MOLECULE PAGE

Rab3

John A Williams, Stephen A Ernst and Xuequn Chen

University of Michigan and Wayne State University, Ann Arbor and Detroit MI

e-mail: jawillms@umich.edu


Gene Symbol: RAB3A, RAB3B, RAB3C, RAB3D

1. General Information

Rab proteins constitute the largest family of Ras-related small G proteins and play a role in regulating the specificity of membrane trafficking (63, 78). Because of its potential importance in acinar cell digestive enzyme secretion Rab3 is an important subject for review. Rab3 was originally identified in brain and shown to be localized to synaptic vesicles (24). Because of its localization and homology to the yeast protein Sec4, it was believed to play a role in the terminal steps of secretion. Subsequent molecular cloning revealed four members of the Rab3 family termed 3A (the form originally found in brain), 3B, 3C, and 3D. These four forms show 75-80% amino acid identity with the amino and carboxyl terminal regions being most distinct. All four forms are present in brain, but most other tissues contain one or two forms. Rab3B and 3 D have been found in exocrine, endocrine, epithelial and adipose cells (60). A single form of Rab3 exists in C. elegans and is believed to play a role in neurotransmitter release (47), while in sea urchin eggs a Rab3 is believed to play a role in cortical granule exocytosis (13). Rab3 isoforms have been identified in different tissues and cell types where it has been suggested to play a role in secretion (Table 1). Note that in some cases Rab3 inhibits and in others it stimulates secretion.

Structurally, Rab3 species contain conserved functional domains similar to other Rabs. These include four regions participating in guanine nucleotide binding, and effector region corresponding to the effector region in Ras, and a CXC prenylation motif at the C-terminal (45). The latter is used for the addition of two geranylgeranyl groups to the cysteine residues which attach the Rab3 to membranes (35). The crystal structure of Rab3A in the active configuration or bound to Rabphilin has been determined (19, 50) and Rab3D has been fit to the same model with 6 β-strands and 5 α-helices (45). The structure of the Rabs changes upon binding GTP and mutant Rab3 species have been described similar to Ras that are locked into active and inactive configurations (6). Rab3A and 3D mouse genes possess 5 exons and Rab3D has been localized to chromosome 13 (1,5).

Similar to other small G proteins, the guanine binding state of Rab3 proteins is regulated by guanine nucleotide exchange proteins or factors (GEPs or GEFs) and GTPase activating proteins

This work is subject to a Creative Commons Attribution 3.0 license.
(GAPs). To date, two Rab3 GEFs have been identified. Rab3 GEP was purified from brain and acted on Rab3A, 3C and 3D (72). Although it did not regulate Rab 2, Rab 5, Rab 10 or Rab 11, a recent study indicates it can act on Rab 27A and 27B (23). Rab3 GEP is identical to the human DENN/MADD protein and contains a death domain which can bind the TNFR1 (15). Rab3 GEP knockout mice die at birth and embryonic synaptic transmitter release is impaired (64). The second Rab3 GEF, known as GRAB, interacts with inositol hexakisphosphate kinase and Rab3A and its protein expression is primarily in brain (41). There are no known GEFs specific for individual Rab3 isoforms such as Rab3D. There is one known Rab3 GAP which acts on all four Rab3 isoforms and is broadly distributed in different tissues (26). It is now known to have two subunits including a p130 catalytic subunit. Mutations in p130 cause Warburg Micro syndrome in humans and deletion in mice affects transmitter release and neuroplasticity (59). There is no known specific Rab3 GDI, but rather a common family of Rab GDI isoforms which bind prenylated Rabs in the cytoplasm and participates in insertion and removal from membranes (75). Likewise, Rab Escort Protein plays a role in the recycling of many or all Rab proteins (2). Rab3A activity is also specifically regulated by calcium-calmodulin complex (15).

Considerable attention has been paid to understanding how Rab3A regulates secretion from neurons and neuroendocrine cells. Gene deletion of Rab3A had only subtle electrophysiological effects on transmitter release

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Secretion</th>
<th>Rab3 Isoform</th>
<th>Effect Shown</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Pituitary</td>
<td>Various Hormones</td>
<td>3B</td>
<td>Antisense to Rab3D inhibits secretion</td>
<td>40</td>
</tr>
<tr>
<td>ACTH Cell line</td>
<td>ACTH</td>
<td>3D</td>
<td>N135I mutant blocks secretion</td>
<td>3</td>
</tr>
<tr>
<td>Adrenal Chromaffin Cells</td>
<td>Catecholamines</td>
<td>3A</td>
<td>Overexpression Rab3A inhibits secretion</td>
<td>38</td>
</tr>
<tr>
<td>Hepatocyte</td>
<td>Transcytotic Pathway</td>
<td>3D</td>
<td>Overexpression of Rab3A inhibits while Rab3B enhanced secretion</td>
<td>73</td>
</tr>
<tr>
<td>Islet Beta Cells</td>
<td>Insulin</td>
<td>3A</td>
<td>Localization to vesicles</td>
<td>38</td>
</tr>
<tr>
<td>Lacrimal Acinar Cell</td>
<td>Secretory Component</td>
<td>3D</td>
<td>Overexpression of DN 3D inhibits secretion of secretory component</td>
<td>21</td>
</tr>
<tr>
<td>Mast Cells</td>
<td>Granules</td>
<td>3D</td>
<td>Translocation to plasma membrane upon stimulation</td>
<td>66</td>
</tr>
<tr>
<td>Melanotrophs</td>
<td>MSH</td>
<td>3A, B</td>
<td>Increased capacitance when Rab3A but not 3B injected into cells; DN Rab3A inhibited</td>
<td>58</td>
</tr>
<tr>
<td>Neurons</td>
<td>Neurotransmitter Release</td>
<td>3A, B, C, D</td>
<td>KO of all 4 forms leads to decreased transmitter release</td>
<td>61</td>
</tr>
<tr>
<td>Pancreatic Acinar Cell</td>
<td>Amylase</td>
<td>3D</td>
<td>KO of Rab3A increased quantal release</td>
<td>28</td>
</tr>
<tr>
<td>Parotid Acinar Cell</td>
<td>Amylase</td>
<td>3D</td>
<td>DN Rab3D inhibits amylase release</td>
<td>8, 9</td>
</tr>
<tr>
<td>PC-12 Cell Line</td>
<td>Catecholamines</td>
<td>3A, D</td>
<td>Overexpression of all forms of Rab3D inhibits secretion</td>
<td>46</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>Lysosomal Enzymes, Acid</td>
<td>3D</td>
<td>Rab 3D ** mice show defective bone resorption</td>
<td>52</td>
</tr>
<tr>
<td>RBL-2H3 Cell Line</td>
<td>β-hexosaminidase</td>
<td>3D</td>
<td>DN N135I Rab 3D inhibits secretion</td>
<td>17, 55</td>
</tr>
<tr>
<td>Sperm</td>
<td>Acrosomal Reaction</td>
<td>3A</td>
<td>Rab3D inhibits in permeabilized sperm; Active 3A triggers acrosomal reaction</td>
<td>44</td>
</tr>
<tr>
<td>STC-1 Cell Line</td>
<td>CCK</td>
<td>3A</td>
<td>Overexpression of active Rab3A Q81L mutant inhibits secretion</td>
<td>29</td>
</tr>
<tr>
<td>Endothelial Cells</td>
<td>Weibel-Palade bodies</td>
<td>3D</td>
<td>Overexpression of both WT and mutant Rab3D inhibits secretion of von-Willebrand factor</td>
<td>37, 79</td>
</tr>
</tbody>
</table>
and long term potentiation (7, 28). Because of the presence of multiple forms of Rab3 in neuronal cells, the group of T. Sudhof prepared a mouse model in which all four isoforms of Rab3 were deleted. Although these mice died shortly after birth from respiratory failure (61), their cultured hippocampal neurons showed normal synaptic structure, but reduced transmitter release. This defect could be rescued by a single allele of Rab3A. In a study of chromaffin cells from the same compound knockout mice, the number of large dense core granules was reduced and the number of morphologically docked granules was normal, but the releasable pool was reduced indicating an altered function in priming (62). In a study of C. elegans which has a single Rab3 species, deletion reduced the number of synaptic vesicles at the neuromuscular junction and the amount of transmitter released (47). Another role of Rab3 mediated by its effector Rim is to localize secretion to sites of calcium channels in the presynaptic membrane (27). Overall these studies in neuronal cells indicate Rab3 is involved in vesicle formation and transmitter release. Rab3 has also been reported as a presynaptic target for ethanol sensitivity (4).

Little is known as to how Rab3 exerts its actions. Earlier work focused on the effects of Rab3 effector domain peptides, but these effects were probably the result of peptide insertion into membranes and may not reflect the activity of the intact Rab3 molecule. Current work focuses on the identification of specific Rab effector molecules and how they affect secretion at the molecular level. These possible effectors include syaptotagmin like proteins (Slp1-5), Slp proteins lacking C2 domains (Slac2), rabphillin, Rim, and Noc2 (25). These proteins contain a Rab-binding domain (RBD) which is sometimes referred to as a Slp homology domain. These proteins interact with Rab3 and Rab 27 isoforms and participate in the docking of secretory granules to the plasma membrane.

2. Rab3 and the Exocrine Pancreas

Small G proteins are known to be key regulators of pancreatic digestive enzyme secretion and Rab3 was one of the first to be studied (74). The primary form of Rab3 present in pancreatic acinar cells and on pancreatic ZG is Rab3D based on RT-PCR, Western blotting and mass spectrometry (11, 48, 69). Rab3D is also present in other exocrine glands with large secretory granules including parotid and other salivary glands, lacrimal acinar and gastric chief cells (12, 22,46,48,54,65). Immunohistochemistry localizes Rab3d to the ZG region of acinar cells with no obvious localization to ducts. High resolution confocal microscopy, immunogold electron microscopy, and mass spectrometry localized Rab3D to the outer surface of the ZG membrane where it is attached by isoprenyl groups (10,48,69). Over 90 % of Rab3D in mouse acini is particulate and most of this partitions into Triton X-114, indicating a hydrophobic component, in this case isoprenylation (9,69). Both immunohistochemistry of intact pancreas or acini and staining of granules after isolation indicates that essentially all ZG bear Rab3D as shown for rat and mouse pancreas in Figs 1 and 2. Following stimulation of secretion in rat pancreatic lobules, Rab3 (isoform unknown) was shown to redistribute from ZG to Golgi by EM immunohistochemistry (33). In contrast to rat and mouse pancreatic acinar cells, the AR42J cell line expresses all four forms of Rab3 and these show different density distributions (36,53). Subsequently, Rab3D in AR42J cells was shown to localize to ZG when dexamethasone was used to induce an acinar phenotype (39). Islet beta cells and beta cell derived cell lines also express all four forms of Rab3 (31,56).

Developmental changes in acinar cell Rab3D have also been studied. Rab3D was first detectable in the embryonic rat pancreas at day
18, on day 20-21 was primarily cytosolic, with redistribution to a membrane fraction after birth (68). For reference, ZG appear between

Figure 1. Immunoflourescence localization of Rab 3D in rat pancreas. A. Low power view (bar = 40 µm). B. High power shows outline of individual zymogen granules as indicated by arrowheads (bar = 10 µm) Reproduced from Reference (48).
embryonic day 16-17 and regulated secretion becomes prominent shortly after birth. Rab3D also undergoes developmentally regulated carboxymethylation that decreases at the time when secretion begins (67). The same study showed that dexamethasone induced maturation of AR42J cells was accompanied by an increase in the unmethylated Rab3D protein.

As discussed in Section 1, most studies point to a role for Rab3 in regulating secretion and possibly exocytosis. The secretory granule localization of Rab3D also suggests a role in the secretory process. Early work showed putative effector domain peptides from Rab3A stimulated secretion from permeabilized acini and that this action synergized with GTPyS suggesting an effect on a terminal step in secretion (51). A similar effect was reported when ZGs were mixed with plasma membrane in vitro (20). Subsequent work however has focused on the intact structure and configuration of the G protein. A transgenic mouse study reported enhanced regulated amylase secretion from pancreatic acini of mice overexpressing epitope tagged Rab3D which was targeted to the zymogen granule (49). Because small G proteins can be locked into distinct guanine nucleotide bound configurations, X. Chen et al carried out studies using adenoviral vectors to overexpress mutant Rab3D forms in mouse acini (8). Overexpression of a dominant negative mutants Rab3D T36N or Rab3D N135I inhibited amylase secretion without affecting intracellular Ca2+ signaling while the constitutively active mutant Rab3D Q81L had no effect. Furthermore, the dominant negative mutants did not localize to the ZG, but rather in the basolateral region and upon cell fractionation to the cytosol. This combined with the fact that essentially 100% of Rab3D in acini under basal conditions is already in the GTP bound state, and that the dominant negative mutants reduced this to 25-35% as determined by a GST-Rim pull down assay, suggested that the mutants blocked GDP/GTP exchange (9). Thus the dominant negative Rab3D species could have been binding to a GEF acting on the related Rab protein, Rab 27B, raising questions of specificity that could better be answered by siRNA knockdown. However, such
techniques are difficult on primary cells. In contrast to acini, only about 20% of Rab3A in islet beta cell is in the GTP bound active configuration, but can be increased by overexpressing Rab3 GEP (15).

A Rab3D knockout mouse has also been described (57). These mice did not have an obvious abnormal secretory function and both pancreas and parotid glands responded appropriately to secretagogues. However, the size of the secretory granules were increased and granule volume was calculated to be doubled. The authors concluded that rather than regulating secretion, Rab3D regulated granule formation. Mast cell granule exocytosis was also unaffected. Other evidence supporting a role for Rab3D in Golgi function and granule biogenesis is the finding of Rab3d on cis Golgi of goblet cells and Brunners gland acinar cells of the intestine (71). Another possible explanation of the findings in the Rab3D knockout mouse is that another Rab, such as Rab27B, was redundant with Rab3D and adapted to replace it. It will be interesting to see the result of a double Rab3D/Rab27B knockout.

Several putative effectors have been identified for mammalian pancreas Rab3 isoforms (25). Possible effectors for Rab3D include Noc2 (No C2 domain) and Slp1; Rabphillin and Slp-4/granulophillin are potential effectors for Rab3A in islet beta cells, but have not been reported to be present in acinar cells. GST-Noc2 interacts with GTP-liganded Rab3 and Noc2 knockout mice show acinar cells filled with zymogen granules, but with reduced amylase secretion; the mice also showed reduced insulin secretion (43). Antibody to Noc2 blocks secretion in permeabilized parotid acinar cells (32). However Noc2 may be mediating the effect of Rab27 rather than Rab3. Slp 1 can interact with Rab3D and also with Myosin 5 isoforms and may thereby regulate motility of granules; however, other Rabs also can play this role (18). Valentijn, Valentijn et al (70) suggested that Rab3D is involved in actin polymerization around exocytosing granules, but whether this is related to myosin is unknown. In islet beta cells, Rim2 interacting with Rab3A is required for granule docking and priming (77) while Slp-4 also known as granulophillin binds Rab3A and Munc-18 a SNARE protein regulator (14). Future work is necessary to establish the targets of Rab3D in pancreatic acinar cells.

3. Tools for study of Rab3

a. Antibodies

A number of antibodies are available including ones that react with all Rab3 isoforms and ones specific for individual species. To study Rab3D in pancreatic acini for Western blotting and immunofluorescence in frozen sections we have use a rabbit polyclona antisera prepared by Mark McNiven (Mayo Medical School) against a mouse peptide sequence from the c terminus of Rab3D (48). A similarly prepared antibody is available from Fitzgerald Industries (2OR-1343). Other antipeptide antibodies are available against the amino terminal sequence. A rabbit antibody against recombinant Rab3D has been prepared and used by Robert Raffaniello (39).

b. Plasmid and Viral Vectors

Plasmids coding for human Rab3A, 3B, and 3D with a 3xHA amino terminal epitope tag in pcDNA3.1 are available from Missouri Science & Technology cDNA Resource center (www.cdna.org). Adenoviral vectors coding for WT Rab3D and Q81L, N135I, and T36N mutants have been prepared by us and described (8).

c. Mouse Models

Knockout mice with Rab3D deleted on a C57BL6/J background have been prepared and described (57).
4. References


50. Ostermeier C, and Brunger AT. Structural basis of Rab effector specificity: crystal structure of the small G protein Rab3A complexed with the effector domain of Rabphilin-3A. *Cell* 96: 363-374, 1999. PMID: 10025402


