Small G-protein

There is a larger family of small GTP-binding switch proteins, related to Gα.

G-protein: heterotrimeric G proteins

In addition to heterotrimeric G-proteins, there is a superfamily of small monomeric G-proteins. Their size is around 21 kDa, approximately one half of the average size of the α subunits of heterotrimeric proteins.

These small proteins also operate as switches, which are “ON” when they bind GTP, and “OFF” after the GTP has been hydrolyzed to GDP (which in turn remains bound).

Monomeric G-proteins are activated by proteins which induce a conformational change resulting in reduced affinity to GDP, and thus in GDP release.

The general term for such proteins is GEF (guanine nucleotide exchange factor). The GEFs of the small G-proteins are not the activated receptors, but rather proteins downstream the signaling cascade. GEFs vary with regard to their mode of activation and their selectivity for specific monomeric G-proteins.

Small GTP-binding proteins include (roles indicated):
- initiation & elongation factors (protein synthesis).
- Ras (growth factor signal cascades).
- Rab (vesicle targeting and fusion).
- ARF (forming vesicle coatomer coats).
- Ran (transport of proteins into & out of the nucleus).
- Rho (regulation of actin cytoskeleton)

All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

Generally, GTP binding induces the active state.

Ras was the first small G-protein discovered.
Most GTP-binding proteins depend on helper proteins: GAPs, GTPase Activating Proteins, promote GTP hydrolysis.

A GAP may provide an essential active site residue, while promoting the correct positioning of the glutamine residue of the switch II domain.

Frequently a (+) charged arginine residue of a GAP inserts into the active site and helps to stabilize the transition state by interacting with (−) charged O atoms of the terminal phosphate of GTP during hydrolysis.

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Compared to the α subunit of heterotrimeric G-proteins, GTPase activity of the monomeric G-proteins is very low in the absence of interference. However, association with a protein of the GAP (GTPase-activating protein) type results in very rapid GTP hydrolysis.

As in the case of GEFs, the activation state of GAPs can be regulated, and GAPs are selective with regard to the small G-proteins they affect.

Ras

A single amino acid mutation of Ras is found in more than 30% of all cancers! As many as 90% of certain human tumors like pancreatic carcinomas have mutant Ras!

Ras controls both proliferation and differentiation pathways.

GEF=Guanine nucleotide Exchange Factor
GAP=GTPase Activating Proteins

The cytosol concentration of GTP is 10x that of GDP
**G proteins and Ras-Common**

Signaling switch  
GTP/GDP binding protein  
GTP-binding form is active, while GDP-binding form is inactive  
Intrinsic GTPase activity  
GAPs increase GTPase activity  
Tethered to cell membrane

**G proteins and Ras-Difference**

<table>
<thead>
<tr>
<th>G Proteins</th>
<th>Ras</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subunit:</td>
<td>Trimeric (α, β and γ)</td>
</tr>
<tr>
<td>Activation:</td>
<td>Dissociation (α and βγ)</td>
</tr>
<tr>
<td>Receptor:</td>
<td>Direct binding</td>
</tr>
<tr>
<td>Signaling:</td>
<td>cAMP (PKA)</td>
</tr>
<tr>
<td>PLC-β: DAG (PKC)</td>
<td>IP3 (Ca²⁺)</td>
</tr>
</tbody>
</table>

Dissociated βγ may also activate MAP kinase, and Ras may indirectly induce PLC-γ (via PI3K).

**The regulation of small monomeric G-proteins.**

Ras was the first small G-protein discovered, and is presented here as an example.

**Ras activation**

Discovered  
In Dros.: Sevenless Drk SOS

**Mutations of the ras oncogene are associated with a wide range of human cancers**

The ras oncogene causes oncogenic transformation. Mutations in the ras oncogene are found in a wide range of human cancer.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Number of samples tested</th>
<th>% samples with a mutated ras gene</th>
<th>ras gene found to be mutated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreas adenocarcinoma</td>
<td>156</td>
<td>84</td>
<td>K</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>45</td>
<td>33</td>
<td>K</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>277</td>
<td>44</td>
<td>K</td>
</tr>
<tr>
<td>Thyroid follicular carcinoma</td>
<td>15</td>
<td>53</td>
<td>H, K, N</td>
</tr>
<tr>
<td>Myeloid disorder (AML)</td>
<td>412</td>
<td>35</td>
<td>N, K</td>
</tr>
</tbody>
</table>

Bos, JL (1989) Cancer Res. 49, 4682

These mutations lead to constitutive activation of the Ras signaling pathway.
Ras plays critical roles in the signaling pathway leading to transformation


Ras plays critical roles in the signaling pathway leading to transformation.

Oncogenic Ras mutations in ~30% of all cancers.
- Oncogenic Raf mutations in ~70% of malignant melanomas.
- Over-expression / activation of RTK in many cancers

Downstream of Ras


Ras/MAP Kinase Cascade

MAP kinase cascade
- Serine/threonine kinases
- Activate transcription factor
- Highly conserved cascade
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- Rho (regulation of actin cytoskeleton)

All GTP-binding proteins differ in conformation depending on whether GDP or GTP is present at their nucleotide binding site.

Generally, GTP binding induces the active state.

*Ras was the first small G-protein discovered*
Role of signal transduction pathways in cell locomotion and the organization of the cytoskeleton

Active nuclear transport is driven by Ran

Ran is a small GTPase- binds and hydrolyses guanine nucleotide triphosphate (GTP)
- Ran exists in two conformational forms: Ran - GTP and Ran-GDP
- Ran has weak GTPase activity that is stimulated by RanGAP (GTPase activating protein)
- A guanine nucleotide exchange factor (GEF), called RCC1 stimulates the release of GDP and binding of GTP (higher concentration of GTP in the cell assures preferential binding of GTP)
- The cycle of GTP binding, hydrolysis, release provides energy for nuclear transport
- asymmetric localization of RanGAP and RCC1 assure directionality of transport

Ran = GTPase
GAP = GTPase-activating protein
GEF = Guanine exchange factor
Conformal change → high affinity to NES
Interact with FG-nucleoporins

Exportin-t: bind to tRNA and interact with Ran → export tRNA to cytoplasm

TABLE 13–1 Subcellular Locations of Some Rab Proteins

<table>
<thead>
<tr>
<th>PROTEIN</th>
<th>ORGANELLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rab1</td>
<td>ER and Golgi complex</td>
</tr>
<tr>
<td>Rab2</td>
<td>cis Golgi network</td>
</tr>
<tr>
<td>Rab3A</td>
<td>synaptic vesicles, secretory granules</td>
</tr>
<tr>
<td>Rab4</td>
<td>early endosomes</td>
</tr>
<tr>
<td>Rab5A</td>
<td>plasma membrane, clathrin-coated vesicles</td>
</tr>
<tr>
<td>Rab5C</td>
<td>early endosomes</td>
</tr>
<tr>
<td>Rab6</td>
<td>medial and trans Golgi cisternae</td>
</tr>
<tr>
<td>Rab7</td>
<td>late endosomes</td>
</tr>
<tr>
<td>Rab8</td>
<td>secretory vesicles (basolateral)</td>
</tr>
<tr>
<td>Rab9</td>
<td>late endosomes, trans Golgi network</td>
</tr>
</tbody>
</table>

Plasma Membrane: Vesicle Transport and Fusion

A. Steps in Vesicular Targeting:
1. Transport vesicle with v-SNARE is tethered to target mb by a Rab GTPase.
2. If v-SNARE on vesicle and t-SNARE on target match, then loosely tethered vesicle becomes tightly "docked".

Rab proteins (monomeric GTPase) help ensure the specificity of vesicle docking

B. Machinery Involved:
Rab-GTPases - small GTP binding proteins on vesicles.
Related to the oncogene product Ras.
Act as tethering factors that mediate initial interaction between membrane
Bind to Rab effectors on target membrane.
Over 30 different Rab proteins specific to different membranes.
Another protein (guanine-nuc. exchange factor) catalyzes exchange of GDP bound to cytosolic Rab for GTP, which allows Rab to bind to the transport vesicle.
NSF - (N-ethylmaleimide sensitive factor) a tetramer of identical subunits that binds and hydrolyzes ATP. Required for disassembly of SNARE complex.
SNAPs - (soluble NSF attachment protein). Act as a cofactor mediating NSF attachment to SNAREs.
SNAP NSF Receptors (SNAREs) - a family of cognate membrane proteins. Vesicular (v)-SNAREs on vesicles form complexes with target (t)-SNAREs on target membranes, either on the same membrane (cis) or different membranes (trans). SNAREs alone can cause fusion of membranes, although most likely in cells they act as direct catalysts of fusion along with other regulatory and triggering proteins.
A conserved set of GTPase switch proteins controls assembly of different vesicle coats.

All three coated vesicles contain a small GTP-binding protein

- COP I and clathrin vesicle: ARF (ADP-ribosylation factors), retrograde
- COP II vesicle: Sar I protein, anterograde (ER to golgi)
- ARF and Sar I protein can switch GTP (GDP-protein → GTP-protein active)

There are two sets of small GTP-binding proteins for vesicle secretion. One is ARF and Sar I; another is Rab protein

**ARF (ADP Ribosylation Factor) protein exchanges bound GDP for GTP and then binds to its receptor on Golgi membrane**

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**COP II coated formation**

GTP → Sar1 conformational change → Sar1-GTP binding to membrane → polymerization of cytosolic complexes of COPII subunit on the membrane → formation of vesicle buds

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**Sar1 membrane binding, GTP exchange**

Cytosol → Sar1 attached to Sec23/24 coat protein complex → cargo protein are recruited to the formation vesicle bud by binding of specific short sequence in their cytosolic regions to sites on the Sec23/24 → assembly to second type of coat complex composed of Sec13/31 → completed → Sec23 promotes Sar1-GTP hydrolysis → release Sar1-GDP → disassembly of the coat → transport vesicle

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**Vesicle formation**

Coat assembly controlled by monomeric G-protein (SAR1 or ARF) with fatty acid tail

- GDP-bound SAR1 or ARF are free in cytosol
- Membrane-bound G-protein recruits coat protein subunits
- Assembly of coat pulls membrane into bud → Leads to exposure of fatty acid tail membrane binding Donor membrane contains guanine nucleotide-releasing factor → causes Sar1-GDP SAR1-GTP
Different Rab GTPases & Rab effectors control docking of different vesicles on target membranes: vesicle docking controlled by Rab protein.

Vesicle docking controlled by Rab proteins
Monomeric GTPases attach to surface of budding vesicle
Rab-GTP on vesicle interacts with Rab effector on target membrane
After vesicle fusion GTP hydrolysed, triggering release of Rab-GDP
Different Rab proteins found associated with different membrane-bound organelles

Monomeric Rab-GTPases
A guanine nucleotide exchange factor (GEF) recognizes a specific rab protein and promotes exchange of GDP for GTP.
GTP bound rabs have a different conformation that is the “active” state.
Activated rabs release GDI, attach to the membrane via covalently attached lipid groups at their C-termini and are incorporated into transport vesicles.
Rab-GTP recruits effectors that can promote vesicle formation, vesicle transport on microtubules, and vesicle fusion with target membranes.
After fusion Rab-GTP hydrolyzes GTP to GDP and is released from the membrane. GTPase activating proteins proteins accelerate hydrolysis, reducing the availability of active rabs.

Paired sets of SNARE proteins mediate fusion of vesicles with target membranes.

Analysis of yeast sec mutants defective in each of the >20 SNARE genes.
In vitro liposome fusion assay.
SNARE-mediated fusion → exocytosis → secretory protein
In this case, v-SNARE as VAMP (vesicle associated membrane protein)
 t-SNAREs are syntaxin
 SNAP-25 attached to membrane by hydrophobic anchor.
Formation of four-helix bundle: VAMP (1) / Syntaxin (1) and SNAP-25 (2) has provide one helix SNARE complex had specificity

COP II vesicle formation is mediated by a monomeric GTPase. A GEF in the donor membrane interacts with the GTPase, Sar1, causing GDP/GTP exchange. Sar1-GTP extends a fatty acid tail that inserts into the membrane. COP II assembles on the Sar1 to form a vesicle.
COPII vesicle formation involves a protein called ARF that is analogous to Sar1.
Uncoating of the COPI and COP II vesicles occurs when the G-protein (ARF or Sar1) hydrolyzes GTP and retracts that fatty acid tail. This may not require a GAP but is instead dictated by the rate of hydrolysis intrinsic to the G-protein.
Small G proteins Or small GTPases

<table>
<thead>
<tr>
<th>Subfamily</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ras</td>
<td>Regulates cell growth through serine-threonine protein kinases</td>
</tr>
<tr>
<td>Rho</td>
<td>Reorganizes cytoskeleton through serine-threonine protein kinases</td>
</tr>
<tr>
<td>Arf</td>
<td>Activates the ADP-ribosyltransferase of the cholera toxin A subunit; regulates vesicular trafficking pathways; activates phospholipase D</td>
</tr>
<tr>
<td>Rab</td>
<td>Plays a key role in secretory and endocytic pathways</td>
</tr>
<tr>
<td>Ran</td>
<td>Functions in the transport of RNA and protein into and out of the nucleus</td>
</tr>
</tbody>
</table>

Cycle between an active GTP-bound form and inactive GDP-bound form