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RESEARCH COMMUNICATION

Identification of rab2 as a tubulovesicle-membrane-associated protein in rabbit gastric parietal cells

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Rab proteins, which are ras-like low-molecular-mass GTP-binding proteins, are postulated to act as specific regulators of membrane trafficking in exocytosis and endocytosis. Previously, we reported a 23 kDa tubulovesicle-associated GTP-binding protein in rabbit gastric parietal cells [Basson, Goldenring, Tang, Lewis, Padfield, Jamieson & Modlin (1991) Biochem. J. 279, 43–48]. The major component of the 23 kDa protein is now identified as rab2. Rab2 was co-localized in tubulovesicle membranes from parietal cells. Consistent with GTP-binding activity (as documented before), upon maximal stimulation of parietal cells, rab2 immunoreactivity was redistributed from a 50000 *g* to a 4000 *g* subcellular membrane fraction. The tubulovesicle-associated rab2 behaved as an integral membrane protein, since both 0.5 M-NaCl and 0.1 M-carbonate extraction failed to remove the protein from the tubulovesicle membrane. Utilizing a PCR the rab2 cDNA sequence from rabbit parietal cells was obtained, and it showed only one amino acid difference compared with the human sequence. The results of the present study provide strong evidence that parietal cells possess a rab2 protein which is tightly associated with tubulovesicle membranes.

INTRODUCTION

The classical oligomeric GTP-binding proteins (G-proteins) are well-recognized plasma membrane components that regulate signal transduction through interactions with second messenger systems, such as adenylate cyclase and phospholipases [1]. An increasing number of low-molecular-mass ras-like GTP-binding proteins have also been described in association with various cell structures or components [2,3]. To perform their cellular functions, all GTPases (G-proteins, elongation and initiation factors, and ras-like small GTP-binding proteins) undergo the same unidirectional cycle, in which an active GTP-bound state cycles with an inactive GDP-bound state through a GTPase activity and GDP/GTP exchange [4]. GTP and non-hydrolysable GTP analogues profoundly alter vesicular transport in experimental models of endocytosis and exocytosis [5,6]. These effects have been suggested to be mediated by small GTP-binding proteins. Two such proteins, YPT1p and SEC4p, are required at specific stages in the secretory pathway of yeast [7,8]. Proteins encoded by the *rab* gene family apparently represent the mammalian counterparts of these yeast proteins [9,10]. Recent studies indicate that the rab proteins may act to facilitate or direct the movement of intracellular vesicles [3,11]. Different rab proteins have been localized to particular compartments along both the exocytotic and endocytotic pathways: rab1 and rab2 to an intermediate compartment between endoplasmic reticulum and Golgi [12], rab3A to synaptic vesicles [13], rab4 to early endosomes [11,14], and rab5 and rab7 to early and late endosomes [15]. All of the rab proteins associate with the specific subcellular membranes through polyisoprenylation with geranylgeranyl moieties on *C*-terminal cysteines [16–18].

Gastric acid is secreted from parietal cells by a H^+/K^+-ATPase. In the resting state, this enzyme is incorporated in the tubulovesicles occupying the cytoplasm. Upon stimulation via histaminergic, cholinergic or gastrinergic pathways, the tubulovesicles fuse with target canalicular membranes, generating a massively amplified apical secretory surface. The secretory process of the parietal cell is unique because of this prominent reversible recruitment of the membrane. Both fusion of tubulovesicles with the canalicular membrane and recycling of tubulovesicles out of the secretory surface must be highly regulated. However, the mechanisms which underlie this process remain obscure.

Previously, we have identified a 23 kDa tubulovesicle-associated GTP-binding protein in rabbit parietal cells which redistributed with H^+/K^+-ATPase during parietal cell stimulation [19]. We have now identified this protein as rab2. The results indicate that rabbit gastric parietal cells possess a tubulovesicle-membrane-associated rab2 protein.

MATERIALS AND METHODS

Materials

[α-^{32}P]GTP and ^{125}I-labelled goat anti-mouse IgG were purchased from DuPont/NEN (Boston, MA, U.S.A.). All other chemicals were routinely obtained from Sigma and were of the highest purity available. Monoclonal antibodies against H^+/K^+-ATPase (HK 12.18) were provided by Dr. Adam Smolka (Medical University of South Carolina, Charleston, SC, U.S.A.). Oligonucleotide primers were synthesized by the Yale Department of Pathology DNA Synthesis Laboratory on an Applied Sciences Model 380A synthesizer with subsequent cartridge purification (Rab2FP, GTCCATGGCGTACGCCTATCTCTT; Rab2RP, CACTCAGCAGCAGCCTCCCCCTGCCCT). Polyclonal affinity-purified rabbit antisera against recombinant human rab1p and rab2p were a gift from Dr. N. Touchot [10,20]. Monoclonal antibodies against recombinant human rab3Ap were a gift from Dr. T. Sudhof [21]. Polyclonal antibodies against

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human rab4p and rab6p were provided by Dr. P. van der Sluijs [22]. All anti-rab antibodies demonstrated staining of a single species of appropriate molecular mass on Western transfers of rabbit brain protein (results not shown).

Preparation of rabbit parietal cells and chief cells

Rabbit parietal cells were prepared from rabbit fundic mucosa by collagenase and Pronase digestion followed by Nycodenz (Accurate Chemicals) gradient enrichment by the modified method of Mazzeo *et al.* [23]. Parietal cell preparations contained more than 85% pure parietal cells. Parietal cells showed greater than 95% viability for 4 h after isolation, as assessed by Trypan Blue exclusion. Parietal cells of greater than 95% purity were prepared by further centrifugal elutriation of Nycodenz-isolated parietal cells, as previously described [24].

Preparation of subcellular fractions

Subcellular fractions were prepared from stimulated and unstimulated parietal cells by a modification [19] of the methods of Forte and colleagues [25]. Briefly, the cells were incubated at 37 °C for 45 min in the presence of 100 μM-cimetidine (unstimulated) or 100 μM-histamine plus 10 μM-forskolin (maximally stimulated). After 45 min, the cells were pelleted at 50 *g* for 3 min at 4 °C. The resulting cell pellets were homogenized. Parietal cell subfractions were obtained by differential centrifugation at speed 40 *g* (P0), 4000 *g* (P1), 14500 *g* (P2) and finally 50000 *g* (P3). The final high-speed supernatant (S3) was utilized as the cytosolic fraction. Each pellet was resuspended in homogenization buffer and re-centrifuged to provide washed pellets. Each fraction was then resuspended in buffer containing sucrose (300 mM), EDTA (0.2 mM) and Tris/HCl (5 mM), pH 7.4. Protein concentrations in each fraction were determined by the method of Bradford [26] with BSA as a standard. The 50000 *g* vesicles were further separated into an enriched tubulovesicle fraction and crude microsomal membranes on two sequential discontinuous 20% sucrose gradients, as previously described [19,25]. Coomassie Blue staining of the parietal cell tubulovesicle preparation revealed that greater than 90% of protein staining was accounted for by the α and β subunits of H^+/K^+-ATPase.

Membrane extraction

Utilizing a modification of the method of Cameron & Castle [27], tubulovesicle membrane fraction, prepared as above, was resuspended in either control buffer (sucrose 300 mM, EDTA 0.2 mM, Tris/HCl 5 mM, pH 7.4), 0.5 M-NaCl solution or bicarbonate extraction buffer (Na_2CO_3 100 mM, KCl 100 mM, EDTA 5 mM, pH 10.5). The membrane suspensions were then incubated at 4 °C for 30 min. The soluble and insoluble membrane fractions were separated on an Airfuge (150000 *g*, 5 min). The pelleted membranes were then resuspended in the same respective extraction buffers.

Western blotting

Membrane proteins were resolved on SDS/15%-PAGE gels and transferred to nitrocellulose [28]. The blots were then probed with [α-^{32}P]GTP as described previously [19,29]. GTP-binding bands were visualized by autoradiography (Kodak XRP-1 or XAR-5 film). For two-dimensional blots, proteins were resolved in two-dimensional gels by the method of O'Farrell [30] with pH 3–10 Ampholytes (Pharmacia).

In the case of studies on immunoreactivities of rab2 or H^+/K^+-ATPase, samples of parietal cell membrane proteins were resolved on 15% or 8% gels and electrophoretically transferred to Immobilon-P (Millipore). The blots were then probed with anti-rab antisera at 1:500 dilution or HK 12.18 at 1:2000 dilution respectively, followed by incubation with a secondary ^{125}I-IgG. The specific proteins were visualized by autoradiography.

Rab2 cloning and sequencing

3′ and 5′ oligonucleotide primers were constructed according to sequences for rat and human rab2. The primers were utilized to amplify the sequence from mRNA prepared from > 95% pure parietal cells (FastTrack, Invitrogen) in an RNA/PCR reaction (Perkin–Elmer, Danbury, CT, U.S.A.). Reverse transcription was primed with oligo-dT followed by amplification primed by 150 nM-Rab2FP and -Rab2RP primers (five cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 60 s; 30 cycles at 92 °C for 20 s, 52 °C for 30 s and 72 °C for 45 s). A 630 bp single band was obtained, gel-purified (GCG agarose; FMC, Rockland, ME, U.S.A.) and ligated into a TA cloning pCR1000 vector (Invitrogen). Positive clones were confirmed using PCR amplification of plasmid minipreps. Double-stranded sequencing of plasmids was performed on both strands using Sequenase primed with flanking M13 primers and the original Rab2FP and Rab2RP oligonucleotides. Three separate clones were sequenced and compared with existing sequences in the GenBank registry (GCG, Version 7).

Northern blotting

PCR-amplified rab2 insert (25 ng) was labelled with [α-^{32}P]CTP using a standard random-primer extension reaction (Promega). Total RNA was prepared from rabbit brain and > 95% parietal cells using RNAzol (BIOTECX, Friendswood, TX, U.S.A.). Formaldehyde/agarose gel electrophoresis was carried out with 30 μg of total RNA per lane. The RNA was transferred to Magnagraph (MSI) probed with $(5–10) \times 10^6$ c.p.m. of rab2 probe/ml overnight at 42 °C. Blots were washed to high stringency (65 °C, 0.1 × SSPE/0.5% SDS) and exposed to X-ray film at −70 °C.

RESULTS

Association of rab2 with parietal cell tubulovesicles

A 23 kDa GTP-binding protein was previously described in association with parietal cell tubulovesicles [1]. This 23 kDa protein was resolved as one major and two minor isoelectric

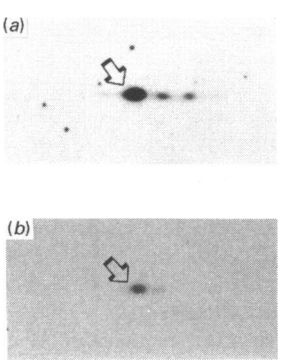

Fig. 1. Rab2 immunoreactivity in tubulovesicles

Identical two-dimensional blots of tubulovesicle protein were evaluated for GTP binding (*a*) and immunoreactivity for rab2 (*b*). The arrows indicate the co-migration of the major species of GTP-binding proteins with rab2 immunoreactivity. The results are representative of four separate experiments.

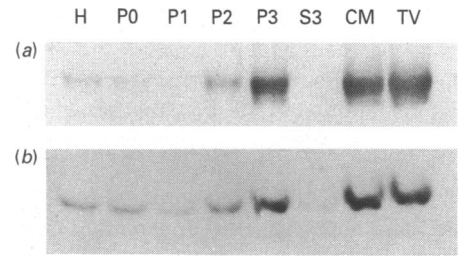

Fig. 2. H^+/K^+-ATPase and rab2 immunoreactivity in parietal cell tubulovesicle subfractions

Tubulovesicles were prepared from isolated parietal cells. The subfractions (30 μg of protein/lane) obtained from differential centrifugation were evaluated for H^+/K^+-ATPase (*a*) and rab2 (*b*) immunoreactivity. H, parietal cell homogenate; P0, P1, P2 and P3, 40 *g*, 4000 *g*, 14500 *g* and 50000 *g* centrifugal membranes pellets respectively; S3, 50000 *g* supernatant; CM, crude microsomes; TV, tubulovesicles. The results are representative of three separate experiments.

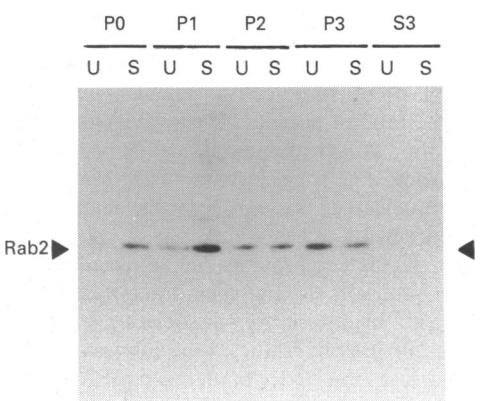

Fig. 3. Rab2 immunoreactivity in parietal cell subfractions during stimulation

Parietal cells were incubated in the presence of 100 μM-cimetidine (U) or 100 μM-histamine plus 10 μM-forskolin (S). Following subfractionation, proteins were resolved by SDS/PAGE (15% gels; 25 μg of protein/lane) and subsequently transferred to Immobilon-P. Rab2 immunoreactivity was then evaluated and was found to be redistributed from light microsomes into the heavier membrane fractions. See the legend to Fig. 2 for definition of fractions. The results are representative of three separate experiments.

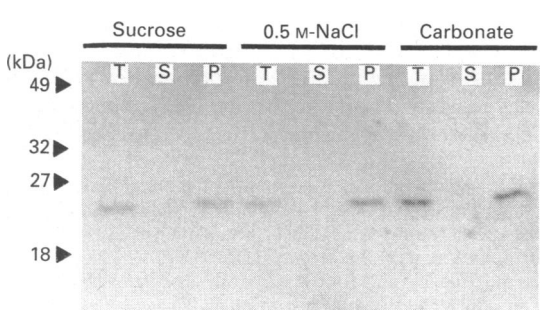

Fig. 4. Rab2 immunoreactivity in parietal cell tubulovesicle membrane extracts

Parietal cell tubulovesicle membrane (20 μg) was incubated in sucrose buffer, 0.5 M-NaCl or carbonate extraction buffer at pH 10.5 (see the Materials and methods section). Rab2 immunoreactivity was evaluated in total membrane proteins (T), soluble membrane proteins (S) and pelleted membranes (P). The results are representative of two separate experiments.

(*a*)

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          201                                                  250
Rab2Rabbit .......AT GGCGTACGCC TATCTCTTCA AGTACATCAT CATCGGCGAC
Rab2Human  CCGCGGCCAT GGCGTACGCC TATCTCTTCA AGTACATCAT AATCGGCGAC
Rab2Rat    .......AT GGCGTACGCC TATCTCTTCA AGTACATCAT CATCGGCGAC
          251                                                  300
Rab2Rabbit ACAGGTGTTG GTAAATCATG CTTATTGCTA CAGTTTACGG ACAAGAGGTT
Rab2Human  ACAGGTGTTG GTAAATCATG CTTATTGCTA CAGTTTACAG ACAAGAGGTT
Rab2Rat    ACAGGTGTTG GTAAATCGTG CTTATTGCTA CAGTTTACAG ACAAGAGGTT
          301                                                  350
Rab2Rabbit TCAGCCAGTG CAGGACCTTA CTATTGGTGT AGAGTTTGGT GCTCGAATGA
Rab2Human  TCAGCCAGTG CATGACCTTA CTATTGGTGT AGAGTTCGGT GCTCGAATGA
Rab2Rat    TCAGCCGGTG CATGACCTCA CAATGGGTGT AGAGTTTGGT GCTCGAATGA
          351                                                  400
Rab2Rabbit TAACGATTGA TGGGAAACAG ATAAAACTAC AGATATGGGA TACAGCAGGT
Rab2Human  TAACTATTGA TGGGAAACAG ATAAAACTTC AGATATGGGA TACGGCAGGG
Rab2Rat    TAACCATTGA TGGGAAACAG ATAAAACTCC AGATCTGGGA TACAGCAGGG
          401                                                  450
Rab2Rabbit CAAGAGTCCT TTCGTTCCAT CACAAGGTCA TATTACAGAG GTGCAGCAGG
Rab2Human  CAAGAATCCT TTCGTTCCAT CACAAGGTCG TATTACAGAG GTGCAGCAGG
Rab2Rat    CAGGAGTCCT TTCGTTCTAT CACAAGGTCA TATTACAGAG GTGCAGCGGG
          451                                                  500
Rab2Rabbit GGCTTTACTA GTGTATGATA TTACAAGGAG AGATACATTC AACCACTTGA
Rab2Human  AGCTTTACTA GTTTACGATA TTACACGGAG AGATACATTC AACCACTTGA
Rab2Rat    GGCTTTACTA GTGTATGATA TTACAAGGAG AGACACGTTC AACCACTTGA
          501                                                  550
Rab2Rabbit CAACATGGTT AGAAGATGCC CGCCAGCATT CCAATTCCAA CATGGTCATT
Rab2Human  CAACCTGGTT AGAAGATGCC CGCCAGCATT CCAATTCCAA CATGGTCATT
Rab2Rat    CAACCTGGTT AGAAGACGCC CGTCAGCATT CCAATTCCAA CATGGTCATC
          551                                                  600
Rab2Rabbit ATGCTTATTG GAAATAAAAG TGATTTAGAA TCTAGAAGAG AAGTAAAAAA
Rab2Human  ATGCTTATTG GAAATAAAAG TGATTTAGAA TCTAGAAGAG AAGTAAAAAA
Rab2Rat    ATGCTTATTG GAAATAAAAG TGACTTAGAA TCTAGGAGAG AAGTGAAAAA
          601                                                  650
Rab2Rabbit AGAAGAAGGT GAAGCGTTGC CAAGAGAACA TGGACTTATC TTCATGGAAA
Rab2Human  AGAAGAAGGT GAAGCTTTTG CACGAGAACA TGGACTCATC TTCATGGAAA
Rab2Rat    GGAAGAAGGT GAAGCTTTTG CACGAGAGCA TGGACTTATC TTCATGGAAA
          651                                                  700
Rab2Rabbit CGTCTGCTAA GACCGCTTCC AATGTAGAAG AGGCATTTAT TAATACAGCA
Rab2Human  CGTCTGCTAA GACTGCTTCC AATGTAGAAG AGGCATTTAT TAATACAGCA
Rab2Rat    CTTCTGCCAA GACTGCTTCT AATGTAGAGG AGGCATTTAT TAACACAGCA
          701                                                  750
Rab2Rabbit AAAGAGATTT ATGAAAAAAT CCAAGAAGGA GTCTTTGACA TTAATAATGA
Rab2Human  AAAGAAATTT ATGAAAAAAT TCAAGAAGGA GTCTTTGACA TTAATAATGA
Rab2Rat    AAAGAAATTT ATGAAAAAAT CCAAGAAGGG GTCTTTGACA TTAATAATGA
          751                                                  800
Rab2Rabbit GGCAAATGGT ATTAAAATTG GCCCTCAGCA TGGTGCTACT AATGCAACAC
Rab2Human  GGCAAATGGC ATTAAAATTG GCCCTCAGCA TGCTGCTACC AATGCAACAC
Rab2Rat    GGCAAACGGC ATCAAAATTG GCCCTCAGCA TGCTGCTACC AATGCATCTC
          801                                                  850
Rab2Rabbit ATGCCGGCAA TCAAGGAGGA CAGCAGGCTG GGGGTGGCTG CTGTTGA...
Rab2Human  ATGCAGGCAA TCAGGGAGGA CAGCAGGCTG GGGGCGGCTG CTGTTGAGTC
Rab2Rat    ACGGAGGCAA CCAAGGAGGG CAGCAGGCAG GGGGAGGCTG CTGCTGA...
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(*b*)

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          1                                                    50
Rab2Rabbit MAYAYLFKYI IIGDTGVGKS CLLLQFTDKR FQPVHDLTIG VEFGARMITI
Rab2Human  MAYAYLFKYI IIGDTGVGKS CLLLQFTDKR FQPVHDLTIG VEFGARMITI
Rab2Rat    MAYAYLFKYI IIGDTGVGKS CLLLQFTDKR FQPVHDLTMG VEFGARMITI
          51                                                   100
Rab2Rabbit DGKQIKLQIW DTAGQESFRS ITRSYYRGAA GALLVYDITR RDTFNHLTTW
Rab2Human  DGKQIKLQIW DTAGQESFRS ITRSYYRGAA GALLVYDITR RDTFNHLTTW
Rab2Rat    DGKQIKLQIW DTAGQESFRS ITRSYYRGAA GALLVYDITR RDTFNHLTTW
          101                                                  150
Rab2Rabbit LEDARQHSNS NMVIMLIGNK SDLESRREVK KEEGEAFARE HGLIFMETSA
Rab2Human  LEDARQHSNS NMVIMLIGNK SDLESRREVK KEEGEAFARE HGLIFMETSA
Rab2Rat    LEDARQHSNS NMVIMLIGNK SDLESRREVK KEEGEAFARE HGLIFMETSA
          151                                                  200
Rab2Rabbit KTASNVEEAF INTAKEIYEK IQEGVFDING EANGIKIGPQ HGATNATNAG
Rab2Human  KTASNVEEAF INTAKEIYEK IQEGVFDING EANGIKIGPQ HAATNATNAG
Rab2Rat    KTASNVEEAF INTAKEIYEK IQEGVFDING EANGIKIGPQ HAATNASHGG
          201  212
Rab2Rabbit NQGGQQAGGG CC
Rab2Human  NQGGQQAGGG CC
Rab2Rat    NQGGQQAGGG CC
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Fig. 5. cDNA sequence and deduced amino acid sequence for parietal cell rab2

(*a*) cDNA sequence of rabbit parietal cell rab2-coding region compared with sequences from human and rat. Numbering designations refer to full-length sequence from human. The rabbit and human sequences differ at only 27 bases (noted in bold/underlined). (*b*) Deduced amino acid sequence of rabbit parietal cell rab2 compared with the amino acid sequences from human and rat. There is only a single amino acid difference between the rabbit and human sequences (position 192; noted in bold/underlined).

species on two-dimensional SDS/PAGE gels (Fig. 1*a*). The same sample resolved on a two-dimensional gel was then transferred to an Immobilon-P membrane and sequentially probed with polyclonal anti-rab2 serum (Fig. 1*b*). The most prominent species visualized with [^{32}P]GTP binding was co-labelled with anti-rab2 antiserum. The two minor species were not consistently labelled with the antiserum. Polyclonal and monoclonal antibodies against rab1, rab3a, rab4 and rab6 did not recognize any of the 23 kDa GTP-binding protein species (results not shown).

These results suggested that the major species of the tubulo-

vesicle-associated small GTP-binding proteins was rab2. We therefore studied the co-fractionation of the H^+/K^+-ATPase and rab2 immunoreactivity in parietal cells. Fig. 2 demonstrates that, among all the parietal cell subfractions, the tubulovesicle fraction showed the most H^+/K^+-ATPase immunoreactivity. Furthermore, rab2 immunostaining completely paralleled the labelling for H^+/K^+-ATPase throughout the tubulovesicle preparation subfractions. Additionally, rab2 immunostaining in parietal cell tubulovesicle membranes co-migrated with immunostaining for rab2 in rabbit brain (results not shown). These results suggest that a rab2-like protein is exclusively associated with tubulovesicles in rabbit parietal cells.

Redistribution of rab2 protein during parietal cell stimulation

In our original studies, we observed a redistribution of the 23 kDa GTP-binding proteins in the subcellular fractions of resting (incubated with 100 μM-cimetidine) compared with maximally stimulated (incubated with 100 μM-histamine and 10 μM-forskolin) parietal cells [19]. Consistent with the GTP-binding activity, rab2 immunoreactivity was found to decrease after stimulation in P3 (50000 *g*) light membranes while increasing in P1 (4000 *g*) heavy membranes (Fig. 3). The P3 membrane fraction is relatively enriched in tubulovesicles, as documented using H^+/K^+-ATPase immunostaining as a marker (Fig. 2). The heavy membrane fraction (P1) is enriched in canalicular membrane, as evidenced by the lack of H^+/K^+-ATPase immunoreactivity and enrichment of ezrin, an 80 kDa apical membrane marker [31]. As previously described [19], the redistribution of rab2 immunoreactivity showed a similar pattern to the translocation of H^+/K^+-ATPase during parietal cell stimulation. The results suggest that rab2 is associated with tubulovesicles and is translocated with H^+/K^+-ATPase during tubulovesicle fusion with the canalicular membrane.

Rab2 as an integral membrane protein associated with tubulovesicles

The rab2 *C*-terminal Cys-Cys sequence has been shown to be modified by geranylgeranyl moieties [18]. We therefore examined the association of rab2 with tubulovesicles. In Fig. 4, purified tubulovesicle membranes (T) were extracted with either 0.5 M-NaCl or 0.1 M-carbonate, pH 10.5, extraction buffer. Rab2 immunostaining remained with the pelleted membrane (P) after extraction at 4 °C for 30 min. No staining was observed in the extraction supernatants (S). The results are consistent with the tight attachment of rab2 into tubulovesicle membranes.

Cloning of parietal cell rab2 cDNA

Rab2 sequences have been determined for rat, human and canine species [9,10]. We therefore constructed oligonucleotide primers based on the known rab2 sequences of rat and human for use in PCR amplification of a rabbit parietal cell cDNA. The reaction yielded a 637 bp product (Fig. 5). The deduced rabbit rab2 sequence diverged from the human product at only one amino acid, at position 192. The derived rabbit rab2 sequence was used to probe total RNA from rabbit brain and parietal cells. The rab2 cDNA probe revealed a major 2.3 kb and a minor 1.4 kb mRNA transcript in both rabbit brain and parietal cells (results not shown).

DISCUSSION

We have previously reported that a 23 kDa GTP-binding protein in rabbit gastric parietal cells was associated with tubulovesicles. The present studies have identified this protein as rab2. Rab2 co-purified in membrane fractionation with H^+/K^+-ATPase. The rab2 protein was tightly associated with the tubulovesicle membrane and was redistributed during parietal cell stimulation, consistent with its constitutive association with proton-pump-containing tubulovesicles. The redistribution of rab2 during stimulation suggests that this protein may be an important candidate regulator of canalicular membrane trafficking.

The ras-like small GTP-binding proteins are localized in distinct intracellular compartments, apparently through signals at their *C*-termini determining specific patterns of isoprenylation-dependent anchoring to cellular membranes. The differential localization of rab protein suggests that these proteins may bear targeting signals regulating vesicular trafficking between components either directly, or indirectly through the activity of their cognate effector proteins. Rab2 has been localized to a post-endoplasmic reticular, pre-Golgi membrane compartment in MDCK cells and NRC cells [3,12]. This localization suggests that rab2 in these cells may play a role in intermediate recycling between the endoplasmic reticulum and the Golgi apparatus. However, the exact function of rab2 in these cells remains unclear. Expression of rab2 was also found to increase during neuronal differentiation in the central nervous system, especially in growth cones [20]. The results of the present study suggest that the rab2 protein is not necessarily localized in the endoplasmic reticulum/Golgi intermediate compartment as seen in MDCK cells. Indeed, the rab2 in parietal cells appears to be intimately involved with the 'exocytotic vesicle' of the parietal cell, the tubulovesicle. These data suggest that localization of specific rab proteins in particular cell systems may be determined by the functional requirements of the system.

Some cellular regulatory proteins, including rab proteins [21], are present as both soluble and membrane-bound forms and exert their function by association–dissociation with the specific membranes. In our present studies, rab2 immunoreactivity was absent from both resting and stimulated parietal cell cytosolic fractions. In addition, the membrane-associated rab2 could not be dissociated by high concentrations of salt or carbonate extraction. Thus it is likely that rab2 is post-translationally modified and is present as a resident protein of the tubulovesicle membrane, which recycles as a membrane-bound component.

The gastric parietal cell is responsible for stimulated acid and intrinsic factor secretion. Stimulation requires the active movement and fusion of intracellular tubulovesicles with a target canalicular membrane surface. Unlike other classical secretory cells, such as pancreatic acinar cells, parietal cells do not possess a prominent endoplasmic reticular/Golgi membrane recycling apparatus. Nevertheless, in this cell system massive yet reversible membrane movement is central to the physiological functioning of the parietal cell. Given the important hypothesized role of these low-molecular-mass GTPases in the sorting of both endocytotic and exocytotic membranes, the presence of rab2 as on tubulovesicles suggests that it may be involved in the processing of membranes into or out of the parietal cell secretory canaliculus.

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