

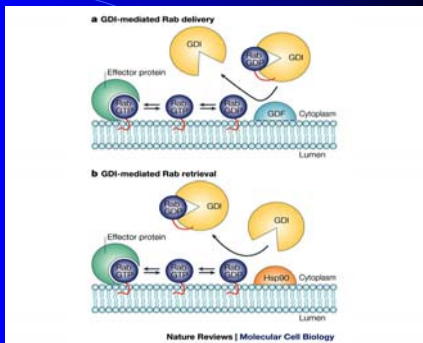
# TARGETING RAB GTPases TO DISTINCT MEMBRANE COMPARTMENTS

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Presented by  
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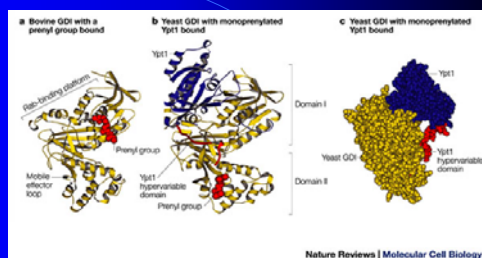
## How are the Rab proteins delivered to distinct membranes?

- Rab proteins associate tightly with membranes by virtue of the double prenylation of their C termini
- Prenylated Rab GTPases are also present in the cytosol, where they are bound to a protein known as GDP-dissociation inhibitor (GDI).
- Complexes of prenylated Rab proteins bound to GDI contain all the information that is needed for accurate membrane delivery.



Rab delivery to and retrieval from intracellular membranes

## How does GDI bind prenylated Rabs?



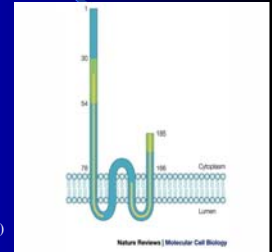
The structures of the bovine and yeast GDP-dissociation inhibitors (GDIs).

- In summary, Ypt1 drapes over the surface of GDI, and is held in place by switch-domain recognition at the top and prenyl-group binding at the bottom.
- This places the targeting, information-rich, hypervariable domain of Ypt1 on the GDI surface for presentation to target membranes during the process of membrane delivery.
- When bound to GDI, the differences in hypervariable-domain length and sequence will create a distinct surface for recognition by membrane components during the delivery process.

- In vitro reconstituted Rab proteins delivery onto membranes show GDP release and GTP binding followed the membrane association of Rab proteins by a significant lag. Both the initial rate and the overall extent of the membrane binding were saturable, which indicates that the process might be enzyme catalysed.
- There might be a 'GDI-DISPLACEMENT FACTOR' (GDF) that catalyses the dissociation of Rab-GDI complexes.

## Protein family YIPS (Ypt-interacting proteins) are strong candidates for potential GDFs.

- The Yip-protein-family members share a common topology — their N and C termini face the cytosol, and the remaining bulk of their sequence is membrane embedded<sup>35</sup>



The topology of human Yip3/prenylated Rab acceptor-1 (PRA1)

## Yip3 is a GDI-displacement factor

- Femtomoles of Yip3/PRA1 could quantitatively dissociate picomoles of pure, stable prenylated-Rab9-GDI complexes.
- anti-Yip3/PRA1 antibodies blocked the recruitment of Rab9 onto endosomes
- the expression of Yip3/PRA1 in cells yielded endosomes with a greater capacity for Rab9 recruitment
- RNA-interference-mediated depletion of Yip3/PRA1 in cells led to a decrease in membrane-associated (but not total) Rab9 protein.

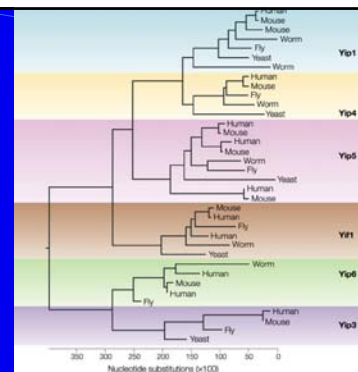
Table 1 Characterized Yip-family members and their interactions

Protein	Location	Essential in <i>S. cerevisiae</i> ?	Size (kDa)	Binding partners	References
Yip1	Golgi and ER-Golgi vesicles	Yes	27.1	Yip1, multiple Yips	33
Yip1	Golgi and ER-Golgi vesicles	Yes	35.5	COP1 (Sec23/Sec24), Bos1 v-SNARE, Sec22	45-47
Yip2 (Yip1)	Golgi, ER	No	20.3	Yip1, Yip7, Yip10, Yip11, Yip12, Yip13, Yip14, Yip15, Yip16, Yip17, Yip18, Yip19, Yip20, Yip21, Yip22, Yip23, Yip24, Yip25, Yip26, Yip27, Yip28, Yip29, Yip30, Yip31, Yip32, Yip33, Yip34, Yip35, Yip36, Yip37, Yip38, Yip39, Yip40, Yip41, Yip42, Yip43, Yip44, Yip45, Yip46, Yip47, Yip48, Yip49, Yip50, Yip51, Yip52, Yip53, Yip54, Yip55, Yip56, Yip57, Yip58, Yip59, Yip60, Yip61, Yip62, Yip63, Yip64, Yip65, Yip66, Yip67, Yip68, Yip69, Yip70, Yip71, Yip72, Yip73, Yip74, Yip75, Yip76, Yip77, Yip78, Yip79, Yip80, Yip81, Yip82, Yip83, Yip84, Yip85, Yip86, Yip87, Yip88, Yip89, Yip90, Yip91, Yip92, Yip93, Yip94, Yip95, Yip96, Yip97, Yip98, Yip99, Yip100	35, 36
Yip3	Unknown	No	19.4	Yip1, multiple Yips	25
Yip4	Unknown	No	26.0	Yip1, Yip5, Yip11, Yip12, Yip13, Yip14, Yip15, Yip16, Yip17, Yip18, Yip19, Yip20, Yip21, Yip22, Yip23, Yip24, Yip25, Yip26, Yip27, Yip28, Yip29, Yip30, Yip31, Yip32, Yip33, Yip34, Yip35, Yip36, Yip37, Yip38, Yip39, Yip40, Yip41, Yip42, Yip43, Yip44, Yip45, Yip46, Yip47, Yip48, Yip49, Yip50, Yip51, Yip52, Yip53, Yip54, Yip55, Yip56, Yip57, Yip58, Yip59, Yip60, Yip61, Yip62, Yip63, Yip64, Yip65, Yip66, Yip67, Yip68, Yip69, Yip70, Yip71, Yip72, Yip73, Yip74, Yip75, Yip76, Yip77, Yip78, Yip79, Yip80, Yip81, Yip82, Yip83, Yip84, Yip85, Yip86, Yip87, Yip88, Yip89, Yip90, Yip91, Yip92, Yip93, Yip94, Yip95, Yip96, Yip97, Yip98, Yip99, Yip100	34
Yip5	Unknown	No	34.8	Yip1, Yip4, Yip7, Yip10, Yip11, Yip12, Yip13, Yip14, Yip15, Yip16, Yip17, Yip18, Yip19, Yip20, Yip21, Yip22, Yip23, Yip24, Yip25, Yip26, Yip27, Yip28, Yip29, Yip30, Yip31, Yip32, Yip33, Yip34, Yip35, Yip36, Yip37, Yip38, Yip39, Yip40, Yip41, Yip42, Yip43, Yip44, Yip45, Yip46, Yip47, Yip48, Yip49, Yip50, Yip51, Yip52, Yip53, Yip54, Yip55, Yip56, Yip57, Yip58, Yip59, Yip60, Yip61, Yip62, Yip63, Yip64, Yip65, Yip66, Yip67, Yip68, Yip69, Yip70, Yip71, Yip72, Yip73, Yip74, Yip75, Yip76, Yip77, Yip78, Yip79, Yip80, Yip81, Yip82, Yip83, Yip84, Yip85, Yip86, Yip87, Yip88, Yip89, Yip90, Yip91, Yip92, Yip93, Yip94, Yip95, Yip96, Yip97, Yip98, Yip99, Yip100	35
Human Yip3 (PRA1)	Golgi and endosome	NA	20.7	Rab5, Rab6	54
				Phenyl/Rab3A, leucine/Rab3A, prenyl/Rab1, VAMP2, syntaxin membrane domain <sup>1</sup> (not Rac1, RhoA, JNM1)	38
				Rab1a, Rab1b, Rab1c, Rab1d, Rab1e, Rab1f, Rab1g, Rab1h, Rab1i, Rab1j, Rab1k, Rab1l, Rab1m, Rab1n, Rab1o, Rab1p, Rab1q, Rab1r, Rab1s, Rab1t, Rab1u, Rab1v, Rab1w, Rab1x, Rab1y, Rab1z, Rab1aa, Rab1ab, Rab1ac, Rab1ad, Rab1ae, Rab1af, Rab1ag, Rab1ah, Rab1ai, Rab1aj, Rab1ak, Rab1al, Rab1am, Rab1an, Rab1ao, Rab1ap, Rab1aq, Rab1ar, Rab1as, Rab1at, Rab1au, Rab1av, Rab1aw, Rab1ax, Rab1ay, Rab1az, Rab1ba, Rab1bb, Rab1bc, Rab1bd, Rab1be, Rab1bf, Rab1bg, Rab1bh, Rab1bi, Rab1bj, Rab1bk, Rab1bl, Rab1bm, Rab1bn, Rab1bo, Rab1bp, Rab1bq, Rab1br, Rab1bs, Rab1bt, Rab1bu, Rab1bv, Rab1bw, Rab1bx, Rab1by, Rab1bz, Rab1ca, Rab1cb, Rab1cc, Rab1cd, Rab1ce, Rab1cf, Rab1cg, Rab1ch, Rab1ci, Rab1cj, Rab1ck, Rab1cl, Rab1cm, Rab1cn, Rab1co, Rab1cp, Rab1cq, Rab1cr, Rab1cs, Rab1ct, Rab1cu, Rab1cv, Rab1cw, Rab1cx, Rab1cy, Rab1cz, Rab1da, Rab1db, Rab1dc, Rab1dd, Rab1de, Rab1df, Rab1dg, Rab1dh, Rab1di, Rab1dj, Rab1dk, Rab1dl, Rab1dm, Rab1dn, Rab1do, Rab1dp, Rab1dq, Rab1dr, Rab1ds, Rab1dt, Rab1du, Rab1dv, Rab1dw, Rab1dx, Rab1dy, Rab1dz, Rab1ea, Rab1eb, Rab1ec, Rab1ed, Rab1ee, Rab1ef, Rab1eg, Rab1eh, Rab1ei, Rab1ej, Rab1ek, Rab1el, Rab1em, Rab1en, Rab1eo, Rab1ep, Rab1eq, Rab1er, Rab1es, Rab1et, Rab1eu, Rab1ev, Rab1ew, Rab1ex, Rab1ey, Rab1ez, Rab1fa, Rab1fb, Rab1fc, Rab1fd, Rab1fe, Rab1ff, Rab1fg, Rab1fh, Rab1fi, Rab1fj, Rab1fk, Rab1fl, Rab1fm, Rab1fn, Rab1fo, Rab1fp, Rab1fq, Rab1fr, Rab1fs, Rab1ft, Rab1fu, Rab1fv, Rab1fw, Rab1fx, Rab1fy, Rab1fz, Rab1ga, Rab1gb, Rab1gc, Rab1gd, Rab1ge, Rab1gf, Rab1gg, Rab1gh, Rab1gi, Rab1gj, Rab1gk, Rab1gl, Rab1gm, Rab1gn, Rab1go, Rab1gp, Rab1gq, Rab1gr, Rab1gs, Rab1gt, Rab1gu, Rab1gv, Rab1gw, Rab1gx, Rab1gy, Rab1gz, Rab1ha, Rab1hb, Rab1hc, Rab1hd, Rab1he, Rab1hf, Rab1hg, Rab1hh, Rab1hi, Rab1hj, Rab1hk, Rab1hl, Rab1hm, Rab1hn, Rab1ho, Rab1hp, Rab1hq, Rab1hr, Rab1hs, Rab1ht, Rab1hu, Rab1hv, Rab1hw, Rab1hx, Rab1hy, Rab1hz, Rab1ia, Rab1ib, Rab1ic, Rab1id, Rab1ie, Rab1if, Rab1ig, Rab1ih, Rab1ii, Rab1ij, Rab1ik, Rab1il, Rab1im, Rab1in, Rab1io, Rab1ip, Rab1iq, Rab1ir, 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<sup>1</sup>These interactions require membrane 30-54 and 175-180 of human Yip3. <sup>2</sup>Human Yip3 might prefer the GTP-bound form of these Rab proteins. Bos1, Sec17, syntaxin, GTP, cytosolic protein, ER, endoplasmic reticulum, GDI, GDI-displacement inhibitor, NA, not applicable, PRA, prenylated Rab acceptor, Sec, secretory protein, v-SNARE, vesicle-associated membrane protein-2, Yip, vesicular protein acting as v-SNARE, vesicle membrane acidic v-SNARE (with phosphatidylethanolamine-binding protein) accessory protein (SNARE) receptor, Yip, Yip-interacting factor, Yip, Yip-interacting protein.

## In yeast, does Yip1 participate in membrane recruitment of Ypt1?

- Some positive results:
- antibodies against the N terminus of Yip1 blocked the formation of coatomer protein-II (COPII)-coated transport vesicles
- the formation of fusion-competent vesicles that are derived from the ER was inhibited by anti-Yip1 antibodies
- Yip1 binds Bos1 and Sec22, the ER/Golgi vesicle-membrane (v)-SNARE proteins
- the human homologue of Yip1 binds to Sec23/24, which are components of ER-derived transport-vesicle coats



The phylogenetic-tree analysis shows strong conservation of each Yip-family member throughout evolution.

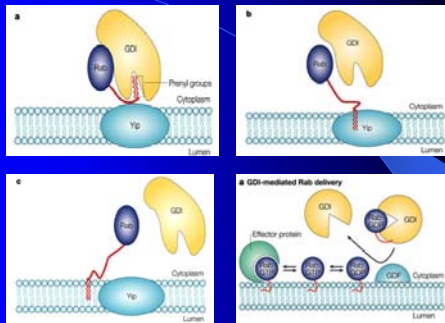
Table 2 | Mammalian Yip proteins

Protein	Human locus*	Location	Ubiquitous expression?	Pattern of expression (species and tissues of highest expression)
Yip1	81555	ER, Golgi	Yes	Mouse ovary, thyroid and salivary gland
Yip2a	65555	ND	Yes	Human brain, testis, parotid, pituitary and kidney
Yip2b	221095	ND	Yes	Mouse small intestine, gall bladder, snout epidermis and trachea (not tested)
Yip2c	50546	ND	Yes	Mouse bone marrow and snout epidermis
Yip2d	51308	ND	ND	Specific for human brain and pituitary
Yip2e	7905	ND	Yes	Human brain, thyroid, parotid, pituitary, adrenals and prostate
Yip2f	60340	ND	ND	Specific for mouse liver, testis, gall bladder, small intestine and eye
Yip3 (PFA1)	10567	Golgi, endosomes	Yes	Human brain, placenta, salivary gland, spleen, kidney, liver and heart
Yip4	265451	ND	Yes	Mouse lung, testes, small intestine, eye and gall bladder
Yip5a	54432	ND	Yes	Human thyroid, prostate, salivary gland, liver, spleen, testis, pituitary and placenta
Yip5b	25044	ND	Yes	Human placenta, spleen, pituitary and brain
Yip5c	78002	ND	ND	Human brain, spleen, placenta and uterus
Yip5a (PFA2)	11220	ER	Yes	Human brain, pituitary, spleen and heart
Yip5b	10550	ND	Yes	Human brain, blood, prostate, ovary, lung, thymus, adrenals and pituitary
Yip1a	10897	ER, Golgi	Yes	Human liver, placenta, spleen, pituitary, kidney and heart
Yip1b	60522	ND	ND	ND

\*Human loci are shown as GeneID numbers from Ensembl. Gene names are shown in all caps. ND, not determined. PFA, preformed Rab acceptor; ER, endoplasmic reticulum; ND, not determined; PFA, preformed Rab acceptor; YP, Yip1 interacting protein; YP, Yip1 interacting protein.

	Yip proteins	Rab proteins
Human cells	12	60
fly	9	29
worm	10	29
yeast	6	11

Yip proteins interact with numerous Rab proteins to mediate their initial membrane targeting.



A model for the membrane recruitment of Rab proteins.

- Rab proteins would be delivered to the membrane by GDI, and the complex would be dissociated by a GDF that might be a Yip-family member.