class Molecules / Protein Classes of RGS family members**. These class molecules are classified as SGMP defined and AUTHOR defined. SGMP defined class molecules generally contain all the members of the protein family and AUTHOR defined contains limited number of protein members with known interactions.

S.No	Name	Description	Class Type
1	Rgs[1,3-4]	RGS protein	AUTHOR
2	Rgs[1,4,7,11,14,16,19-20]	RGS proteins (Go)	AUTHOR
3	Rgs[1,4,7,11,14,16-17,19-20]	RGS proteins (Go)	AUTHOR
4	Rgs[1-2,4-5,8,13,16,19]	R4 family of the RGS	AUTHOR
5	Rgs[1-4,18-19]	RGS	AUTHOR
6	Rgs[1-5,10,12-14,16-20]	RGS	AUTHOR

7	Rgs[1-5,7-8,13,16-18]	RGS proteins (Gq)	AUTHOR
8	Rgs[1-5,7-8,13,16-19]	RGS proteins (Gq)	AUTHOR
9	Rgs[1-5,7-8,13,16-20]	RGS proteins (Gq)	AUTHOR
10	Rgs[1-5,8,10,12-14,16-20]	RGS family 1 (canonical RGS's)	SGMP
11	Rgs[1-5,8,13,16,18,21]	R4 RGS family	AUTHOR
12	Rgs[1-5,8,13,16,18]	RGS	AUTHOR
13	Rgs[1-5,8,13,16,19]	R4 family of the RGS	AUTHOR
14	Rgs[2-5,7-8,13,16-18]	RGS proteins (Gq)	AUTHOR
15	Rgs[2-5,8,13,16,18]	R4 RGS family	AUTHOR
16	Rgs[4,10,17,19-20]	Gz RGSs	AUTHOR
17	Rgs[5,7-8,13,16-17]	RGS proteins (Gq)	AUTHOR
18	Rgs[5,8,13,16-17]	RGS proteins (Gq)	AUTHOR
19	Rgs[6-7,9,11]	RGS family 2 (R7 members)	SGMP
20	Rgs1[0,7] | Rgs20	Gz RGSs	AUTHOR
21	Rgs1[2,4]	RGS12,14	AUTHOR
22	Rgs1[7,9] | Rgs20	Gz binding RGSs	AUTHOR

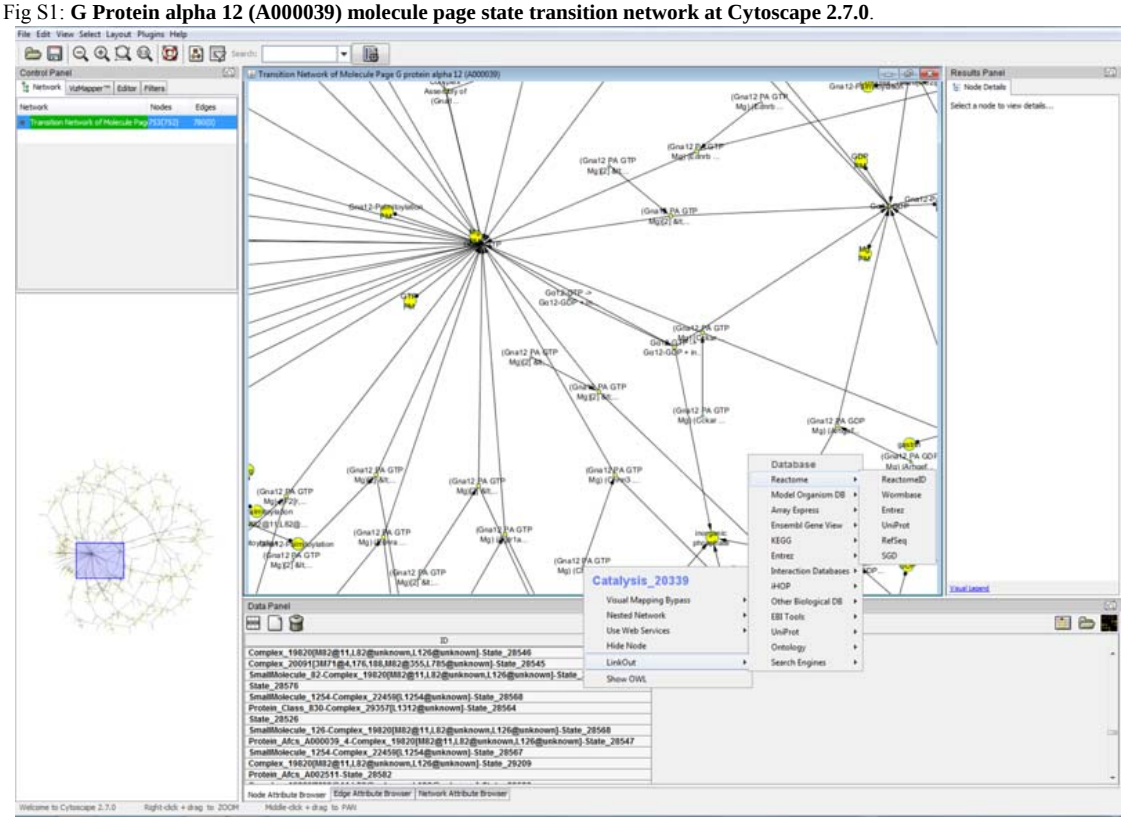
DATA EXPORT:

BioPAX provides more detailed information on individual entities and interactions, while SBML is targeted at modeling dynamic biochemical networks that are described by reactions. Furthermore, by using BioPAX level3 format one can also visualize, process and manipulate data through other external network visualization/analysis software, such as Cytoscape (Shannon, et al., 2003) (see Fig S1 and Table S12).

Table S12: **Some BioPAX level3 classes used to define SGMP data**. BioPAX is an abstract data (ontology) model to describe all biological processes. BioPAX provides more detailed information on individual entities and interactions.

S.No	SGMP Term	BioPAX Term
1	State	Protein / Complex
2	Transition	pathwayStep
3	Transition Network	Pathway
4	Protein/Ligand association/dissociation	ComplexAssembly
5	Change of Cellular Location	Transport
6	Channel/Transporter function	Transport
7	Enzymatic/Catalytic reaction	Catalysis
8	Transcription Factor Function	TemplateReactionRegulation
9	Class Molecule / Protein Class	Protein (ProteinReference with a list of memberReferenceEntity)

Fig S1: **G Protein alpha 12 (A000039) molecule page state transition network at Cytoscape 2.7.0**.



The above Fig S1 was generated by exporting SGMP BioPAX file for 'G Protein alpha 12' and then importing it into cytoscape**.** The figure demonstrates the utility of SGMP data to facilitate visualization and analysis using Cytoscape, which is the definitive pathway analysis resource in the community. Once, exported the SGMP initialized Cytoscape graph can be enriched further by adding pathway data from another database or vice versa.

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PDF export:

Presently this feature can be accessed at http://biome.sdsc.edu:8004/molecule.

Molecule Page ID	A001997
Primary Name	Rab8a
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Accession	NP_075615.2
Entez Gene ID	17274
Species	Mouse
Sequence	1 MAKTYDYLFK LLLIGDSGVG KTCVLFRFSE DAFNSTFIST IGIDFKIRTI 51 ELDGKRIKLQ IWDTAGQERF RTITTAYYRG AMGIMLVYDI TNEKSFDNIR 101 NWIRNIEEHA SADVEKMILG NKCDVNDKRQ VSKERGEKLA LDYGIKFMET 151 SAKANINVEN AFFTLARDIK AKMDKKLEGN SPQGSSHGVK ITVEQQKRTS 201 FFRCSLL
Sequence Length	207 aa
Molecular Weight	23.669699 kD
Isoelectric Point	pH 9.15
Extinction Coefficient	19420 $M^{-1}cm^{-1}$
Absorption Coefficient	0.82
Aliphatic Index	82.95

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Rab8a

Angela Wandinger-Ness[1] and Dusanka Deretic[2]

The Rab8a GTPase is one of two known protein isoforms that have cell-type specific functions during the process of exocytosis and coordinates regulation of the cytoskeleton. Rab8a also appears to interface with endocytic recycling circuits. At the molecular level, Rab8a regulates both the export of vesicles from the trans-Golgi apparatus, and the directed translocation along actin filaments and microtubules. Rab8a also regulates targeted fusion with select plasma membrane domains; including the apical membrane of enterocytes and kidney proximal tubule cells, basolateral membrane of Madin-Darby canine kidney (MDCK) cells, neuronal dendrites and the primary cilium. Numerous regulators of Rab8a activity, such as Rab8a specific guanine exchange factors (GEFs), GTPase-activating proteins (GAPs) and kinases have been shown to modulate activity, while cytoskeletal motors or motor-binding proteins and effectors help coordinate Rab8a function during membrane trafficking. Rab8a has critical roles in the processes of cell morphogenesis, cell signaling and development. Consequently, Rab8a functional deficiencies result in retinal degeneration, microvillar atrophy and an inability to absorb nutrients, as well as developmental and kidney disorders.

PROTEIN FUNCTION

The revolutionary identification of two Ras-like GTPases, Sec4p and Ypt1p, with crucial regulatory functions in *Saccharomyces cerevisiae* secretion rapidly led to the discovery of additional small molecular GTPases in mammalian cells and fission yeast (Salminen and Novick 1987; Segev *et al*. 1988; Zahraoui *et al*. 1989; Haubruck *et al*. 1990). Seven mammalian Rab GTPases were identified in the first cohort and all were shown to be involved in exocytosis (Zahraoui *et al*. 1989). Rab8 was identified soon thereafter as a regulator of membrane trafficking from the trans-Golgi apparatus to the basolateral epithelial plasma membrane or the dendritic plasma membrane of cultured neurons and as a close functional and sequence homolog of Ypt2 in *Schizosaccharomyces pombe* and Sec4 in *S. cerevisiae* (Salminen and Novick 1987; Haubruck *et al*. 1990; Huber *et al*. 1993a; Huber *et al*. 1993b).

Overall, in specialized cell types, Rab8a regulates the polarized trafficking of diverse cargo and serves as an essential interface between membrane transport circuits, the cytoskeleton and cell signaling. After its initial characterization, Rab8 was shown to be mandatory for neurite outgrowth and for the export of exocytic vesicles carrying rhodopsin from the trans-Golgi network to the rod outer segment of photoreceptor cells (Deretic *et al*. 1995; Huber *et al*. 1995). In melanocytes and neurons, Rab8 regulates melanosome trafficking, beta-amyloid precursor protein processing and secretion, and trafficking of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (McConlogue *et al*. 1996; Chakraborty *et al*. 2003; Gerges *et al*. 2004; Chabrillat *et al*. 2005).

In intestinal epithelia, Rab8a is essential for localization of apical proteins and maintenance of intestinal microvilli (Sato *et al*. 2007). The formation of membrane protrusions through extensive rearrangements of both actin-based and microtubule-based networks was first revealed upon overexpression of wild-type Rab8 or a constitutively active Rab8Q67L mutant, thereby linking Rab8-regulated trafficking and cytoskeletal rearrangements (Peränen *et al*. 1996; Kobayashi 2002). On the other hand, the inactivating Rab8T22N mutant expressed in transgenic *Xenopus laevis* inhibited docking and fusion of rhodopsin-containing export vesicles and caused dramatic retinal degeneration (Moritz *et al*. 2001).

Like other Rab GTPases, Rab8a synchronizes multiple events in transport from budding to fusion. In the Golgi apparatus, Rab8a interfaces with the clathrin adapter protein-1B (AP-1B), the optineurin effector and motor protein myosin VI to promote cargo export, possibly through recycling endosomes (Ang *et al*. 2003; Ang *et al*. 2004; Sahlender *et al*. 2005; Au *et al*. 2007). Interaction of Rab8a with the Sec6/8 exocyst components is thought to control vesicle docking and fusion with the basolateral plasma membrane (Ang *et al*. 2004).

The unique capacity of Rab8a to nucleate cytoskeletal-based protrusions and regulate transport is in keeping with its recently identified role in ciliogenesis (Nachury *et al*. 2007; Yoshimura *et al*. 2007). The primary cilium is present on nearly all mammalian cells and the rod outer segment of photoreceptors is considered a modified cilium. Primary cilia are critical for organogenesis, cell signaling and differentiation, thus placing Rab8 centrally in these processes. Further roles for Rab8a in

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controlling macropinosome formation, nuclear shuttling and regulation of apoptosis, and endocytic recycling and endosomal cholesterol removal have also been demonstrated (De Marco *et al*. 2006; Hattula *et al*. 2006; Roland *et al*. 2007; Linder *et al*. 2007).

A second Rab8 isoform with distinct tissue distribution, but with functional commonalities in its regulation of cytoskeletal rearrangements and neurite outgrowth was identified and termed Rab8b (Armstrong *et al*. 1996). Because Rab8a activity is crucial for controlling exocytic and endocytic transport, cytoskeletal rearrangements and cell morphology, it is not surprising that in the absence of proper regulation, a number of human diseases result, such as open angle glaucoma, human microvillus inclusion disease and Bardet Biedl Syndrome (Rezaie *et al*. 2002; De Marco *et al*. 2006; Sato *et al*. 2007; Nachury *et al*. 2007).

REGULATION OF ACTIVITY

Rab8a, like other Rab family members, is regulated through a cycle of nucleotide binding and hydrolysis, as well as membrane binding and release (Schwartz *et al*. 2007). Membrane binding is partly regulated by post-translational modification at the C terminus, via an isoprenoid moiety. Rab8a has a CaaX motif that is modified by geranylgeranyl transferase (Joberty *et al*. 1993; Wilson *et al*. 1998). After prenylation, the tripeptide–AAX motif is cleaved and the terminal cysteine is methylated (Leung *et al*. 2007).

Nucleotide binding is regulated by specific guanine nucleotide GEFs. MSS4 (accession number CAA49904) was the first nucleotide exchange factor shown to act on Rab8 as well as several other Rab GTPases (Burton *et al*. 1994). Subsequently, a Rab8a specific nucleotide exchange factor, human Rabin8 (equivalent to rat Rabin3; accession number AAA67890.1), was identified via a yeast two-hybrid assay and shown to act only on Rab8 and not on Rab3 (Hattula *et al*. 2002). Rabin8 expression resulted in cytoskeletal remodelling and enhanced Golgi to plasma membrane transport, which is analogous to activated Rab8a, further demonstrating the importance of Rabin8 in Rab8a activation. The growing number of human diseases resulting from a defect in this regulation also supports the pivotal role of Rabin8 in the regulation of Rab8a activity (discussed further under Rab8a function).

Nucleotide hydrolysis and Rab inactivation is modulated by GAPs. AS160 (also called TBC1 domain family, member 4 (Tbc1d4); accession number NP_001074747.2) was shown to function as a Rab8a and Rab14 GAP in L6 muscle cells (Ishikura *et al*. 2007). In addition, the Rab8a specific GAP Tbc1d30 (also called XM_037557; accession number BAA76828.1) was identified by screening for GAP activities that were inhibitory in primary cilia formation (Yoshimura *et al*. 2007), mirroring data showing Rab8a activity was required in the process (Nachury *et al*. 2007). Although several regulators of Rab8a activation status are now known, it remains of interest if Rab8a activity is locally regulated, for example, at the lateral membrane or at cilia by distinct sets of regulators.

Rab proteins may be regulated by serine, threonine or tyrosine phosphorylation. In platelets stimulated with thrombin, phosphorylation of Rab8 is reported to enhance alpha granule secretion (Karniguian *et al*. 1993), though the site of phosphorylation and the kinase responsible have not been identified.

INTERACTIONS WITH LIGANDS AND OTHER PROTEINS

Rab GTPases are in part spatially and temporally regulated by nucleotide (ligand) binding and hydrolysis, which is, in turn, controlled by multiple accessory proteins (see also Rab8a Regulation of Activity section). In the regulation of ciliogenesis, the Rab8a GEF, rabin8, is anchored to a large protein complex called the BBSome at the basal body. Here the BBSome specifically recruits and activates GTP binding by Rab8a and promotes extension of the ciliary membrane (Nachury *et al*. 2007). Ciliogenesis is further controlled by the Rab8a effector cenexin 3/ODF2 (accession number NP_038643.1) and a Rab8a specific GAP, XM_037557, that promotes hydrolysis of bound nucleotide to GDP (Yoshimura *et al*. 2007).

The sequential interaction with multiple effector (proteins binding active Rab8a) and interacting proteins provides further spatial and temporal control over Rab8a regulated membrane transport. The stress-activated serine/threonine protein kinase, the germinal center (GC) kinase, was the first Rab8 interacting protein to be identified, but has not received much follow-up (Ren *et al*. 1996). Most interesting in the context of Rab8a function are the connections to both actin and microtubule networks. FIP-2 (14.7 kD interacting protein) was originally identified as a tumor necrosis factor (TNF)-α-inducible coiled-coil protein that interacts with adenovirus protein E3 14.7 kD protein (Li *et al*. 1998; Hattular and Peranen 2000.) FIP-2 (also known as optineurin) (accession number Q96CV9) is a Rab8a effector that links membranes bound to Rab8a to the actin cytoskeleton through myosin VI, a minus-end directed motor (Hattula and Peränen 2000; Sahlender *et al*. 2005). Optineurin interaction with huntingtin (Hdh, Htt or HIP1) links the complex to microtubules via HAP1 (a trio-like protein with a Rac1 GEF domain), dynactin (p150glued) and dynein (Wanker *et al*. 1997; Engelender *et al*. 1997; Sahlender *et al*. 2005). Upon oxidative stress, optineurin dissociates from Rab8a and translocates into the nucleus to prevent apoptosis (De Marco *et al*. 2006). An E50K optineurin mutant associated with glaucoma fails to translocate into the nucleus.

Several synaptotagmin-like proteins, including Slp1/JFC1 (accession number BAB32651), Slp4/granuphilin (accession number AAH26819.1), Noc2 (accession number BAD07030.1), rabphilin (accession number BAD07029.1) and Rim2 (accession number BAD32694.1) have also been shown to interact with Rab8a (Fukuda 2003; Hattula *et al*. 2006) and are of interest because these proteins are likely to function in linking Rab8 to plus end directed myosin motors.

Noc2 preferentially binds Rab27A/B *in vivo*, but also interacts strongly with Rab8a and all isoforms of Rab3A/B/C/D. Noc2 does not interact with Rab8b. Rabphilin, by comparison, interacts almost equally with Rab27A/B and Rab8a. Both Noc2 and rabphilin bind selectively to the dominant active Rab8A, as compared to the dominant inactive Rab8A protein, suggesting that they may behave as Rab8A effector proteins. Tissue and cellular immunolocalization

studies show Noc2 to be highly expressed in amine and peptide secreting endocrine cells where it is associated with secretory granules (Teramae *et al*. 2007). A Noc2 null mouse accumulates secretory granules in exocrine and endocrine cells (Matsumoto *et al*. 2004). At present, it is difficult to judge the extent of Rab8a and Noc2 expression in similar cell types, though it is noteworthy that Noc2 was cloned from a mouse adult brain library and both proteins are expressed in the small intestine (Fukuda 2003; Matsumoto *et al*. 2004; Sato *et al*. 2007). Rab8a has been shown to be most highly expressed in lung, liver, skeletal muscle, kidney and testis, but specific expression and function in endocrine and exocrine cells has not been analyzed (Armstrong *et al*. 1996). Conversely, the expression of Noc2 in tissues with highest Rab8a expression or in cells with known requirements for Rab8a function has not been studied.

Rabphilin was originally identified as a Rab3 specific effector protein (Shirataki *et al*. 1993). However, studies in rabphilin knock-out mice and *C. elegans* did not reveal a requirement for Rab3A and rabphilin interaction in neurotransmitter release, though rabphilin did interact with syntaxin, SNAP25 and SNARE proteins to regulate neurotransmitter release (Schlüter *et al*. 1999; Staunton *et al*. 2001). Careful reassessment by Fukuda in 2003 using co-immunoprecipitation assays showed that rabphilin binds strongly to Rab27A and Rab8A, modestly to Rab3A/B/C/D, marginally to Rab15 and failed to bind any of the other 38 recombinant Rab proteins tested, including Rab8B. The Rab switch I region is crucial for rabphilin binding, although two α-helical Slp homology domains in rabphilin function in Rab binding (SHD1/RBD1 and SHD2/RBD2). Both Noc2 and rabphilin bind selectively to the dominant active Rab8A, as compared to the dominant inactive Rab8A protein, suggesting that they may behave as Rab8A effector proteins. Functional and localization studies on rabphilin suggest that it is required during regulated secretion and expression in neuronal, neuroendocrine, intestinal goblet cells and kidney podocytes (Shirataki *et al*. 1993; Stahl *et al*. 1996; Rastaldi *et al*. 2003; Valentijn *et al*. 2007). At the subcellular level, rabphilin is associated with the cis-Golgi and the actin cytoskeleton (Baldini *et al*. 2005; Valentijn *et al*. 2007). Because most studies, including those cited here, have examined rabphilin expression only in the context of co-expression and complex formation with Rab3A, the *in vivo* function of a rabphilin/Rab8A complex remains enigmatic. Thus, analyses of the *in vivo* functions of rabphilin are of significant interest for further study. The kidney, particularly the podocytes, might represent a good starting point, as both Rab8 and rabphilin are known to be expressed in kidney podocytes (Simons *et al*. 1999).

Rim2 interacts with Rab8a and Rab3A/B/C/D (Fukuda 2003). Multiple isoforms of the Rim1 and Rim2 proteins appear to arise by alternate RNA splicing (Fukuda 2003; Fukuda 2005). Rim2 has a Rab binding domain 1 (RBD1) highly homologous to Rim1 lacking amino acids 56–105. A conserved acidic cluster (KKEEK) in Rim2 and Rim1Δ56-105 is critical for distinguishing Rab3A and Rab27A binding. However, Rim2 and Rim1Δ56-105 exhibit all binding activities for Rab8A and none for Rab26, respectively, suggesting that additional unidentified sequences in Rim2 are required for Rab8A recognition. As is the case for Rabphilin, the Rab switch I domain is critical for Rim2 to recognize Rab. Rim2 function has been primarily studied in regulated secretion in endocrine and exocrine cell types (Shibasaki *et al*. 2004; Fukuda 2005). Further study, therefore, is required to elucidate whether or not Rab8a and Rim2 are co-expressed in similar cell types and have any functional interdependence.

Rab8a-GTP interacts directly with the synaptotagmin-like protein 1/exophilin 7, designated Slp1 or Sytl1 (mouse) or JFC1 (human), to promote membrane protrusions (Hattula *et al*. 2006). There is also a functional interaction between Rab8a and Arf6 in the formation of protrusions (Hattula *et al*. 2006). Slp1 interacts with Akt, Rab27a and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and is proposed to serve as an adapter to regulate vesicle secretion or NADPH oxidase activity in response to stimulus and protein kinase β (AKT) or phosphatidylinositol-3-OH kinase activation (McAdara Berkowitz *et al*. 2001; Johnson *et al*. 2005).

Synaptotagmin-like protein 4 a (Slp4a)/granuphilin-a is present on insulin containing vesicles of pancreatic β cells, as well as on dense core vesicles of neuroendocrine cells (Fukuda *et al*. 2002). On dense core vesicles, Slp4a specifically forms complexes with Rab27A and a minor fraction of Rab8A; the major fraction of Rab8A being present in a Slp4a-free state in the cell body. The Slp4a/Rab27A complex serves to negatively regulate neuropeptide secretion, due to the specific interaction of Slp4a with Rab27A in its GDP bound form (Fukuda 2003). The Slp4a/Rab8a complex had no apparent function in regulated secretion (Fukuda *et al*. 2002); thus, the function of Slp4a as a Rab8a negative regulator or effector remains in question. Rabphilin, Noc2 and Rim2 are more likely candidates for being Rab8A effectors (Fukuda 2003). Careful analyses of the localization of various Slp protein family members with respect to Rab8a, as well as their *in vivo* functions as Rab8A effectors will be required.

On the endocytic recycling circuit, Rab8a is involved in non-clathrin mediated endocytosis, where interactions with either myosin Vb or Vc may regulate distinct routes (Roland *et al*. 2007).

These interactions are presented in tabular format in Table 1 and in graphical format in Figure 1.

REGULATION OF CONCENTRATION

There is limited information on the regulation of Rab8a protein concentration. The levels of Rab8 are reported to decrease in Alzheimer's disease or upon expression of mutant presenillin (Shimohama *et al*. 1999; Kametani *et al*. 2004). In the small intestine, Rab8a was most highly expressed in 2-week-old mice, but its expression decreased afterwards (Sato *et al*. 2007). Rab8a levels were also decreased in human microvillus inclusion disease. Conversely, levels of Rab8a and several other exocytic Rab GTPases are increased during egg development (Bruscalupi *et al*. 1998). The mechanisms regulating increases or decreases in Rab8a protein levels remain undefined.

SUBCELLULAR LOCALIZATION

Rab8a has multiple subcellular localizations depending on its activation status and cellular function. Like most Rab GTPases, inactive protein is found in the cytoplasm in a complex with GDP dissociation inhibitor

Table 1 | Functional States of Rab8a

Description	Components	Location
Rab8a	Rab8a	Unknown
Rab8a-GDP	Rab8a GDP	cytoplasm
Rab8a-GDP/Rep1	Rab8a GDP, Chm	cytoplasm
Rab8a-GDP-GG/Rep1	Rab8a GG GDP, Chm	cytoplasm
Rab8a-GDP-GG/Rep1	Rab8a GG GDP, Chm	endoplasmic reticulum
Rab8a-GDP-GG (ER)	Rab8a GG GDP	endoplasmic reticulum
Rab8a-GDP-GG-PR	Rab8a GG PR GDP	endoplasmic reticulum
Rab8a-GDP-GG-PR-Me	Rab8a GG ME PR GDP	endoplasmic reticulum
Rab8a-GDP (mem)	Rab8a GG ME PR GDP	membrane
Rab8a-GDP/Rabin3	Rab8a GG ME PR GDP, Rab3ip	cilium membrane
Rab8a-GTP/Rabin3	Rab8a GG ME PR GTP, Rab3ip	cilium membrane
Rab8a-GDP/Rabin3/Actin	Rab8a GG ME PR GDP, Rab3ip	cortical actin cytoskeleton
Rab8a-GDP/Rabin3/BBsome	Rab8a GG ME PR GDP, Rab3ip, Bbs1, Bbs9, Bbs4, Ttc8, Bbs7, Bbs2, Bbs5, Pcm1	basal body
Rab8a-GTP/Rabin3/BBsome	Rab8a GG ME PR GTP, Rab3ip, Bbs1, Bbs9, Bbs4, Ttc8, Bbs7, Bbs2, Bbs5, Pcm1	basal body
Rab8a-GDP/Mss4	Rab8a GG ME PR GDP, Rabif	membrane
Rab8a-GTP/Mss4	Rab8a GG ME PR GTP, Rabif	membrane
Rab8a-GTP	Rab8a GG ME PR GTP	membrane
Rab8a-GTP (Golgi)	Rab8a GG ME PR GTP	Golgi apparatus
Rab8a-GTP (TGN)	Rab8a GG ME PR GTP	trans-Golgi network membrane
Rab8a-GTP (mel)	Rab8a GG ME PR GTP	melanosome membrane
Rab8a-GTP/Optineurin	Actb, Hap1, Myo6, Dynll1, Rab8a GG ME PR GTP, Htt, Dctn1, Optn	trans-Golgi network membrane
Rab8a-GTP/Optineurin	Ap1b1, Ap1g1, Ap1m2, Ap1s1, Myo6, Rab8a GG ME PR GTP, Optn	endosome
Rab8a-GTP (endo)	Rab8a GG ME PR GTP	endosome
Rab8a-GTP/Myo5b	Rab8a GG ME PR GTP, Myo5b, Ehd1, Ehd3	endosome
Rab8a-GTP (sec ves)	Rab8a GG ME PR GTP	secretory vesicle membrane
Rab8a-GTP/Noc2	Rab8a GG ME PR GTP, Rph3al	secretory vesicle membrane
Rab8a-GTP/Rabphilin	Rab8a GG ME PR GTP, Rph3a	secretory vesicle membrane
Rab8a-GTP/Rim2	Rab8a GG ME PR GTP, Rims2	secretory vesicle membrane
Rab8a-GTP (baso PM)	Rab8a GG ME PR GTP	basolateral plasma membrane
Rab8a-GTP (ciliary mem)	Rab8a GG ME PR GTP	cilium membrane
Rab8a-GTP/Tbc1d30	Rab8a GG ME PR GTP, TBC1D30	cilium membrane
Rab8a-GDP/Tbc1d30	Rab8a GG ME PR GDP, TBC1D30	cilium membrane
Rab8a-GTP-P	Rab8a GG ME P PR GTP	platelet alpha granule
Rab8a-GTP/Tbc1d4	Rab8a GG ME PR GTP, Tbc1d4	membrane
Rab8a-GDP/Tbc1d4	Rab8a GG ME PR GDP, Tbc1d4	membrane
Rab8a-GTP/ODF2	Rab8a GG ME PR GTP, Odf2	membrane
Rab8a-GTP/Gck	Map4k2, Rab8a GG ME PR GTP	membrane
Rab8a-GTP/Slp1	Rab8a GG ME PR GTP, Sytl1	membrane
Rab8a-GTP/Slp4a	Rab8a GG ME PR GTP, Sytl4	membrane
Rab8a-GDP/GDI1	Rab8a GG ME PR GDP, Gdi1	cytoplasm
Rab8a-GTP/Myo5c	Myo5c, Rab8a GG ME PR GTP	membrane

GG, geranylgeranyl; ME, methyl; PR, proteolysis; GTP, Guanosine-5'-triphosphate; GDP, Guanosine-5'-diphosphate

(GDI; accession number P21856). Isoprenylation by protein geranylgeranyltransferase enzymes facilitates membrane association.

Prenylation by GGTaseI represents the minor pathway of Rab8a geranylgeranylation (Wilson *et al*. 1998). The nucleotide status of Rab8a that permits its interaction with GGTaseI has not been rigorously tested, but is suggested to be rab8a-GDP, based on the requirements for rab protein binding to the related proteins, REP1 and Rab GDI.

The majority of Rab8a (60–70%) is geranylgeranylated by the REP1/GGTaseII system *in vivo* (Wilson *et al*. 1998). Rab proteins form a ternary complex with REP1/GGTaseII via two possible routes (Goody *et al*. 2005). The first such route involves the rab protein first binding to REP1; this binary complex then associates with GGTaseII bound to geranylgeranyl pyrophosphate. REP1 also exhibits significant affinity for GGTaseII bound to geranylgeranyl pyrophosphate, so a second possible pathway could involve the REP1/GGTaseII-GGpp complex subsequently recruiting the rab protein. After geranylgeranylation, the GGTase dissociates and REP1 delivers the modified protein to the membrane via a classical pathway (Alexandrov *et al*. 1994). GGTaseII does not have any detectable affinity for rab proteins in the absence of REP1, suggesting that a binary complex between GGTaseII and rab does not exist (Pereira-Leal *et al*. 2003; Goody *et al*. 2005). Mutation of the α-subunit of GGTaseII results in pigmentation and immune defects in mice (Detter *et al*. 2000) and may serve as a regulatory subunit (Zhang *et al*. 2000). The beta subunit of GGTaseII binds to geranylgeranyl pyrophosphate and serves as the catalytic subunit (Zhang *et al*. 2000). Following prenylation, Rab8a is initially delivered to the endoplasmic reticulum membrane, where it undergoes proteolytic processing and carboxymethylation before being rerouted to its site of function (Leung *et al*. 2007).

Rab8a is found to be associated with the actin cytoskeleton, BBSomes, the basolateral or dendritic plasma membrane, the trans-Golgi network, recycling endosomes, melanosomes and the primary cilium membrane (Huber *et al*. 1993a; Huber *et al*. 1993b; Deretic *et al*. 1995; Peränen *et al*. 1996; Gomez *et al*. 2001; Chakraborty 2003; Ang *et al*. 2004; Chabrillat *et al*. 2005; Hattula *et al*. 2006; Nachury *et al*. 2007; Roland *et al*. 2007; Sato *et al*. 2007; Yoshimura *et al*.

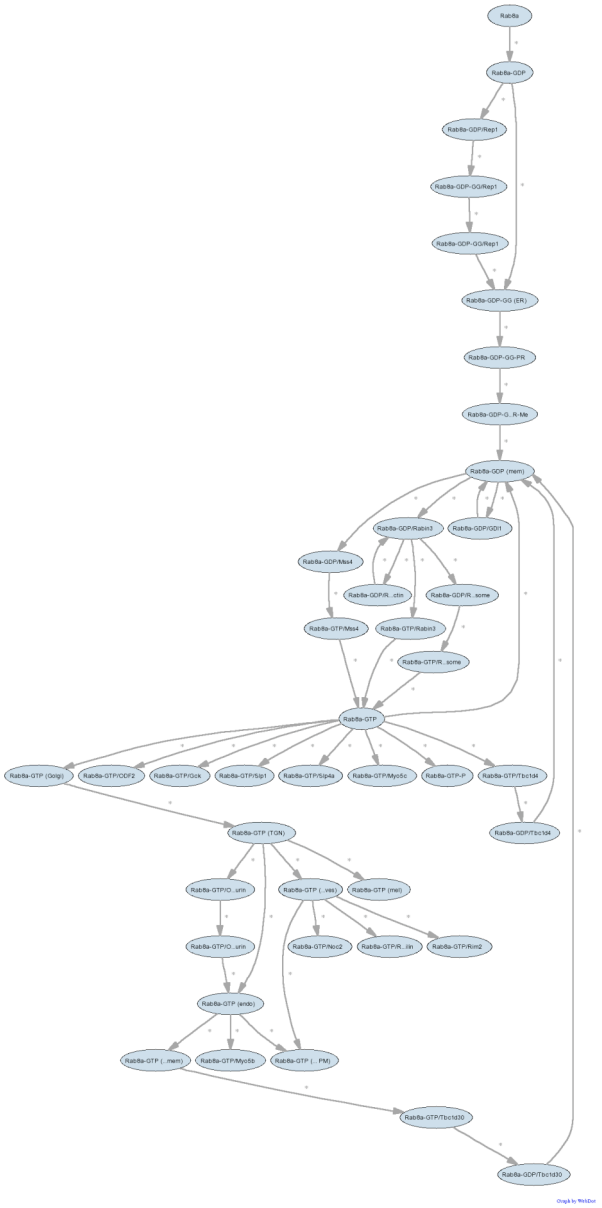

Figure 1 | Functional states of Rab8a (blue ovals). Arrows depict transitions between the different functional states.

2007). Mislocalization of Rab8a has been reported in autosomal dominant polycystic kidney disease (Charron *et al*. 2000).

MAJOR SITES OF EXPRESSION

Rab8a is encoded on human chromosome 19. At the messenger RNA (mRNA) level Rab8a is significantly expressed in lung, liver, skeletal muscle, kidney and testis, but only negligibly expressed in heart, brain and spleen (Elferink *et al*. 1992; Armstrong *et al*. 1996). mRNA expression corresponds in a large part to the reported protein expression, which is highly expressed in brain, small intestine, kidney, spleen and thymus, moderately expressed in heart, lung, liver, and pancreas, and scarcely expressed in skeletal muscles (Sato *et al*. 2007). At the cellular level, Rab8a protein is expressed in numerous cell types, including kidney epithelia and podocytes (Chavrier *et al*. 1990; Charron *et al*. 2000; Kobayashi 2002), photoreceptor cells (Deretic *et al*. 1995), neurons and brain (Elferink *et al*. 1992; Ghorbel *et al*. 2005), adipocytes (Cormont *et al*. 1993), platelets (Karniguian *et al*. 1993) and melanocytes (Chakraborty *et al*. 2003). The tissue expression of Rab8a is in keeping with its specific functions in polarized membrane trafficking and process formation in neurons and cilia formation in the epithelia.

PHENOTYPES

No naturally occurring mutations or deletions of Rab8a or phenotypes have been reported in humans, mice, or other organisms, although reduced expression and impaired activation may occur in neurological disease, intestinal and kidney disease (Kametani *et al*. 2004; Sato *et al*. 2007; Nachury *et al*. 2007). Overexpression of mutant presenilin (A260V) in PC12 cells reduced Rab8 expression (Kametani *et al*. 2004), however, in a transgenic mouse model of familial Parkinson's disease (Tg5093 mice), Rab8a exhibits enhanced binding to the mutant human A30P alpha-synuclein (Dalfó *et al*. 2004).

Loss of Barbet-Biedl Syndrome protein 1 prevented Rab8a activation and is an underlying cause of a pleiotropic developmental disorder that causes polydactyly, obesity, cystic kidneys, retinopathy, intestinal fibrosis among other deficits (Nachury *et al*. 2007). In this regard it is interesting to consider the phenotypes of transgenic and null animals. A conditional knockout mouse lacking Rab8a expression in the intestine resulted in the redirection of apical peptidases and transporters to lysosomes and a significant impairment in nutrient absorption that led to premature death showing the importance of Rab8a in the intestine (Sato *et al*. 2007). A transgenic *X. laevis* that expressed the dominant negative Rab8T22N mutant resulted in rapid retinal degeneration, demonstrating the critical function of Rab8a in retinal photoreceptor cells (Moritz *et al*. 2001). The combined data show that loss of Rab8a activation or loss of Rab8a may be functionally equivalent.

SPLICE VARIANTS

There are no known splice variants for Rab8a. The related Rab8b homologue is encoded by a separate gene on human chromosome 15 (Armstrong *et al*. 1996).

ANTIBODIES

Monoclonal anti-Rab8 is available from BD-Biosciences (610844) and, in the authors' experience, works extremely well for immunofluorescence and immunoblotting methods. This mouse IgG2b was generated using amino acids 84–205 of human Rab8. There is significant sequence similarity between Rab8a and Rab8b in this region, with the greatest divergence occurring between amino acids 166–200 in Rab8b. There are Rab8a (anti-MEL) specific antibodies commercially available; however, the authors do not have any experience with these reagents to report.

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