

SUPPLEMENTARY MATERIAL

Methods

Plasmids - *Caenorhabditis elegans* small G protein constructs were either PCR cloned from a *C. elegans* cDNA library (*de novo*) or retrieved from the *C. elegans* ORFeome collection version 1.1 {Reboul, 2003 #12} using Gateway® cloning technology (Invitrogen) as described previously {Caruso, 2005 #10}. Following manufacturer's instructions, amplified ORFs were recombined using BP clonase into Gateway pDONR201 vectors (Invitrogen). This provided entry clones that were subsequently used to transfer the target gene in three different Gateway compatible expression vectors using LR clonase i) pGEX-2TK, a bacterial expression vector to produce N-terminally tagged GST fusion proteins required for the AlphaScreen GTP affinity; ii) pL4440, to perform RNAi by feeding; and iii) pECFP, a mammalian expression vector producing fluorescent proteins suitable for analysis of the sub-cellular localization of the recombinant protein.

Alphascreen – Recombinant GST-GTPases were expressed in bacteria and purified using glutathione Sepharose 4B as described previously {Caruso, 2005 #10}. During the purification step, GST-GTPases were unloaded from their intrinsic nucleotide by adding 5 mM EDTA in lysate before purification. AlphaScreen competition assays were then performed as described previously {Caruso, 2005 #10}. Briefly, nucleotide-free GST-GTPases were incubated with 30 nM biotin-GTP γ S and increasing concentrations of unlabeled GTP (ranging from 0.4 nM to 3 μ M) for 30 minutes in a 384-well plate. Streptavidin Donor beads and GSH Acceptor beads (PerkinElmer) were added simultaneously at a final concentration of 0.02 mg/ml per well and incubated for 1 h at 23°C before reading. Laser excitations were carried out at 680 nm, and readings were performed at 520–620 nm using an Envision plate reader (PerkinElmer). The IC₅₀ value corresponds to the competitor concentration required to reduce the maximal signal (obtained in the absence of competitor) by 50%. The IC₅₀ values for the competition assay represent the average of at least three independent experiments each performed in triplicate. *ND*, not determined.

Sub-cellular localization and siRNA treatments in mammalian cells – Cellular localization was assessed by transfecting ECFP-tagged small G protein constructs

into Vero cells (ATCC CCL81). Transfections and live cell imaging were carried out as described previously {Simpson, 2000 #13}. Downregulation of G proteins was carried out in reverse transfection format. Briefly, siRNAs (Ambion) were mixed with Lipofectamine2000 transfection reagent (Invitrogen) and printed on to chambered glass coverslips. Vero cells were added to these siRNA arrays and gene down-regulation allowed to proceed for 32 hours. Following this, cells were infected with recombinant adenoviruses expressing ECFP-tsO45G for 16 hours at 39.5°C in order to accumulate the tsO45G cargo in the ER. A pulse of tsO45G was then released through the secretory pathway at 32°C for 1 hour, and the cells were fixed and immunostained for cell surface tsO45G. Secretory activity of the cells was calculated from the ratio of cell surface tsO45G to total tsO45G as described previously {Simpson, 2007 #14}. Two independent siRNAs were used to target each small G protein.

Tissue localization - Expression profiles were analyzed using GFP-reporter transgenic worms {Hunt-Newbury, 2007 #15}. Living worms were mounted on agarose pads in M9 buffer containing 1mM Levamisol (Sigma). Fluorescence emission from living worms was observed using an Axiovert-200 microscope (Zeiss) and images analyzed using Northern Eclipse V6.0 (Empix Imaging, Mississauga, Ontario, Canada).

RNAi in C. elegans - RNAi experiments were carried out using a feeding procedure as described previously {Caruso, 2008 #11}. pL4440 vector containing GTPases gene were transformed into HT115(DE3) bacteria. Isolated bacterial clones were grown for 12h at 37°C in Luria-Bertani (LB) medium supplemented by 100 μ g/ml ampicillin. Bacteria were spotted on NGM plates containing 1mM IPTG and 100 μ g/ml ampicillin, dried and induced overnight at room temperature. Synchronized L1 worm populations were transferred to these plates and treated for three days. L4 worms were then analyzed under optical microscope for phenotypic change compare to untreated control worms.

Messenger RNA Co-expression - mRNA co-expression data were retrieved for Wormbase and corresponded to data generated previously {Kim, 2001 #20}.

Hierarchical clustering and Network analyses – To integrate data, a matrix combination approach was undertaken (||A-B||). Matrices were computed from the alignment using PHYLIP ProtDist according to the Jones-Taylor-Thornton model. The hierarchical clustering tree was computed using the PHYLIP Neighbor command based on the UPGMA method and visualized using TreeView. Biochemical values were used to generate distance matrices based on biochemical activity(ies) similarities (alone or in combination). These matrices were calculated based on Euclidian distances, and hierarchical clustering trees were then computed using the PHYLIP Neighbor command based on the UPGMA method and visualized using TreeView. Tree comparison was carried out using PHYLIP Treedist. The amino acid sequences corresponding to these genes were retrieved for Wormbase database (<http://www.wormbase.org/>). Sequence-based hierarchical clustering was performed using ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). All the clustering data analyses were carried out using the PermutMatrix software (<http://www.lirmm.fr/~caraux/PermutMatrix/>) and network analyses were performed using Cytoscape (<http://www.cytoscape.org/>).

Table S1: *C. elegans* small GTPases and their human closest counterpart. Common and WormBase sequence names of the 59 small G proteins identified in *C. elegans* genome with the cloning strategy used and the expression vectors in which the corresponding gene was successfully cloned as well as their function in *C. elegans* when elucidated.

Sub-family	Name	WormBase sequence name	Closest human homolog	Cloning strategy	pGEX-2TK	pECFP	pL4440	Function in <i>C.elegans</i>
Rho (7)	Crp-1	Y32F6B.3		<i>De novo</i>	X	X	X	Regulation of the UPR, membrane trafficking
	Rac-2	K03D3.10	Rac2	ORFeome	X	X	X	CAN and PDE axon pathfinding, cell migration
	Cdc-42	R07G3.1	Cdc42	ORFeome	X	X	X	Control embryonic polarity
	Rho-1	Y51H4A.3	RhoA	ORFeome	X	X	X	Emb polarity, cell migration, cell shape, muscle contraction
	Chw-1	F22E12.2	RhoU	<i>De novo</i>				
	Ced-10	C09G12.8	Rac1	ORFeome	X	X	X	Phagocytosis in apoptosis, migration of distal tip cells
Mig-2	C35C5.4	RhoG	ORFeome	X	X	X	Cell migration, axon outgrowth and guidance	
Ran (1)	Ran	K01G5.4	Ran1	ORFeome	X	X	X	Spindle formation, centrosome connection, nuclear envelope assembly
Ran (29)	rab-1	C39F7.4	Rab1	ORFeome	X	X	X	Intracellular vesicle trafficking, fertility, movement, and survival
	unc-108	F53F10.4	Rab2	ORFeome	X	X	X	Removal of apoptotic cells, neurons locomotion
	rab-3	C18A3.6	rab3	ORFeome	X	X	X	Synaptic transmission, exocytosis
	rab-5	F26H9.6	Rab5	<i>De novo</i>	X	X	X	Membrane fusion, ER morphology, fat storage
	rab-6.1	F59B2.7	Rab6	<i>De novo</i>	X	X	X	Embryonic development and reproduction
	rab-6.2	T25G12.4	rab6	<i>De novo</i>	X	X	X	
	rab-7	W03C9.3	Rab7	<i>De novo</i>		X	X	Vesicular trafficking, regulation of fat storage
	rab-8	D1037.4	Rab8	<i>De novo</i>	X	X	X	Affect ovarian morphology and function
	rab-10	T23H2.5	Rab10	ORFeome	X	X	X	Basolateral endocytic recycling in the intestine
	rab-11.1	F53G12.1	Rab11	ORFeome	X	X	X	Cytokinesis during early embryogenesis, uptake of yolk proteins
	Rab-11.2	W04G5.2		ORFeome	X	X	X	
	rab-14	K09A9.2	Rab14	ORFeome	X	X	X	
	rab-18	Y92C3B.3	Rab18	<i>De novo</i>	X	X		
	rab-19	Y62E10A.9	Rab19	ORFeome	X	X	X	
	rab-21	T01B7.3	Rab21	ORFeome	X	X	X	
	aex-6	Y87G2A.4	Rab27	ORFeome	X	X	X	Insulin branch or the dauer pathway
	rab-28	Y11D7A.4	Rab28	ORFeome	X	X	X	
	rab-30	Y45F3A.2	Rab30	ORFeome	X	X	X	
	rab-33	F43D9.2	Rab33B	ORFeome		X	X	
	rab-35	Y47D3A.25	Rab35	ORFeome	X	X	X	
	rab-37	W01H2.3	Rab37	<i>De novo</i>	X	X	X	
	rab-39	D2013.1	Rab39	ORFeome	X	X	X	
	tag-312	C33D12.6	RASEF1	<i>De novo</i>		X		
		K02E10.1		<i>De novo</i>				
		4R79.2	EFCAB4B	<i>De novo</i>				
		C56E6.2	Rab6A	<i>De novo</i>				
		F11A5.3	Rab2	ORFeome	X	X	X	
		F11A5.4	Rab2A	ORFeome	X	X	X	
	Ras (9)	ras-1	C44C11.1	R-Ras2	<i>De novo</i>	X	X	X
ras-2		F17C8.4	M-ras	<i>De novo</i>	X	X	X	
let-60		ZK792.6	K-ras	ORFeome	X	X	X	
rap-1		C27B7.8	Rap1b	ORFeome	X	X	X	Epithelial integrity
rap-2		C25D7.7	Rap2a	ORFeome	X	X	X	Epithelial integrity
rap-3		C08F8.7	Rap1c	<i>De novo</i>				
Rheb-1		F54C8.5	RheB	<i>De novo</i>	X	X	X	
dnm-1		C54A12.4	Di-ras2	ORFeome				
ral-1		Y53G8AR.3	RalA	ORFeome	X	X	X	
Arf/Arf/Sar (14)	arf-1.1	F45E4.1	Arf1	ORFeome	X	X	X	
	arf-1.2	B0336.2.1	Arf1.2	<i>De novo</i>	X	X	X	Regulate membrane traffic and the actin cytoskeleton
	arf-3	F57H12.1	Arf5	<i>De novo</i>	X	X	X	
	arf-6	Y116A8C.12	Arf6	ORFeome	X	X	X	Regulate receptor-mediated endocytosis
	arl-1	F54C9.10	Arf1	ORFeome	X	X	X	
	evl-20	F22B5.1	Arl2	ORFeome	X	X		Cortical microtubule formation
	arl-3	F19H8.3	Arl3	ORFeome	X	X	X	
	arl-5	ZK632.8	Arl5	ORFeome	X	X	X	
	arl-6	C38D4.8	Arl6	ORFeome	X	X	X	
	arl-8	Y57G11C.13	Arl8b	<i>De novo</i>		X	X	
	sar-1	ZK180.4	Sar1	<i>De novo</i>		X	X	
	arc-1	ZK1320.6	Mid1	<i>De novo</i>				
		Y54E10BR.2		<i>De novo</i>		X	X	
		Y37E3.5		<i>De novo</i>		X	X	

Table S2: *C. elegans* small G protein cellular localization. Cellular localizations were determined using ECFP tagged GTPases transfected into Vero cells and visualized using fluorescence microscopy. Empty slots correspond to localizations that were not determined.

<i>C. elegans</i> gene name	Gene designation (WormBase)	Cellular localization									
		Nuclear membrane	ER (minor Golgi)	Intermediate compartment	Golgi	recycling endosome, endo. vesicle	Endocytic vesicles	Microtubules	Plasma membrane	Diffuse cytoplasm	Nucleus
rab-1	C39F7.4	0	0	0	1	0	0	0	0	0	0
rab-2/unc-108	F53F10.4	0	0	0	1	0	0	0	0	0	0
rab-3	C18A3.6	0	0	0	0	0	0	1	1	0	0
rab-5	F26H9.6	0	0	0	0	0	1	0	0	1	1
rab-6.1	F59B2.7	0	1	0	1	0	0	0	0	0	0
rab-6.2	T25G12.4	0	0	0	1	0	0	0	0	1	0
rab7	W03C9.3	0	1	0	0	0	0	0	0	0	0
rab-8	D1037.4	0	1	0	0	0	0	0	1	0	0
rab-10	T23H2.5	0	1	0	1	0	1	1	0	0	0
rab11.1	F53G12.1	0	0	0	0	1	0	0	1	0	0
rab14	K09A9.2	0	0	0	1	0	1	0	0	0	0
rab-18a	Y92C3B.3	0	0	0	0	0	0	0	0	1	1
rab-19	Y62E10A.9	0	1	0	1	0	0	0	0	0	0
rab-21	T01B7.3	0	0	0	0	0	1	0	0	0	0
acx-6	Y87G2A.4	0	1	0	0	0	0	0	0	0	0
rab-28	Y11D7A.4	0	1	0	0	0	0	0	0	0	0
rab-30	Y45F3A.2	0	0	0	1	0	0	0	0	0	0
rab-33	F43D9.2	0	0	0	1	0	0	0	0	0	0
rab-35	Y47D3A.25	0	0	0	0	1	0	0	1	0	0
rab-37	W01H2.3	0	0	0	0	0	1	0	0	0	0
rab-39	D2013.1	0	0	0	0	0	0	0	0	1	1
rabY1/tag312	C33D12.6	0	0	0	0	0	0	0	0	1	1
-	4r79.2										
-	K02E10.1										
-	F11A5.4	0	0	0	0	1	0	0	1	0	0
-	F11A5.3	0	0	0	0	0	0	0	0	0	1
-	C56E6.2										
arl-1	F54C9.10	0	0	0	0	0	0	0	0	1	1
arf-1.2	B0336.2	0	0	0	0	0	0	0	0	1	1
arf-5	ZK632.8	0	0	0	0	0	0	0	0	1	1
arf-3	F57H12.1	0	0	0	0	0	0	0	0	1	1
arf-6	Y116A8C.12	0	0	0	0	0	0	0	0	1	1
arf-1.1	F45E4.1	0	1	0	0	0	0	0	1	0	0
evl-20	F22B5.1	0	0	0	0	0	0	0	0	1	1
ari-8	Y57G11C.13	0	0	0	0	0	0	0	0	1	1
arl-6	C38D4.8	0	0	0	0	0	0	0	0	1	1
arl-3	F19H8.3	0	0	0	0	0	0	0	0	1	1
-	ZK180.4	1	0	0	0	0	0	0	0	1	1
-	Y54E10Br.2	0	0	0	0	0	0	0	0	1	1
-	Y37E3.5	0	0	0	0	1	0	0	1	0	0
arc-1	ZK1320.6										
ran-1	K01G5.4	1	0	0	0	0	0	0	0	0	0
let-60	ZK792.6	0	0	0	0	0	0	0	0	1	1
ras-1	F17C8.4	0	0	0	0	1	0	0	1	0	0
ras-2	C44C11.1	0	0	0	0	0	0	1	1	0	0
Drm-1	C54A12.4										
rap-1	C27B7.8	0	0	0	0	1	0	0	1	0	0
rap-2	C25D7.7	0	0	0	0	1	0	0	1	0	0
rap-3	C08F8.7										
rheb-1	F54C8.5	0	0	0	0	0	0	0	0	1	1
ral-1	Y53G8Ar.3	0	0	0	0	1	0	0	1	0	0
rho-1	Y51H4A.3	0	0	0	0	0	0	0	0	1	1
rac2	K03D3.10	0	0	0	0	0	0	0	0	1	1
cdc42	r07G3.1	0	0	0	0	0	0	0	1	0	0
crp-1	Y32F6B.3	0	0	0	0	1	0	0	1	0	0
mig-2	C35C5.4										
chw-1	F22E12.2										
ced-10	C09G12.8										
rab-11.2	W04G5.2										

