

## Molecular Cloning of YPT1/SEC4-Related cDNAs from an Epithelial Cell Line

PHILIPPE CHAVRIER, MARTIN VINGRON, CHRIS SANDER, KAI SIMONS, AND MARINO ZERIAL\*

*European Molecular Biology Laboratory, Postfach 10.2209, Meyerhofstrasse 1, 6900 Heidelberg,  
Federal Republic of Germany*

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**Molecular analysis of *Saccharomyces cerevisiae* secretion mutants has led to the identification of two Ras-like GTP-binding proteins, Ypt1p and Sec4p, which are essential for transport along the exocytic route. To study the regulation of membrane traffic in epithelial cells, a set of 11 clones encoding proteins similar to the YPT1/SEC4 products were isolated from an MDCK (Madin-Darby canine kidney) cell cDNA library. Four of these proteins, Rab8, -9, -10, and -11, are novel members of this subfamily of Ras-like proteins, and two of them are closely related to Ypt1p and Sec4p. The ratio of the number of clones isolated over the total number screened reveals a high level of complexity for this subfamily of GTP-binding proteins. This diversity supports their proposed function in controlling different steps in membrane traffic.**

Ras and Ras-like proteins constitute a superfamily of GTP/GDP-binding proteins sharing regions of structural homology highly conserved through evolution (3). Different members of this superfamily can be grouped in individual subfamilies on the basis of their structural features. These characteristics suggest that the members of each subfamily, besides the common biochemical property of binding guanine nucleotides, carry out similar biological functions.

Recent *in vivo* and *in vitro* studies have indicated that the superfamily of Ras-related proteins includes proteins involved in controlling membrane traffic. Molecular analysis of *Saccharomyces cerevisiae* secretion mutants has led to the identification of two low-molecular-weight GTP-binding proteins, Ypt1p (13, 18, 19, 29-32) and Sec4p (15, 28, 39), which are structurally similar to the *ras* gene products and are essential for transport along the exocytic route.

Ypt1p is a 23-kDa GTP-binding protein which displays 38% homology with the human *c-ras* gene products (13, 30). Conditional lethal mutants of the *ypt* gene expressed at the nonpermissive temperature cause growth arrest and impaired sporulation, changes in microtubule organization, abnormal proliferation of endoplasmic reticulum and Golgi membranes, incomplete protein glycosylation, and a partial defect in secretion (29-32). Recent studies have shown that Ypt1p is involved in membrane transport from the endoplasmic reticulum to the Golgi or within the Golgi apparatus (2). Two other GTP-binding proteins, SAR1 (25) and ARF (33, 34), have also been implicated in early steps of protein secretion in *S. cerevisiae*.

Sec4p is also a Ras-like 23.5-kDa GTP-binding protein, and it controls a late step in secretion. Temperature-sensitive and dominant SEC4 mutants lead to a block in transport from the Golgi apparatus and the cells accumulate post-Golgi secretory vesicles (28, 39).

In higher eucaryotes, there is growing evidence for the involvement of GTP-binding proteins in controlling membrane traffic. The nonhydrolyzable GTP analog, GTP- $\gamma$ -s, inhibits transport in several different cell-free systems assaying intracellular protein transport (14, 22, 23, 36).

Altogether, these results suggest that GTP-binding pro-

teins are involved in distinct steps in membrane traffic (4). Indeed, a number of YPT1/SEC4-related proteins have been identified in mammalian cells (5, 7, 10, 21, 27, 35, 41, 42). Among them, a protein structurally and functionally equivalent to Ypt1p has been found in mouse (19), rat (35) and human (42) cells. However, attempts to isolate a mammalian homolog of the yeast SEC4 gene have so far failed. In this report, we describe the isolation of 11 clones encoding YPT1/SEC4-related products from a kidney epithelial (MDCK) cell cDNA library. Of the cDNAs isolated, four are new members of this subfamily of Ras-related proteins and two of these are distinguished by the high sequence similarity they share with YPT1 and SEC4. The ratio of the number of cDNAs isolated over the total number of cDNAs screened reveals a higher level of complexity for this subfamily of GTP-binding proteins than previously determined.

### MATERIALS AND METHODS

**Cell culture, RNA extraction, and analysis.** All cells were grown in medium supplemented with 2 mM glutamine, 100 U of penicillin per ml, and 10  $\mu$ g of streptomycin (GIBCO) per ml at 37°C in 5% CO<sub>2</sub>. MDCK strain II cells were grown in minimum essential medium plus 5% fetal calf serum, NIH 3T3 cells were grown in Dulbecco modified minimum essential medium plus 10% fetal calf serum, and BHK21 cells were grown in Glasgow minimum essential medium plus 10% fetal calf serum supplemented with 10% tryptose phosphate broth.

Total RNA was prepared from confluent cells by using the guanidine hydrochloride procedure (9). Polyadenylated RNA was purified by binding to oligo(dT) cellulose (Pharmacia). For Northern (RNA) blot analyses, polyadenylated RNAs (1  $\mu$ g per slot) were separated by 1% agarose gel electrophoresis and transferred to nylon GeneScreen membranes. Filters were prehybridized in a solution containing 5 $\times$  SSC (1 $\times$  SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 50% formamide, 5 $\times$  Denhardt solution, and 1% sodium dodecyl sulfate at 42°C for 1 h. Hybridization was carried out in the same solution supplemented with <sup>32</sup>P-labeled probes (2  $\times$  10<sup>6</sup> cpm/ml) for 18 h at 42°C. Washings were performed in 0.1 $\times$  SSC-0.5% sodium dodecyl sulfate at 60°C. X-ray films were exposed for 1 to 2 days at -70°C with intensifying screens.

\* Corresponding author.

	rab3a		rab3b		rab4		rab6		47=rab1		21=rab2		1=rab5		2=rab8		14=rab7		15=rab9		32=rab10		34=rab11		38=rab4b		YPT		YPT3		SEC4		H-ras				
rab3a	100																																				
rab3b	85	100																																			
rab4	39	40	100																																		
rab6	42	40	39	100																																	
47=rab1	52	50	47	41	100																																
21=rab2	42	42	59	40	49	100																															
1=rab5	37	38	44	44	47	40	100																														
2=rab8	56	53	44	45	59	49	41	100																													
1=rab7	31	32	34	34	34	35	34	35	100																												
15=rab9	29	29	34	28	32	30	27	32	54	100																											
32=rab10	54	54	44	44	59	49	39	77	34	32	100																										
34=rab11	41	40	52	36	52	55	46	48	34	32	47	100																									
38=rab4b	38	40	87	39	47	58	43	43	33	32	44	53	100																								
YPT	49	50	50	42	78	45	45	56	34	35	59	52	50	100																							
YPT3	42	40	50	40	53	55	47	50	32	30	47	80	50	52	100																						
SEC4	53	52	44	40	57	48	40	59	35	31	60	46	43	53	46	100																					
H-ras	30	31	32	30	35	33	30	35	28	27	35	35	32	35	34	33	100																				

FIG. 1. Percentages of amino acid identities between MDCK proteins Rab1, -2, -5, -7, -8, -9, -10, -11, -4b; yeast YPT1, SEC4, and YPT3; and human H-ras, Rab3a, -3b, -4, and -6 in the sequence spanning region 1 (amino acid position 23 in the alignment of Fig. 3) to region 4 (position 187).

**cDNA library screening.** An oriented lambda MDCK cDNA library was constructed in the lambda cloning vector UNI-ZAP XR (Stratagene). Inserts had an average size of 2 kb. Fifty thousand bacteriophage plaques were screened by hybridization with a degenerate oligonucleotide (GTP-1 oligonucleotide) based on the conserved sequence Trp-Asp-Thr-Ala-Gly-Gln-Glu present in Sec4p (28), Ypt1p (13), and the different Rab proteins (35, 42). The sequence of the GTP-1 oligonucleotide was as follows: 5'-TGGGA(C<sub>50</sub>/T<sub>50</sub>)AC(A<sub>70</sub>/C<sub>10</sub>/T<sub>10</sub>/G<sub>10</sub>)GC(T<sub>30</sub>/A<sub>70</sub>)GG(A<sub>25</sub>/G<sub>25</sub>/C<sub>25</sub>/T<sub>25</sub>)CA(G<sub>20</sub>/A<sub>80</sub>)GAA-3' (numbers in subscript refer to the relative frequency of each base at a given position). Duplicate filters were prehybridized for 1 h at 42°C in a 6× SSC-5× Denhardt solution-0.05% sodium pyrophosphate-0.5% sodium dodecyl sulfate solution containing 100 µg of boiled herring sperm DNA per ml. Hybridization was carried out for 18 h at 42°C in a 6× SSC-1× Denhardt solution-0.05% sodium pyrophosphate solution containing 100 µg of yeast tRNA per ml with 25 pmol of GTP-1 oligonucleotide labeled with T4 polynucleotide kinase (Biolabs) per ml. Filters were then washed in 6× SSC-0.05% sodium pyrophosphate for 3 h at 44°C. Phage DNAs from the positive plaques were extracted, digested with *Eco*RI and *Xho*I restriction endonucleases, and transferred to a GeneScreen Plus membrane (Du Pont Co., Wilmington, Del.). Duplicate filters were hybridized in the previous conditions with GTP-1 oligonucleotide at 42 or 37°C with FLET-1 oligonucleotide; the latter corresponded to the fourth conserved domain in Ras-like proteins, and its sequence was as follows: 5'-TT(T<sub>50</sub>/C<sub>50</sub>)(T<sub>50</sub>/A<sub>50</sub>)T(G<sub>50</sub>/T<sub>50</sub>)GA(A<sub>25</sub>/G<sub>75</sub>)(A<sub>75</sub>/G<sub>25</sub>)C(A<sub>50</sub>/C<sub>30</sub>/G<sub>10</sub>/T<sub>10</sub>)(A<sub>75</sub>/T<sub>25</sub>)(G<sub>75</sub>/C<sub>25</sub>)(T<sub>75</sub>/C<sub>25</sub>)GC-3'.

The GTP-1 oligonucleotide-hybridized filter was washed for 1 h at 60°C under the conditions described above, while in the case of FLET-1, oligonucleotide washing was performed at 25°C. Eleven clones hybridizing with both oligonucleotides were further characterized. Cross-hybridization analysis performed at high-stringency conditions with the human Rab cDNAs as probes (42) revealed that clones 1, 21, and 47 were highly homologous to Rab5, Rab2, and Rab1, respectively. In vivo excision of the cDNA inserts from the UNI-ZAP XR vector was performed according to the manufacturer's procedure (Stratagene). Phagemid DNAs were prepared and used directly for double-stranded DNA sequencing with the T7 Sequencing kit (Pharmacia), using a set of different primers including the GTP-1 and FLET-1 gen-

erate oligonucleotides. The region of clone 2 upstream nucleotide 163 was obtained by using the anchored polymerase chain reaction procedure (20).

**Computer methods.** The multiple sequence alignment was done by using the MALI and PRALI programs (37) and was improved manually. On the basis of the alignment, the number of mismatches between the conserved parts of the sequences was calculated (Fig. 1). The phylogenetic tree was computed by using the program KITSCH from the PHYLIP package by J. Felstein (12). This program not only calculates the branching pattern of the tree but also estimates the evolutionary distance between adjacent branching points or end nodes. To represent the information in Fig. 1, we have depicted the tree together with a scale of sequence similarities in percent identical residues. In this way, one can read an approximate similarity between species from the height of the branching point linking them. Other methods (Fitch-Margoliash and parsimony) to calculate trees were also used and largely agree with these results.

**Nucleotide sequence accession number.** The sequences of Rab1, -8, -9, -10, -11, and -4b; Rac2; and Rho1 have been submitted to GenBank as accession numbers X56384 to X56391, respectively.

## RESULTS

**Isolation of YPT1/SEC4-related cDNAs.** In order to isolate cDNA clones encoding proteins belonging to the Ypt1p/Sec4p subfamily, 50,000 recombinant plaques of an MDCK cell cDNA library were screened by using an oligonucleotide corresponding to the amino acid sequence WDTAGQE. These seven residues are strictly conserved in Ypt1p, Sec4p, Rho, and all the proteins of this subfamily identified so far (5, 7, 10, 21, 27, 35, 41, 42). Fifty positive clones were isolated under low-stringency-hybridization conditions. A second screening at higher stringency reduced the number of positive clones to 19. These also hybridized under low-stringency hybridization conditions, with a second oligonucleotide corresponding to another conserved region between the Ras-related proteins (FLET-1 oligonucleotide). Cross-hybridization analysis using the phage inserts as probes lowered the number of distinct cDNAs to 11.

**Structural features of YPT1/SEC4-related proteins.** We next determined the nucleotide sequences of these cDNAs and the deduced amino acid sequences corresponding to

**2 = rab8**

GAGTGTAAATATGGCGAAGACCTACGATTACCTGTTCAAGCTGCTGATCGGGACCTCG MetAlaLysThrTyrAspTyrLeuPheLysLeuLeuLeuIleGlyAspSer	60 17
GGGGTGGGAAGACCTGTGTCTCCGCTCTCCGAGGACGCTTCAACTCAACTTTC GlyValGlyLysThrCysValLeuPheArgPheSerGluAspAlaPheAsnSerThrPhe	120 37
ATCTCCACTATAGGAATTGACTTTAAATAGGACCATAGAGCTCGATGGCAAGAGAATT IleSerThrIleGlyIleAspPheLysIleArgThrIleGluLeuAspGlyLysArgIle	180 57
AAGCTACAGATATGGGACACAGCTGGTCAAGAACGGTTTCGGACGATCACAACAGCCTAT LysLeuAlaIleTrpAspThrAlaIleGlyGlnGluArgPheArgThrIleThrAlaTyr	240 77
TACAGGGCGCAATGGGCATCATGTGCTATGACATCACCAACGAGAAATCCTTTGAC TyrArgGlyAlaMetGlyIleMetLeuValTyrAspIleThrAsnGluLysSerPheAsp	300 97
AATATCCGAACTGGATTCCGAACATTGAGGAGCATGCTTCTGCAGATGTCGAAAGATG AsnIleArgAsnTrpIleArgAsnIleGluGluHisAlaSerAlaAspValGluLysMet	360 117
ATACTCGAAACAAGTGTGTGTAACGACAAAAGACAAGTTTCCAAGGAACGGGGAGAA IleLeuGlyAsnLysCysAspValAsnAspLysArgGlnValSerLysGluArgGlyGlu	420 137
AAGCTGGCCCTGGACTATGGAATCAAGTTCATGGAGACAGTGGCAAGCCAACATCAAT LysLeuAlaIleAspTyrGlyIleLysPheMetGluThrSerAlaLysAlaAsnIleAsn	480 157
GTGGAGAACGCATTTTCTACTCTCCGACAGACATCAAGCAAGATGGACAAAAATG ValGluAsnAlaPhePheThrLeuAlaArgAspIleLysAlaLysMetAspLysLysLeu	540 177
GAAGCAACAGTCCCAAGGGAGCAACAGGAGTCAAAATCACACCAGACAGCAGAAG GluGlyAsnSerProGlnGlySerAsnGlnGlyValLysIleThrProAspGlnGlnLys	600 197
AGGAGCAGCTTTTCCGATGTGTCTTCTGTGAGGAACACTGCCTTACTCTGAGCCTCGC ArgSerSerPhePheArgCysValLeu *	660 207
TCAGCTGAGCTGACTGTGCTGTCCCTGAGTGAGCCCTCTCACTCAGCCGGGGCCCTCC CCTCAACTACTCCCTGCCGTGCCAGCCACTGGGCCCATG	720 760

**4 = rac2**

ATGCAGGCCATCAAGTGTGGTGGTGGGAGACGGAGCTGTAGTAAAACCTGTCTACTG MetGlnAlaIleLysCysValValValGlyAspGlyAlaValGlyLysThrCysLeuLeu	60 20
ATCAGTTACACAACCAATGCATTTCTCGGAAATACATCCCACTGTCTTGGACAATTAC IleSerTyrThrThrAsnAlaPheProGlyGluTyrIleProThrValPheAspAsnTyr	120 40
TCTGCCAATGTTATGGTAGATGAAAACCAAGTGAATCTGGGCTTATGGATACAGCTGGA SerAlaAsnValMetValAspGlyLysProValAsnLeuGlyLeuTrpAspThrAlaGly	180 60
CAAGAAGATTATGACAGATTACGTCCTTATCCTATCCGCAACAGATGTTATTTAATT LysLeuAlaIleTrpAspThrAlaIleGlyGlnGluArgProLeuSerTyrProGlnThrAspValPheLeuIle	240 80
TGCTTTCTCTTGTGAGTCTGCATATTGAAAATGTTTCGAGCAAAGTGGTACCTGAA CysPheSerLeuValSerProAlaSerPheGluAsnValArgAlaLysTrpTyrProGlu	300 100
GTGGACACCCTGTCCCAACACCCCTATCATCTTGGTGGGACTAACTTGATCTCAGG ValArgHisHisCysProAsnThrProIleIleLeuValGlyThrLysLeuLeuIleGly	360 120
GACGACAAAGACACAGATTGAGAACTGAAGGAGAAGAGCTGACTCCCATCACCTACCCA AspAspLysAspThrIleGluLysLeuLysGluLysLysLeuThrProIleThrTyrPro	420 140
CAGGTCTGGCCATGGCTAAGGAGATCGGTGCTGTAATACTGGAGTGTCTGCTCTC GlnGlyLysAlaMetAlaLysGluIleGlyAlaValLysTyrLeuGluCysSerAlaLeu	480 160
ACGCAGCGAGGCCCAAGACAGTGTGTGATGAGCTATTCGAGCGGTTCTCTGCCCCCT ThrGlnArgGlyLeuLysThrValPheAspGluAlaIleArgAlaValIleLeuCysProPro	540 180
CCCGTCAAGAAGAGGAAGAAAATGCCTGTGTGTAATGTTGGGTTCCCTGCCCCA ProValLysLysArgLysArgLysCysLeuLeu *	600 192
TCCCCCAGAACCTTTGTACGGTTTGGCTCAAAAATGGTGGAGCCTTCGCACCTAATGCC AAGTTTTTGTACAGATTAGTTTTTCCATAAACCATTTGAACCAATCAGTAATTTT	660 716

**15 = rab9**

AATAAGTTTGATACCCAGCTCTTCCATACAATAGTGTAGAATTTTTAAATAAGATTTG AsnLysPheAspThrGlnLeuPheIleThrIleGlyValGluPheLeuAsnLysAspLeu	60
GAGGTGGATGGACATTTTGTACCATGCAGATTTGGGACACAGCCGGTCAAGAGCGATT GluValAspGlyHisPheValThrMetGlnIleTrpAspThrAlaGlyGlnGluArgPhe	120
AGAAGCCTGAGGACCGGTTTACAGAGTTCTGACTGTTGCTGCTCACTTTTAGTGT ArgSerLeuArgThrProPheTyrArgGlySerAspCysCysLeuLeuThrPheSerVal	180
GATGATTCTCAGAGCTTCCAGAACTTGAGTAACTGGAAGAAAGAAATTCATATATTGCA AspArgSerGlnSerPheGlnAsnLysSerAsnTrpLysLysGluPheIleTyrAla	240
GATGTGAAAGAGCCGAAAGCTTCTCTTTGTGATTTTGGGCAACAAGATCGACATAAGT AspValLysGluProGluSerPheProPheValIleLeuGlyAsnLysIleAspIleSer	300
GAACGCAAGTGTCTACAGAAGAAGCCCAAGCTTGGTGCAGGGACAACGGCGACTATCT GluArgValLeuSerThrGluGluAlaGlnAlaTrpCysArgAspAsnGlyAspTyrPro	360
TACTTTGAACAAGTGCAAAAGATGCCACAAATGTCCGACGAGCCTTTGAGGAAGCTGTT TyrPheGluThrSerAlaLysAspAlaThrAsnValAlaAlaPheGluGluAlaVal	420
CGAAGAGTCTTGTACTGAGGATAGGTCAGATCACCTGATTCAGACAGACAGCTCAGC ArgArgValLeuAlaThrGlnAsnLysSerAsnTrpLysLysLeuIleGlnThrAspThrAla	480
CTGCACCGAAAGCCCAAGCTAGCTCATCTTGTCTGTGATCATTAGAGAGTTCCCTGTG LeuHisArgLysProLysProSerSerSerCysCys *	540
CGATCTAACCAACTTACACACACTACACGAAATACAGGGTGGAGAAGAGAATTAGCA TTTGACGATGGATCACTACTCATAAAATTAACAACTAACCATATCTGCTTGTGTAGT GGGGTGGAGAAGGACACATCCACTCAATGGAAGAATCAATTTACTCAGTAGTGGCACT TACATTTAAATTTGTAATGGTGTCTAATACGTTTAAATTTAAATATGTAAGTTACAGA GCTATAACGAGATGATCAAGACTTTAATTAACAATTAACACTTGAATTTCTGGAAAT TAATGCTTTTTTCCCTGGGAAAATGGAGAACTACTTTTATATGTTATATTTTGT GTAATTAGCATTAAATCTGTGTTGAGGGGGGAAAAGATTCCCTAAAGCAATAATGTTAA ATAATAAAGATTAAATCTAAAAAATAAAAAAAAAAAAAA	600 660 660 720 780 840 900 960 1001

**32 = rab10**

GACTCGAGCCCTCGTTTTTCCACGCTACCCCGGCTCCCGGCTGAGAAGCCCAAGTG AGGAGTTGGCCGCTGTGAGAGGGACCGATCCCTTGGGGCCCGGGCGGAGAGCCTGAG CCGCTCTCCCAATGGCGAAGACGTACGACCTGCTTTTCAAGCTGCTCCTGATCGGA MetAlaLysLysThrTyrAspLeuValPheLysLeuLeuIleGly	60 120 180 16
GACTCGGAGTAGGGAAGACCTGCTCTTTTTCTGTTTTTCGGATGATGCCTTCAATACC AspSerGlyValGlyLysThrCysValLeuPheArgPheSerAspAlaPheAsnThr	240 36
ACCTTTATTTCCACCATAGGAATAGTTTTAAGATCAAAACAGTTGAATTCACAGGAAG ThrPheIleSerThrIleGlyIleAspPheLysIleLysThrValGluLeuGlnGlyLys	300 56
AAGATCAAGCTACAGATATGGATACAGACGGCAGGAGCGATTCACACCATCACAAC LysIleLysLeuGlnIleTrpAspThrAlaGlyGlnGluArgPheHisThrIleThr	360 76
TCCTACTACAGAGGACCAATGGGTATCATGCTAGTATATGACATCACCATGGTAAAAGT SerTyrTyrArgGlyAlaMetGlyIleMetLeuValTyrAspIleThrAsnGlyLysSer	420 96
TTGAAAACATCAGCAATGGCTTAGAAAATAGATGAGCATGCAATGAAGATGTGGAA PheGluGlnIleSerLysTrpLeuArgAsnValAspIleSerSerGlyGlyValAlaGlu	480 116
AGAATGTACTAGGAAAACAAATGTGATATGGACGATAAAAAGAGTTGACTTAAAGAAA ArgMetLeuLeuGlyAsnLysCysAspMetAspAspLysArgValValProLysGlyLys	540 136
GGAGAGCAGATTGCAAGGGAGCATGGTATTAGATTTTTGAGACTAGTGCAAAAAGTAA GlyGluAsnIleSerAlaArgGluHisGlyIleArgPhePheHisAlaSerAlaLysValGlu	600 156
ATAAACATCGAAAAGGCTTTCCTCACATTAGCTGAAGATATCCTCGAAAAGCCCTGTA IleAsnIleGluLysAlaPheLeuThrLeuAlaGluAspIleLeuArgLysThrProVal	660 176
AAAGAGCCCAACAGTGAATGATATACAGCTGGAGCGGCTGACAGGCTGGAG LysGluProAsnSerGluAsnValAspIleSerSerGlyGlyValThrGlyTrpLys	720 196
AGCAAAATGTGCTGAGCGTCTCTGTTCCGTCAGTGGCATCCACCCTGTTTTTTTC SerLysCysCys *	780 200
CTCTTGCTGCAAAAATAAACCACTGTGCTCAATTTAAGTCTAAACAGATATTTTTGTCTCT CATCTTAAGTCTCAATCCACTGTTTATTGTTCTTTCATCCGTA	840 888

their largest open reading frames were analyzed. The polypeptides encoded by clones 1, 21, and 47 showed 98, 99, and 100% amino acid conservation with the human Rab5, Rab2, and Rab1 proteins, respectively (8). Protein 14 showed 99% amino acid conservation with BRL-ras=Rab7 but additionally the sequence was extended in its N terminus by MTSRKKV (5, 8). Figure 2 shows the nucleotide and predicted amino acid sequences of the rest of the clones (the N terminus of protein 15 is missing).

The encoded proteins range from 192 to 216 amino acids in length, and they all contain the WDTAGQE sequence corresponding to the oligonucleotide used in the cDNA library screening. Clones 4 and 41 encode the canine homologs of Rac2 (100% sequence identity; 10) and Rho1 (99%; 41). The

amino acid sequences of the other proteins were aligned with those of YPT1, SEC4, human Rab3A, -3b, -4, -6, and human H-ras as shown in Fig. 3. Since most of the mammalian proteins belonging to this subfamily have been called Rab, to avoid further terminology problems we use the same nomenclature for the MDCK proteins. Therefore, proteins 2, 15, 32, 34, and 38 will be referred to as Rab8, Rab9, Rab10, Rab11, and Rab4b, respectively (see below). The recently identified *Schizosaccharomyces pombe* protein Ypt3 (24) equivalent to SP8 (11) was also included in this comparison.

All proteins display the typical structural features of Ras-like GTP-binding proteins. The four regions which have been shown to participate in the formation of the GTP-binding site in the case of H-ras (26) are highly conserved

**34 = rab11**

CTCCCCGGCCGCGCAATGGCCACCCGCGACGACGAGTACGACTATCTCTCAAAGTTGTC 60  
 MetGlyThrArgAspGluTyrAspTyrLeuPheLysValVal 15  
 CTTATTGGAGATCTGGTGTGGAAAGAGTAATCTCTGTCGATTACTCGAAATGAG 12^  
 LeuIleGlyAspSerGlyValGlyLysSerAsnLeuLeuSerArgPheThrArgAsnGlu 35  
 TTTAATCTCGAAAGTAAGAGCACCATTGGAGTAGAGTTGCAACAAGAAGCATCCAGGTT 180  
 PheAsnLeuGluSerLysSerThrIleGlyValGluPheAlaThrArgSerIleGlnVal 55  
 GATGGAAAACAATAAAGCCACAGATATGGGACACAGCGGGCAGGAGCGATACCGAGCT 240  
 AspGlyLysThrIleLysAlaGlnIleTrpAspThrAlaGlyGlnGluArgTyrArgAla 75  
 ATAACATCAGCATATATTCGTGGAGCTGAGTGCCTTACTGGTTTATGACATTGCTAAG 300  
 IleThrSerAlaTyrTyrArgGlyAlaValGlyAlaLeuLeuValTyrAspIleAlaLys 95  
 CATCTCACATATGAAAATGTAGAAGCATGGCTGAAAAGAACTGAGAGATCATGCTGATAGT 360  
 HisLeuThrTyrGluAsnValGluArgTrpLeuLysGluLeuArgAspHisAlaAspSer 115  
 AACATTGTTATCATGCTTGGGCAATAAGAGTGATTTGGCTCATCTCAGGCGAGTTCTC 420  
 AsnIleValIleMetLeuValGlyAsnLysSerAspLeuArgHisLeuArgAlaValPro 135  
 ACAGATGAAGCAAGAGCTTTTCAGAAAAGAATGGTTGTCATTCATTGAGACTTCCGCT 480  
 ThrAspGluAlaArgAlaPheAlaGluLysAsnGlyLeuSerPheIleGluThrSerAla 155  
 CTAGACTCTACAATGTAGAAGCTGTTTTAGCAAACTCTGACAGAGATATACCGCATT 540  
 LeuAspSerThrAsnValGluAlaAlaPheGlnThrIleLeuThrGluIleTyrArgIle 175  
 GTTCCCAAGAAACAATGTGACACAGCGTGAATAATGACATGCTCCAAGCAACAATGG 600  
 ValSerGlnLysGlnMetSerAspArgGluLeuAsnAspMetSerProSerAsnVal 195  
 GTTCCTATTTCATGTTCCCAACCAAGCTGAAAACAAGGTCAGTGCTGTGAGAG 660  
 ValProIleHisValProProThrThrGluAsnLysProLysValGlnCysCysGlnAsn 215  
 ATAAAGGCATTTCTCTCTCCCTAGAAAGCTGGGGATAGTCCATTCCAGGCTGAGA 720  
 ILe \* 216  
 TTAATATATTGTAATCTTGTGGTCACTTTGTGTTTTATTACTTCTCATACTTATGA 780  
 ATTTCCCATGCTTAAAGCTTTTGGATTTAGCTTTATAAAATCATCCACTTGTCCCGAA 840  
 TGACTGCAGCTTTTTCATGCTATGGCTTACTAGCCTTAGTTTAAATAACTGAATGTT 900  
 TGGATTCTCTCAAAAAAAAAAAAAA 926

**41 = rho1**

ATGGCTGCCATCCGGAAGAACTGGTATGTTGGTGTATGGACCTGTGGTAAAGACTTGT 60  
 MetAlaAlaIleArgLysLysLeuValIleValGlyAspGlyAlaCysGlyLysThrCys 20  
 TTGCTCATCGTCTTTAGCAAGGACCAGTCTCCAGAGGTGTATGTACCACAGTGTGTGAG 120  
 LeuLeuIleValPheSerLysAspGlnPheProGluValTyrValProThrValPheGlu 40  
 AACTATGTGGCAGATATTGAAGTGTATGAAAGCAGGTAGAGTTGGCTTTGTGGGATACA 180  
 AsnTyrValAlaAspIleGluValAspGlyLysGlnValGluLeuAlaLeuTrpAspThr 60  
 GCTGGGAGGAAGATTATGATCGCTTAGAGCCTCTCTCCTATCAGACACTGATGTTATA 240  
 AlaGlyGlnGluAspTyrAspArgLeuArgProLeuSerTyrProAspThrAspValIle 80  
 CTGATGTGTTCTCTATTGACAGCCCTGATAGCTTAGAAAACATCCAGAAAATGGACC 300  
 LeuMetCysPheSerIleAspSerProAspSerLeuGluAsnIleProGluLysTrpThr 100  
 CCAGAAGTCAAGACTTCTGTCCCAACGTCGCCATCATCTGTTGGGAACAAGAAGGAT 360  
 ProGluValLysHisPheCysProAsnValProIleIleLeuValGlyAsnLysLysAsp 120  
 CTTCCGAATGATGAGCACACAAGGCGGGAGCTAGCCAAAGATGAAGCAGGAGCCGGTAAA 420  
 LeuArgAsnAspGluHisThrArgArgGluLeuAlaLysMetLysGlnGluProValLys 140  
 CCGACAGAAGCAGAGATATGGCAACAGGATTTGGTGTCTTTGGGTACATGGAGTGTTC 480  
 ProThrGluGlyArgAspMetAlaAsnArgIleGlyAlaPheGlyTyrMetGluCysSer 160  
 GCAAAGCAAAAGATGGAGTGGAGGAGTTTTTGAATGGCCACGAGAGCTGCTCGCAA 540  
 AlaLysThrLysAspGlyValArgGluValPheGluMetAlaThrArgAlaAlaLeuGln 180  
 GCCAGACGTGGGAAGAAAAAATCTGGGTGCCTTGCTTTGTGAAACCCCTGCTGCAAGCAC 600  
 AlaArgArgGlyLysLysLysSerGlyCysLeuValLeu \* 193  
 GCCTCATCGGTTAATTTGAAGTGCTGTTTAAATCTTAGTGTATGATTACTGGCCT 660  
 TTTTCATTTATCTATAATTTACCTAAGATTACAATC 697

and are as follows: (i) the GXXXXGKS/T sequence (positions 29 to 36 of the sequence alignment) constituting the phosphate-binding loop L1 (nomenclature according to Pai et al. [26]); (ii) the DTAG sequence on L4 (positions 77 to 80) in the highly conserved WDTAGQE motif shared by Rab and Rho proteins, which interacts with the  $\gamma$  phosphate; (iii) the NKXD sequence (positions 139 to 142) at the junction between beta-strand 5 and loop L8, called the guanine specificity region; (iv) the SAK/L sequence in L10 (positions 169 to 171) interacting with the D residue (position 142). The region spanning these four conserved domains contains other identical residues (shown in boldface type) as well as several conservative changes.

**38 = rab4b**

GAGTAGGAAGGACGGGGCTGCAGCCGGAGTGGAGCGGCTGCCAGCCGAGGAGCAGGGG 60  
 CGGCCGCGGCCATATTGCGGCCCGAGCGGGCCGCGCAGTCAATGGCCGAGACTAC 120  
 MetAlaGluThrTyr 5  
 GACTTCTCTTCAAATTCCTGGTATTGGCAGTGCAGGAAGTGGCAATCATGTCTCCTT 180  
 AspPheLeuPheLysPheLeuValIleGlySerAlaGlyThrGlyLysSerCysLeuLeu 25  
 CATCAGTTCATTGAGAATAAGTTCAACAGGACTCCAACACACAATCGGCTGGAGTTT 240  
 HisGlnPheIleGluAsnLysPheLysGlnAspSerAsnHisThrIleGlyValGluPhe 45  
 GGATCTCGGGTAGTCAACGTGGTGGGAAGACTGTGAAGCTCCAGATTGGGACACAGCC 300  
 GlySerArgValValAsnValGlyGlyLysThrValLysLeuGlnIleTrpAspThrAla 65  
 GGCAAGAGCGGTTTCGGTCCGTGACACGGAGTTACTACCGAGGGCGGCTGGAGCCCTG 360  
 GlyGlnGluArgPheArgSerValThrArgSerTyrTyrArgGlyAlaAlaGlyAlaLeu 85  
 CTGGTGTACGACATCACCGCCGGGAGACATACAACCTGTTGGCTGCCTGGCTGACGGAC 420  
 LeuValTyrAspIleThrSerArgGluThrTyrAsnSerLeuAlaAlaTrpLeuThrAsp 105  
 GCCCGACGCTGGTAGCCCAACATCGTGGTCATCTCTGTGGCAACAAGAAAGACCTG 480  
 AlaArgThrLeuAlaSerProAsnIleValIleLeuCysGlyAsnLysLysAlaLeu 125  
 GACCTGAGCGCGAGGTCATTTCTCGAGGCCCTCCCGCTTGTCCAGGAGAATGAGCTA 540  
 AspProGluArgGluValThrPheLeuGluAlaSerArgPheAlaGlnGluAsnGluLeu 145  
 ATGTTCTGGAGACTAGTCCCTCAGGGTGAACCTGGGAAGAGGCTTCTGAAAGTGT 600  
 MetPheLeuGluThrSerAlaLeuThrGlyGluAsnValGluGluAlaPheLeuLysCys 165  
 GCCCGACCATCTGTAACAAGATCGACTCAGGTGAGCTGGACCCGAGAGGATGGGCTCA 660  
 AlaArgThrIleLeuAsnLysIleAspSerGlyGluLeuAspProGluArgMetGlySer 185  
 GGCATTGAGCGGGATGTTCCCTCCGCGAGCTGCGGAGGCTGGAGTGGCCAGGCC 720  
 GlyIleGlnTyrGlyAspAlaSerLeuArgGlnLeuArgGlnProArgSerAlaGlnAla 205  
 GTGGCCCCAGCCCTGTGGCTGTGAGACATGTGGAGCCAGCTACCTGTTCTCCAGGA 780  
 ValAlaProGlnProCysGlyCys \* 213  
 CCAGCCCTGCCCTTCTGGCCGGGGCCAGACCCAGGCC 819

FIG. 2. Nucleotide sequences of the MDCK YPT1/SEC4-like cDNAs and deduced primary sequences of the encoded proteins. Nucleotide and predicted amino acid (in single-letter amino acid code) sequences of cDNAs 2 (Rab8), 4 (Rac1), 15 (Rab9), 32 (Rab10), 34 (Rab11), 38 (Rab4b), and 41 (Rho2) are shown.

A less conserved region is the effector loop L2 (positions 51 to 59) corresponding to amino acids 32 to 40 in the H-ras sequence which are known to mediate the interaction of  $p21^{ras}$  with the GTPase-activating protein (1, 6). All these proteins share a Thr in position 54, which in  $p21^{ras}$  coordinates to the  $Mg^{2+}$  ion and to the  $\gamma$  phosphate. The rest of this region is clearly more divergent from  $p21^{ras}$  and is more similar to the Ypt1p and Sec4p effector loops. In the case of proteins Rab8 and Rab10, this similarity is striking: the FISTIGIDFKIK sequence of Rab10 almost exactly matches that of SEC4 (FITTIGIDFKIK) besides a conservative change (Ser-Thr) in the third position. Rab8 contains the sequence FISTIGIDFKIR, also very similar to that of Ypt1p and Sec4p.

The sequence identity in the region spanning the four conserved domains ranging from alignment positions 23 and 187 (the last conserved hydrophobic amino acid) is shown in Fig. 1. On the basis of these data a phylogenetic-type tree was calculated (Fig. 4) which groups the sequences depending on their sequence similarity. Interestingly, this analysis revealed that the homology between Rab8 and Rab10 proteins and Ypt1 and Sec4 proteins is not limited to the effector loop. The four proteins constitute a group together with Rab1 and, possibly, Rab3a and Rab3b. Besides Rab1, of all mammalian proteins of this subfamily identified so far, Rab8 and Rab10 are the most closely related to the yeast proteins. Within the conserved region, these two proteins share 56 and 59% sequence identity with Ypt1p and 59 and 60% with Sec4p, respectively. The similarity between the two MDCK proteins is 77%, clearly lower than for the highly related Rab3a and Rab3b.

When a mammalian sequence and a yeast sequence have more than 70% identity, one may assume that they perform analogous functions in the two species. This is the case for YPT1 and Rab1 (78%; 19) and, possibly, also for the recently

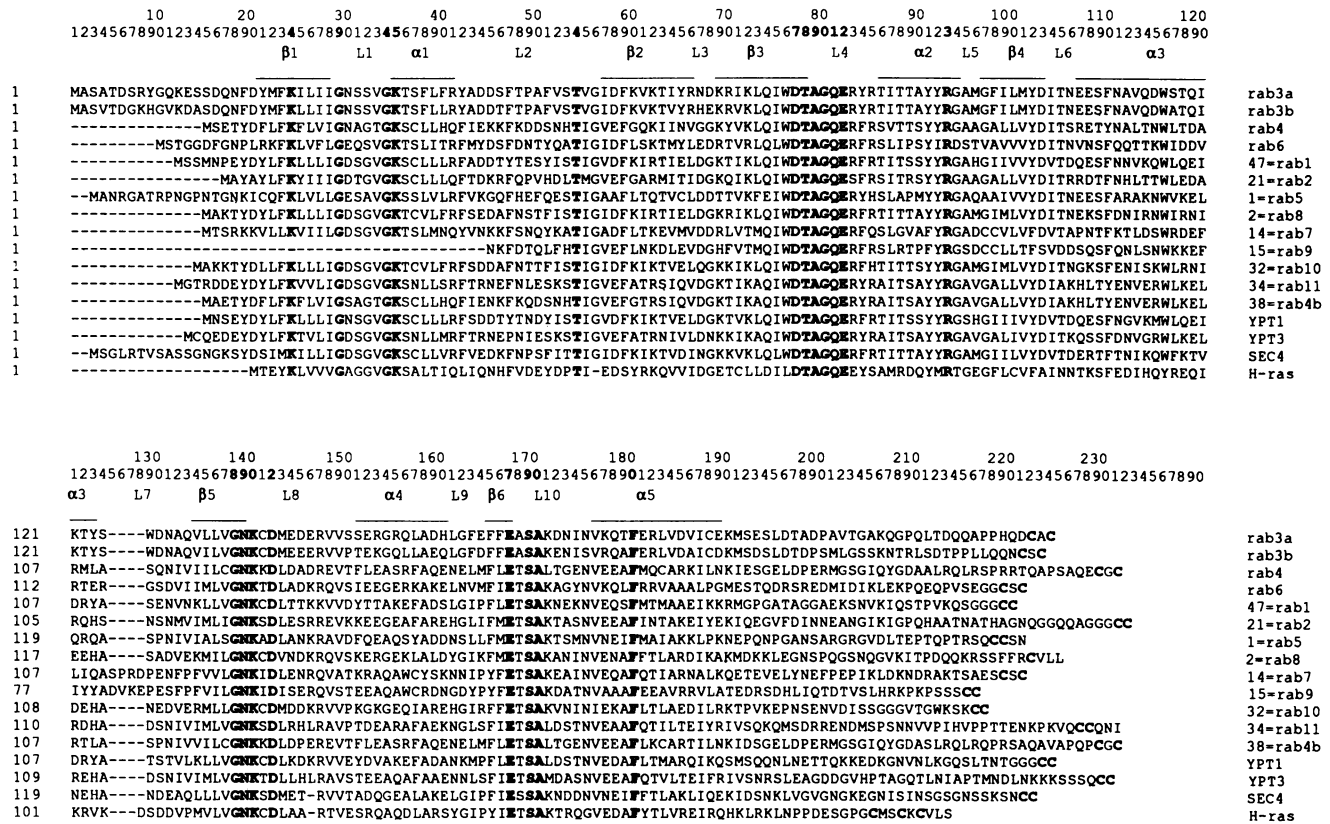


FIG. 3. Amino acid sequence alignment of MDCK proteins Rab1, -2, -5, -7, -8, -9, -10, -11, and -4b with human H-Ras, Rab3a, -3b, -4, -6, and yeast Ypt1p, Sec4p, and Ypt3p. Identical amino acids are shown in boldface type. The reference numbering is determined by the alignment. Alpha-helices, beta-strands, and loops for H-Ras taken from Pai et al. (26) are indicated.

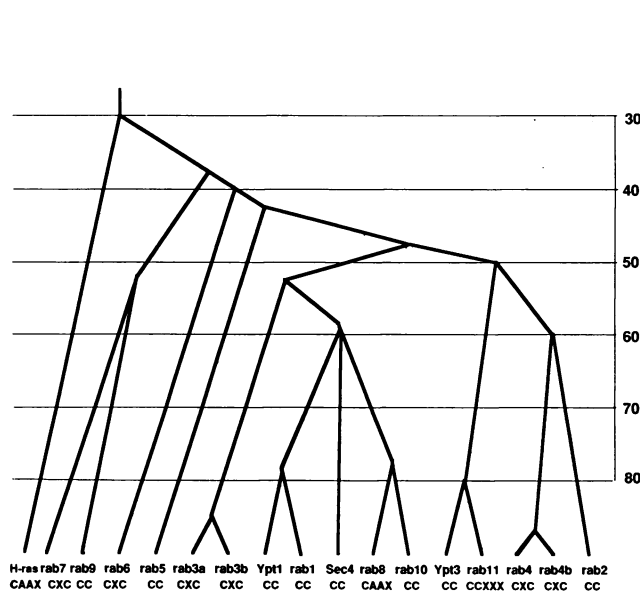


FIG. 4. Evolutionary tree of MDCK proteins Rab1, -2, -5, -7, -8, -9, -10, -11, and -4b; human Rab3a, -3b, -4, and -6; and yeast Ypt1p, Ypt3p, and Sec4p. The tree is drawn such that the height of the branching points roughly indicates the sequence similarity between the most distant sequences in the cluster defined by the branching point. A scale with percent identical residues is given next to the tree. The C-terminal cysteine motifs are also shown for each protein.

identified YPT3=SP8 and Rab11 (80%). MDCK protein 38 shares 87% identical amino acids with human Rab4 in this conserved region. It is not clear whether this divergence is due to species heterogeneity or whether it accounts for distinct, although highly related, proteins. An example of the latter case is proteins Rab3a and Rab3b (85% identity). Until this issue is clarified, we refer to protein 38 as Rab4b. Since Rab2 also shares 59% identity with Rab4 and 58% with protein 38, the three proteins are joined into a subgroup.

Also, Rab7 and Rab9 are subgrouped together due to their 54% sequence identity; in addition, they both have a 4-amino-acid insertion in loop L7 (Fig. 3). There are also proteins in the tree which are clearly separated from the others, like Rab5 and Rab6.

All proteins share one or two cysteine residues at their C termini. In the case of Ras proteins, the cysteine is followed by two aliphatic amino acids and a terminal variable residue. This C-terminal motif, the so-called CAAX box (cysteine-aliphatic-aliphatic-any amino acid), is modified posttranslationally and determines the membrane association of the Ras proteins (16, 17, 40). Analysis of the C termini of the different proteins reveals a sequence heterogeneity at the level of the cysteine motifs. Only Rab8, 4=rac2 and 41=rho1 (Fig. 2) contain the CAAX box. Rab1, Rab2, Rab9, and Rab10 have two consecutive C-terminal cysteines, similar to Ypt1p, Ypt3p, and Sec4p. In Rab5, the two cysteines are followed by serine and asparagine, while Rab11 has an unusual CCQNI C-terminal motif. In Rab3a and Rab3b, Rab4 and Rab4b, and Rab6, and Rab7, the cysteines are separated by glycine, alanine, or serine, respectively.

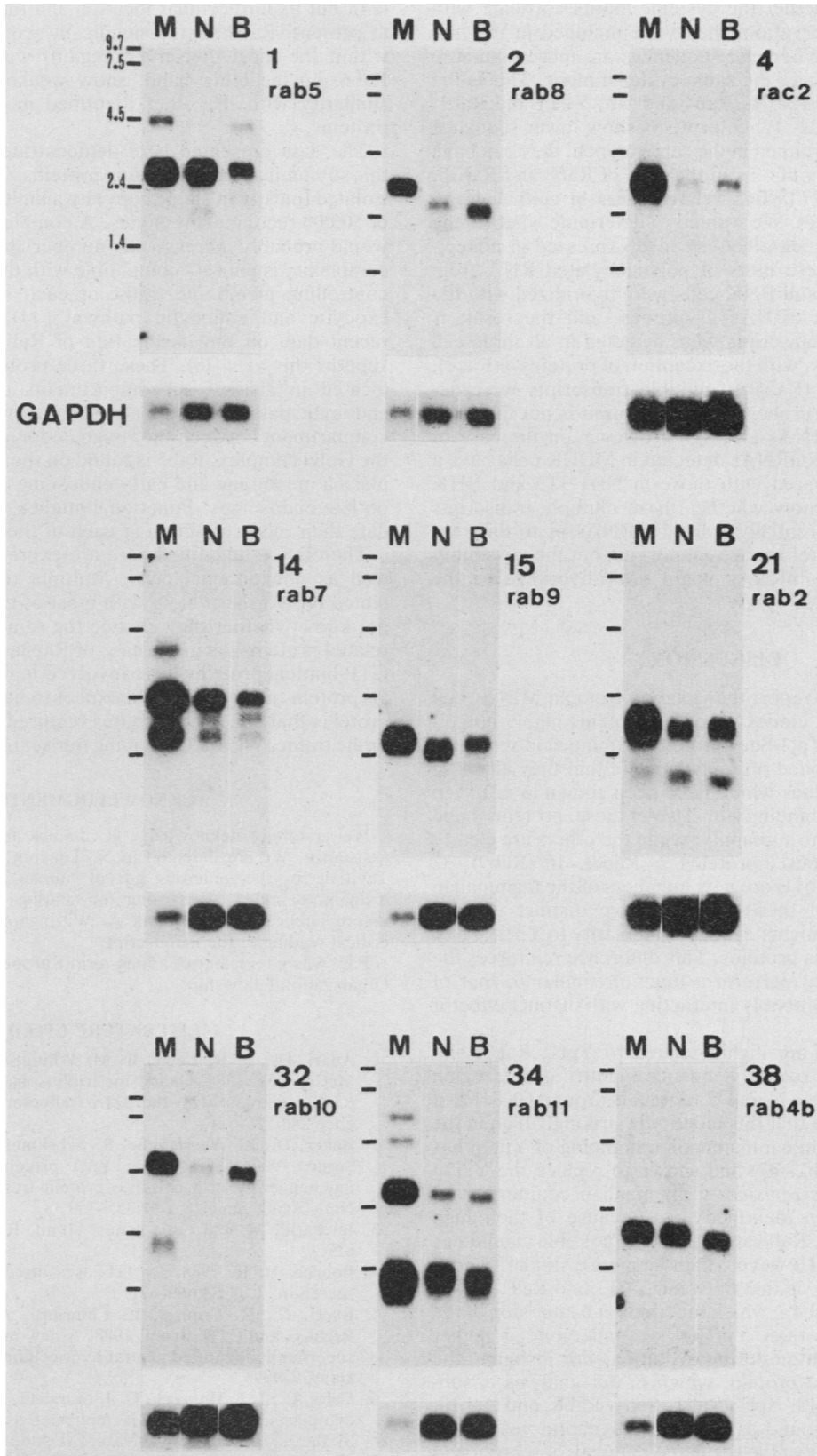


FIG. 5. Northern blot analysis of YPT1/SEC4-like genes expressed in MDCK (M), NIH 3T3 (N), and BHK (B) cells. Polyadenylated RNAs (1  $\mu$ g per lane) electrophoresed on 1% agarose gels and transferred to nylon filters were hybridized with  $^{32}$ P-labeled 1, 2, 4, 14, 15, 21, 32, 34, and 38 cDNAs as probes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control probe. Sizes (in kilobases) were determined by using RNA molecular size standards.



To determine whether the cysteine motifs correlate with the sequence conservation, they were included in the tree scheme of Fig. 4. Whenever sequences are more than 80% similar, they also share the same cysteine motif. This is the case for Rab3a and Rab3b, Rab4 and -4b, YPT1 and Rab1, and YPT3 and Rab11. When proteins show lower sequence similarity, even if grouped in the same branch, they can have different cysteine motifs, as in the case of Rab8 and Rab10.

**Expression of YPT1/SEC4-related genes in epithelial and nonepithelial cell lines.** We wanted to determine whether the sequences we have identified are also expressed in nonepithelial cells. Northern blots of polyadenylated RNA from MDCK, NIH 3T3, and BHK cells were hybridized with the various <sup>32</sup>P-labeled cDNAs as probes, and the result is shown in Fig. 5. Transcripts were detected in all three cell types. In most cases, with the exception of proteins 4 (Rac2), 15 (Rab9), and 38 (Rab4b), multiple transcripts were detected by the same probe. This observation is not unprecedented for Rab mRNAs (35, 42). However, in the case of cDNA 2 (Rab8), the mRNAs detected in MDCK cells have a different size compared with those in NIH 3T3 and BHK cells. We do not know whether these multiple transcripts correspond to differentially spliced mRNAs or to different gene products. Therefore, we cannot rule out the possibility that highly related mRNAs might cross-hybridize to the same probe.

## DISCUSSION

In this paper, we report the isolation from an MDCK cell cDNA library of 11 clones encoding proteins highly homologous to the yeast Ypt1/Sec4 proteins. Amino acid sequence analysis of the encoded proteins indicate that they all share the conserved residues which have been shown in p21<sup>ras</sup> to constitute the GTP-binding site. Two of these, proteins 4 and 41, belong to the Rho subfamily, while the others are clearly Rab proteins: of these, proteins 2 (Rab8), 15 (Rab9), 32 (Rab10), and 34 (Rab11) are new members of the mammalian Rab subfamily. All these proteins have distinct effector regions and show a higher sequence similarity to Ypt1, Ypt3, and Sec4 than to Ras proteins. This difference reinforces the idea that they might perform a function similar to that of Ypt1p and Sec4p, probably interacting with distinct effector proteins.

Rab8 and Rab10 are highly related to Ypt1, Rab1, and Sec4 proteins. The overall sequence identity in the region excluding the variable N and C termini is around 60%. Most important is the fact that this identity is strikingly high in the effector domain. While a mammalian homolog of Ypt1p has been identified (Rab1=47) and shown to replace the Ypt1p functionally in *S. cerevisiae*, a mammalian counterpart of Sec4p has not been identified yet. Because of their high sequence similarity, Rab8 and Rab10 are possible candidates for such a protein. However, their sequence similarity with SEC4 is not as high as that between YPT1 and Rab1 (78%). It is possible that Sec4p, which functions at a later step of the secretory pathway than Ypt1p, has undergone a higher degree of specialization during evolution. For instance, the brain-specific Rab3a protein, which in our analysis is subgrouped together with Ypt1, Rab1, Sec4, Rab8, and Rab10, has recently been found associated to synaptic vesicles, a specialized organelle of the regulated pathway of secretion in neurons (38).

Rab11 is highly homologous to Ypt3p (24) and equivalent to SP8p (11) recently found in *S. pombe*. Deletion of the YPT3 gene causes a temperature-sensitive lethal phenotype

(24), but its intracellular location and function are unknown at present. Rab9 shares significant sequence identity (54% within the most conserved region) with Rab7. Rab5 and Rab6, on the other hand, show weaker (~40%) sequence similarity with the other identified mammalian and yeast proteins.

The data presented here demonstrate the complexity of this subfamily of GTP-binding proteins. The 11 cDNAs were isolated (mostly in single copy) by a limited library screening of 50,000 recombinant clones. A complete library screening would probably increase this number about threefold. Such complexity is entirely compatible with their proposed role in controlling membrane traffic at each specific step of the exocytic and endocytic pathways (4). Furthermore, our recent data on the localization of Rab2, Rab5, and Rab7 support this view (8). These three proteins are specifically located in distinct subcompartments on the exocytic or endocytic pathway. Rab2 is associated with an intermediate compartment between the rough endoplasmic reticulum and the Golgi complex, Rab5 is found on the cytosolic side of the plasma membrane and early endosomes, and Rab7 is found on late endosomes. Functional studies will hopefully elucidate their mode of action at each of these sites.

The cDNAs identified here are expressed in epithelial as well as nonepithelial cells. Multiple transcripts were detected on Northern blots with most of these probes. We do not know whether they encode the same proteins or highly related proteins, as in the case of Rab3a and Rab3b. If these GTP-binding proteins were involved in regulating intracellular protein traffic, we would expect to find epithelial-specific proteins that specify the routes required for polarized membrane traffic. We are continuing our search for such proteins.

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## LITERATURE CITED

- Adari, H., D. R. Lowy, B. M. Willumsen, C. J. Der, and F. McCormick. 1988. Guanosine triphosphatase activating protein (GAP) interacts with the p21<sup>ras</sup> effector binding domain. *Science* **240**:518-521.
- Baker, D., L. Wuesthube, R. Schekman, D. Botstein, and N. Segev. 1990. GTP-binding Ypt1 protein and Ca<sup>2+</sup> function independently in a cell-free protein transport reaction. *Proc. Natl. Acad. Sci. USA* **87**:355-359.
- Barbacid, M. 1987. *ras* genes. *Annu. Rev. Biochem.* **56**:779-827.
- Bourne, H. R. 1988. Do GTPases direct membrane traffic in secretion? *Cell* **53**:669-671.
- Bucci, C., R. Frunzio, L. Chiariotti, A. L. Brown, M. M. Rechler, and C. B. Bruni. 1988. A new member of the *ras* gene superfamily identified in a rat liver cell line. *Nucleic Acids Res.* **16**:9979-9993.
- Cales, C., J. F. Hancock, C. J. Marshall, and A. Hall. 1988. The cytoplasmic protein GAP is implicated as a target for regulation by the *ras* gene product. *Nature (London)* **332**:548-551.
- Chardin, P., and A. Tavitian. 1986. The *ral* gene: a new *ras* related gene isolated by the use of a synthetic probe. *EMBO J.* **5**:2203-2208.
- Chavrier, P., R. G. Parton, H. P. Hauri, K. Simons, and M. Zerial. 1990. Localization of low molecular weight GTP-binding

- proteins to exocytic and endocytic compartments. *Cell* **62**:317–329.
9. Chirgwin, J. M., A. E. Przylyl, R. J. Macdonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299.
  10. Didsbury, J., R. F. Weber, G. M. Bokoch, T. Evans, and R. Snyderman. 1989. *rac*, a novel *ras*-related family of proteins that are botulinum toxin substrates. *J. Biol. Chem.* **264**:16378–16382.
  11. Fawell, E., S. Hook, D. Sweet, and J. Armstrong. 1990. Novel YPT1-related genes from *Schizosaccharomyces pombe*. *Nucleic Acids Res.* **18**:4264.
  12. Felstenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**:783–791.
  13. Gallwitz, D., C. Donath, and C. Sander. 1983. A yeast gene encoding a protein homologous to the human *c-has/bas* proto-oncogene product. *Nature (London)* **306**:704–707.
  14. Goda, Y., and S. R. Pfeffer. 1988. Selective recycling of the mannose 6-phosphate/IGFII receptor to the *trans* Golgi network *in vitro*. *Cell* **55**:309–320.
  15. Goud, B., A. Salminen, N. C. Walworth, and P. J. Novick. 1988. A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. *Cell* **53**:753–768.
  16. Gutierrez, L., A. I. Magee, C. J. Marshall, and J. F. Hancock. 1989. Posttranslational processing of p21<sup>ras</sup> is two-step and involves carboxy-methylation and carboxy-terminal proteolysis. *EMBO J.* **8**:1093–1098.
  17. Hancock, J. F., A. I. Magee, J. E. Childs, and C. J. Marshall. 1989. All *ras* proteins are polyisoprenylated but only some are palmitoylated. *Cell* **57**:1167–1177.
  18. Haubruck, H., C. Disela, P. Wagner, and D. Gallwitz. 1987. The *ras*-related *ypt* protein is an ubiquitous eukaryotic protein: isolation and sequence analysis of mouse cDNA clones highly homologous to the yeast *YPT1* gene. *EMBO J.* **6**:4049–4053.
  19. Haubruck, H., R. Prange, C. Vorgias, and D. Gallwitz. 1989. The *ras*-related mouse *ypt1* protein can functionally replace the *YPT1* gene product in yeast. *EMBO J.* **8**:1427–1432.
  20. Loh, E. Y., J. F. Elliott, S. Cwirla, L. L. Lanier, and M. M. Davis. 1989. Polymerase chain reaction with single-sided specificity: analysis of T cell receptor  $\delta$  chain. *Science* **241**:217–220.
  21. Matsui, Y., A. Kikuchi, J. Kondo, T. Hishida, Y. Teranishi, and Y. Takai. 1988. Nucleotide and deduced amino acid sequences of a GTP-binding protein family with molecular weight of 25,000 from bovine brain. *J. Biol. Chem.* **263**:11071–11074.
  22. Mayorga, L. S., R. Diaz, and P. D. Stahl. 1989. Regulatory role for GTP-binding proteins in endocytosis. *Science* **244**:1475–1477.
  23. Melancon, P., B. S. Glick, V. Malhotra, P. J. Weidman, T. Serafini, M. L. Gleason, L. Orci, and J. E. Rothman. 1987. Involvement of GTP-binding "G" proteins in transport through the Golgi stack. *Cell* **51**:1053–1062.
  24. Miyake, S., and M. Yamamoto. 1990. Identification of *ras*-related, YPT family genes in *Schizosaccharomyces pombe*. *EMBO J.* **9**:1417–1422.
  25. Nakano, A., and M. Muramatsu. 1989. A novel GTP-binding protein, Sar1p, is involved in transport from the endoplasmic reticulum to the Golgi apparatus. *J. Cell. Biol.* **109**:2677–2691.
  26. Pai, E. F., W. Kabsch, U. Krengel, K. C. Holmes, J. John, and A. Wittinghofer. 1989. Structure of the guanine-nucleotide-binding domain of the Ha-ras oncogene product p21 in the triphosphate conformation. *Nature (London)* **341**:209–214.
  27. Polakis, P. G., R. F. Weber, B. Nevins, J. R. Didsbury, T. Evans, and R. Snyderman. 1989. Identification of the *ral* and *rac1* gene products, low molecular mass GTP-binding proteins from human platelets. *J. Biol. Chem.* **264**:16383–16389.
  28. Salminen, A., and P. J. Novick. 1987. A *ras*-like protein is required for a postGolgi event in yeast secretion. *Cell* **49**:527–538.
  29. Schmitt, H. D., M. Puzicha, and D. Gallwitz. 1988. Study of a temperature-sensitive mutant of the *ras*-related YPT1 gene product in yeast suggests a role in the regulation of intracellular calcium. *Cell* **53**:635–647.
  30. Schmitt, H. D., P. Wagner, E. Pfaff, and D. Gallwitz. 1986. The *ras*-related YPT1 gene product in yeast: a GTP-binding protein that might be involved in microtubule organization. *Cell* **47**:401–412.
  31. Segev, N., and D. Botstein. 1987. The *ras*-like yeast *YPT1* gene is itself essential for growth, sporulation, and starvation response. *Mol. Cell. Biol.* **7**:2367–2377.
  32. Segev, N., J. Mulholland, and D. Botstein. 1988. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* **52**:915–924.
  33. Sewell, J. L., and R. A. Kahn. 1988. Sequences of the bovine and yeast ADP-ribosylation factor and comparison to other GTP-binding proteins. *Proc. Natl. Acad. Sci. USA* **85**:4620–4624.
  34. Stearns, T., M. C. Willingham, D. Botstein, and R. A. Kahn. 1990. ADP-ribosylation factor is functionally and physically associated with the Golgi complex. *Proc. Natl. Acad. Sci. USA* **87**:1234–1242.
  35. Touchot, N., P. Chardin, and A. Tavitian. 1987. Four additional members of the *ras* gene superfamily isolated by an oligonucleotide strategy: molecular cloning of YPT-related cDNAs from a rat brain library. *Proc. Natl. Acad. Sci. USA* **84**:8210–8214.
  36. Tuomikoski, T., M.-A. Felix, M. Dorée, and J. Gruenberg. 1989. The cell-cycle control protein kinase *cdc2* inhibits endocytic vesicle fusion *in vitro*. *Nature (London)* **342**:942–945.
  37. Vingron, M., and P. Argos. 1989. A fast and sensitive multiple sequence alignment algorithm. *CABIOS* **5**:115–121.
  38. van Mollard, G. F., G. A. Mignery, M. Baumert, M. S. Perin, T. J. Hanson, P. M. Burger, R. Jahn, and T. Sudhof. 1990. Rab3 is a small GTP-binding protein exclusively localized to synaptic vesicles. *Proc. Natl. Acad. Sci. USA* **87**:1988–1992.
  39. Walworth, N. C., B. Goud, A. Kastan Kabcenell, and P. J. Novick. 1989. Mutational analysis of *SEC4* suggests a cyclical mechanism for the regulation of vesicular traffic. *EMBO J.* **8**:1685–1693.
  40. Willumsen, B. M., K. Norris, A. G. Papageorge, N. L. Hubbert, and D. R. Lowy. 1984. Harvey murine sarcoma virus p21 *ras* protein: biological and biochemical significance of the cysteine nearest the carboxy terminus. *EMBO J.* **3**:2581–2585.
  41. Yeramian, P., P. Chardin, P. Madaule, and A. Tavitian. 1987. Nucleotide sequence of human rho cDNA clone 12. *Nucleic Acids Res.* **15**:1869.
  42. Zahraoui, A., N. Touchot, P. Chardin, and A. Tavitian. 1989. The human Rab genes encode a family of GTP-binding proteins related to yeast YPT1 and SEC4 products involved in secretion. *J. Biol. Chem.* **264**:12394–12401.