Small GTP-Binding Proteins in Parietal Cells: Candidate Modulators of Parietal Cell Membrane Dynamics

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The stimulated fusion of intracellular H/K-ATPase-containing tubulovesicles with a target canalicular membrane surface is central to the process of acid secretion. A super-family of small GTP-binding proteins (smGTPBPs) has been implicated in many aspects of intracellular dynamics and vesicle membrane trafficking. We have investigated the presence of smGTPBPs in isolated rabbit parietal cells. Parietal cells possess a number of smGTPBP species with molecular masses of 18-28 kDa. One 23 kDa smGTPBP has been localized to tubulovesicles and identified immunochemically as rab2. Rab2 redistributes during stimulation in concert with the movement of the H/K-ATPase. The results demonstrate that specific smGTPBPs are associated with the parietal cell secretory apparatus. Small GTP-binding proteins are important candidate regulators of parietal secretory membrane dynamics.

The stimulation of parietal cell secretion is unique among cells because of the massive reversible recruitment of membrane prior to and during the initiation and maintenance of the secretory process [1-3]. The H/K-ATPase ultimately responsible for the secretion of acid is sequestered within the intracellular tubulovesicles of the resting parietal cell. Upon stimulation, the tubulovesicles fuse with the target canalicular membrane, establishing a greatly expanded secretory canalicular surface through which acid may be pumped into the lumen (Fig. 1). In man and rabbit, this fusion event also releases intrinsic factor, which is packaged within the tubulovesicles. It is therefore apparent that the critical physiological events in parietal cell stimulation-secretion coupling revolve around the recruitment of tubulovesicular membrane into and out of the canalicular target surface. The prominent movement of large amounts of membrane into and out of a secretory surface suggests that critical processing signals must be acting at the level of vesicle targeting. Coordinated mechanisms must exist to provide both the organized vectorial fusion of proton pump-containing vesicles into a secretory canalicular surface, as well as the concerted and directed retrieval of membrane from the canalculus.

Parietal cell secretion can be elicited through at least three separate but apparently interrelated pathways involving histaminergic, cholinergic, and gastrinergic stimuli [4]. In the case of stimulation of the H2-histamine receptor, an increase in intracellular cAMP has been correlated with the onset of secretion [5-7]. Histamine activates type I cAMP-dependent protein kinase [8] and can stimulate the phosphor-
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While both histamine- and carbachol-stimulated phosphoproteins have been identified, no common points of phosphorylation have been recognized, and no specific links with the mediation of secretion have been identified for any of the phosphoproteins. The role of gastrin as a direct stimulant of acid secretion is controversial. Although gastrin can elicit an increase in intracellular Ca\(^{2+}\) in both canine and rabbit parietal cells [14,18], especially in rabbit, it is a poor secretagogue in isolated cell systems [14]. While the three secretagogue systems appear to differ in their initial signal-transduction mechanisms, they all induce the morphological changes that are required for delivery of the proton pump to the gastric lumen [3]. It is therefore reasonable to expect that all stimulatory mechanisms must lead to the initiation of the morphological rearrangement necessary for parietal cell secretion.

**SMALL GTP-BINDING PROTEINS**

Recent investigations over the past several years have revealed the existence of a growing super-family of proteins, all related to the transforming virus product ras protein [19]. This protein family, variously referred to as small GTP-binding proteins, low molecular mass (weight) GTP-binding proteins, small G proteins, or low molecular mass GTPases, represents a diverse group of critical cellular regulatory proteins. These small GTP-binding proteins (smGTPBPs) are unrelated to the...
classical membrane-associated signal-transducing GTP-binding proteins, such as $G_\alpha$ and $G_\beta$. Since the smGTPBP proteins are GTPases, the regulation of GTPase activity and GTP/GDP exchange is critical for the function of these proteins. Rather than possessing a prominent intrinsic GTPase activity like the classical $G$ proteins, however, the function of the smGTPBP proteins appears to be regulated through the modulation of both GTPase and GTP/GDP exchange properties [20]. Regulation of the extremely slow intrinsic GTPase activity of smGTPBP proteins is accomplished through interaction with both GTPase activator proteins (GAPs) [21] as well as with GTP dissociation-stimulating (GDS) proteins [22] (Fig. 2). The GAP proteins, by stimulating GTPase activity, cause “inactivation” of the smGTPBP, while the GDS proteins, by stimulating the exchange of GDP for GTP, cause “reactivation” of the smGTPBP proteins. In addition, recent work indicates that smGTPBP proteins are also associated with GDP dissociation inhibitor (GDI) proteins [23]. Thus, at least four critical points of regulation exist, either directly on the smGTPBP proteins or indirectly through the modification of GAP, GDS, or GDI proteins.

All of the smGTPBP proteins also have a similar basic structural composition. The proteins contain three major domain areas: (1) an “effector” domain where GAP proteins are thought to bind, (2) a highly conserved GTP-binding site, and (3) a hypervariable C terminus which usually contains two terminal cysteine sites for isoprenylation which appear to dictate the specific functions and localization of the individual proteins.

The most detailed examination of smGTPBP proteins has been made in yeast cells of *S. cerevisiae* and *S. pombe*. In these studies, two major smGTPBP proteins, SEC4 and YPT1, which possess approximately 40 percent homology with ras protein, were isolated. The YPT1 protein has been associated with microtubular organization in yeast cells and appears to be a key regulator of the cell cycle [24]. SEC4 was originally characterized as one of the 25 genes required for vesicular secretion in yeast [25]. SEC4 appears to function in regulation of the late stages of vesicular secretion from yeast cells [25].

In mammalian cells, a number of smGTPBP proteins have been identified with varying amounts of homology with both YPT1 and the ras oncoproteins (35–75 percent homology) [19]. The mammalian members of this super-family of related proteins can be roughly divided into four sub-families characterized by their homologies with (1) ras, (2) rho, (3) YPT1/SEC4 (rab1), and (4) raf. The members of the ras-related group, including K-ras, N-ras, rap, and ral, are all involved in regulation of cell division and differentiation. The rho-related group, including rac and G25K, appears to be involved in regulation of cytoskeletal structure. Rho, especially, appears to be
involved in the anchoring of actin filaments to the membrane [26]. The rab family of smGTPBPs was originally discovered through homologies with YPT1 and SEC4, and rab proteins appear to be involved in all aspects of membrane vesicle sorting [27-29]. Finally, the ran protein represents a fourth class of smGTPBP, which is involved in mitotic division and is localized to the nucleus [30]. In contrast to the members of the other three families, ran does not possess the C-terminal cysteine residues that are sites for isoprenylation and membrane insertion.

While these smGTPBPs are structurally related, in the few systems where they have been studied carefully (notably MDCK cells), specific GTP-binding proteins are localized to specific intracellular compartments [31,32]. In particular, the rab family of smGTPBPs has been implicated in the regulation of vesicular transport [19,27]. The family of rab-related proteins appears to be extremely diverse, now encompassing at least 20 separate species. Investigations over the past four years indicate that much of the dynamic regulation of vesicle targeting along both the exocytic and the endocytic pathways in a number of cell systems may be mediated by members of the rab family. The rab3A protein localizes in brain exclusively to small synaptic vesicles [33] and is also present on adrenal chromaffin granules [34,35]. In the adrenal system, rab3 appears to redistribute off granules into the cytoplasm upon stimulation of secretion [35]. In MDCK cells, rab1 and rab2 are associated with the exocytic pathway, whereas rab5 and rab7 are associated with the endocytic system [32]. While all of these investigations suggest that rab proteins within particular cells are associated with discrete compartments, no consistent information exists on common functions across cell systems. Indeed, data exist [20] to suggest that the exact localization of particular members of the gene family might vary from cell to cell, depending on the requirements of the cell machinery involved. Under this hypothesis, particular smGTPBPs, through their regulated GTPase activities, may act as "molecular switches" at appropriate points in individual cell systems. Thus, particular smGTPBPs, as putative signal-transducing modulators, might perform quite different functions in different cell systems.

SMALL GTP-BINDING PROTEINS IN PARIETAL CELLS

In light of the central importance of membrane sorting and regulated movement in the parietal cell, we sought to investigate the distribution and identity of parietal cell smGTPBPs. Many, but not all, of the smGTPBPs renature sufficiently after separation on SDS-PAGE and electrophoretic transfer so as to allow detection on overlays with α-[32P]-GTP. The classical membrane signal-transducing GTP-binding proteins (such as Gα and Gβ) as well as, notably among smGTPBPs, the rho protein, fail to renature on blots. Parietal cells demonstrated a number of 18 kDa to 27 kDa smGTPBPs on blot which differed in their distribution among various membrane fractions [36]. In particular, a 23 kDa protein was a prominent binding species in the 50,000 g light microsomal membranes. In order to assess whether any of the smGTPBP species were involved in the stimulated membrane movement, we studied GTP-binding in subfractions prepared from parietal cells incubated with either 100 μM cimetidine (resting) or 100 μM histamine and 10 μM forskolin (maximally stimulated). Upon stimulation, labeling of the 23 kDa GTP-binding species decreased in 50,000 g membranes while increasing in 4,000 g membranes. A similar stimulated redistribution of the H/K-ATPase was also seen, suggesting that the 23 kDa protein might be associated with tubulovesicles [36]. To determine whether the
23 kDa smGTPBP was associated with tubulovesicles, purified preparations of H/K-ATPase-containing tubulovesicles were obtained from isolated rabbit parietal cells and gastric glands. These preparations demonstrated partitioning of the 23 kDa smGTPBP into tubulovesicle membrane fractions. In contrast, the botulinum C3 exotoxin substrate, the rho protein, was completely excluded from the tubulovesicle fractions, although it was present in all other membrane and cytosolic fractions. The 23 kDa smGTPBP separated on two-dimensional gels into one major and two minor species [36]. All three of these species were partitioned into the tubulovesicle fraction with the greatest enrichment seen for the major species (Fig. 3).

Considering the importance of a tubulovesicle-associated smGTPBP, we next considered the possible identity of the 23 kDa species with a number of known smGTPBPs, using monoclonal and polyclonal antisera against rab1, rab2, rab3A, rab4, rab6, and k-ras [37]. A polyclonal antisera raised against recombinant human rab2 showed specific labeling with the major isoelectric component of the 23 kDa smGTPBP (Fig. 3). The two minor species were not labeled with any of the antisera tested. The immunoreactivity for rab2 exactly paralleled that for H/K-ATPase in parietal cell subfractions prepared during the process of isolation of enriched tubulovesicle membranes (Fig. 4). Co-segregation of rab2 immunoreactivity within H/K-ATPase suggested a tight relationship between the localization of rab2 and H/K-ATPase. We therefore studied the localization of rab2 immunoreactivity in subfractions from resting and stimulated parietal cells. Rab2 immunoreactivity in resting cells was predominantly located in the 50,000 g (P3) membranes, as would be expected for its localization in tubulovesicles (Fig. 5). Fractions from stimulated cells demonstrated a decrease in immunoreactivity in the 50,000 g membranes, however, with a concomitant increase in immunoreactivity in the heavier 4,000 g (P1) membranes. These data were similar to the redistribution of H/K-ATPase seen with stimulation [36]. Significantly, no rab2 immunoreactivity was observed in the soluble fraction in either resting or stimulated cells. These data suggest that rab2 redistributes to the canalicular membrane in concert with the fusion of tubulovesicles. These results contrast with those observed for rab3A, which appears to cycle on and off the membrane of neuronal synaptic vesicles during neurotransmitter release [38].

More recently, we have successfully cloned and sequenced rab2 from rabbit
The deduced amino acid sequence differs from the human at only a single conserved site. In addition, Northern blot analysis demonstrates a tenfold higher level of expression for rab2 mRNA in isolated parietal cells compared to mRNA from isolated chief cells. All these results indicate that rab2 may be an important candidate regulator for tubulovesicle sorting in the parietal cell.

Previous investigations in MDCK cells have assigned rab2 to the intermediate endoplasmic reticular-Golgi membrane compartment [32]. Nevertheless, other studies have localized the protein to neuronal growth cones [39], so that a generalized...
function for the protein has not been determined. Indeed, since the actual target effectors for smGTPBP s have not been determined, it is possible that rab2, as well as other smGTPBP s, may act as multi-functional regulators of membrane and cytoskeletal processing. In the parietal cell, one could anticipate a range of regulatory roles including (1) sorting of H/K-ATPase to an apically directed pathway, (2) stimulated movement of tubulovesicles toward fusion with the secretory canaliculus, or (3) coordinated retrieval of membrane back into tubulovesicles at the cessation of the secretagogue stimulus.

FUTURE IMPLICATIONS

It is important to note that rab2 is not the only smGTPBP associated with the tubulovesicles. Enriched tubulovesicle preparations also demonstrate a prominent 25 kDa GTP-binding species [36]. Indeed, we have recently successfully cloned a number of smGTPBP species from parietal cells, including rab1, rab10, rab11, rab14, rhoA, and the nuclear ran protein as well as one previously unidentified, rab-like protein. Rab11, in particular, appears also to be localized to tubulovesicles. Isolation and characterization of the more precise localization and function of these smGTPBP s in the parietal cell may yield critical information into the fine regulation of membrane processing into and out of the secretory canaliculus of the parietal cell.

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