

## G Proteins (Heterotrimeric)

### Key References

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### Overview

Heterotrimeric G proteins, comprising  $\alpha$ ,  $\beta$  and  $\gamma$  subunits, respond to extracellular signals generated by activated seven-transmembrane (7TM) receptors by modulating intracellular effector proteins such as enzymes and ion channels. In the inactive state, GDP is tightly bound to the  $\alpha$  subunit of the heterotrimer. Upon receptor activation GDP is exchanged for GTP, followed by  $\alpha$ -subunit dissociation from  $\beta\gamma$  or alternatively their molecular rearrangement to form active  $\alpha$ GTP and  $\beta\gamma$  complexes. Both  $\alpha$ GTP and  $\beta\gamma$  dimers are capable of regulating downstream effector functions.

The duration of the signal is determined by the intrinsic GTP hydrolysis rate of the  $\alpha$  subunit followed by reassociation of  $\alpha$ GDP with  $\beta\gamma$ . In this way, the heterotrimer is prepared for another round of the activation/deactivation cycle. In addition to the intrinsic GTPase activity of the  $\alpha$  subunit, G protein deactivation is accelerated by GTPase activating proteins (GAPs). GAPs for heterotrimeric G proteins include G protein effectors, such as the  $G\alpha_q$ -dependent phospholipase C $\beta$  and the  $G\alpha_{13}$ -dependent p115RhoGEF, as well as the family of regulators of G protein signaling (RGS proteins). RGS proteins display GAP activity towards either  $G\alpha_{i/o}$  or  $G\alpha_{q/11}$  type G proteins, thereby shortening the duration that  $G\alpha$  is GTP bound and  $\beta\gamma$  is free.

A single ligand occupied receptor is able to activate several G protein molecules during the lifetime of a single  $\alpha$ GTP complex. The signal imparted by the binding of a single agonist to its receptor is thus transduced and amplified leading to generation of several active  $\alpha$ GTP and  $\beta\gamma$  molecules during the lifetime of the first  $\alpha$ GTP. The diversification of the receptor signal comes about from: i) a single receptor has the

ability to affect a group of G proteins, such as the  $G\alpha_i/G\alpha_o$ , the  $G\alpha_{q/11}$ , and/or the  $G\alpha_{12/13}$  class; ii) phosphorylation by kinases of receptors may switch their coupling from one G protein class to another and thus allow coupling to additional sets of effector proteins; iii)  $\alpha$  and  $\beta\gamma$  subunits may have different effects in different cells due to expression of different effectors; iv) G proteins and their effectors can be spatially segregated in a given cell, and; v) effector specificity of  $\beta\gamma$  complexes is not exclusively determined by the nature of the  $\beta\gamma$  subunit combination, but depends on the nature of  $G\alpha$  from which  $\beta\gamma$  is released.

$\alpha$  Subunits are encoded in 15 genes and several transcripts are alternatively spliced (five  $\alpha_s$ , two  $\alpha_{12}$ , two  $\alpha_o$  forms). Receptors may discriminate between splice variants, and splice variants may differ in their ability to regulate effector functions. All  $\alpha$  subunits appear to be palmitoylated near the N-terminus. Palmitate turns over and may affect regulation of GTPase activity by GAPs of  $G\alpha$  subunits as well their subcellular localization.

$\beta\gamma$  Dimers are heterogeneous and encoded in five  $\beta$  and thirteen  $\gamma$  genes. Although some dimers do not form, e.g.  $\beta 1\gamma 3$ ,  $\beta 2\gamma 1$ , and  $\beta 3\gamma 1$ , most  $\beta$  and  $\gamma$  subtypes are able to form distinct  $\beta\gamma$  dimers. Structurally,  $\beta$  subunits are seven-blade propellers, each blade formed of a WD40 motif.  $\gamma$  subunits vary from 68 to 75 amino acids and constitute the most heterogeneous of the three subunit families. All  $\gamma$  subunits are polyisoprenylated at their C-termini.

Although a few reports exist showing that a given receptor may require a specific  $\beta$  or  $\gamma$  subunit within the heterotrimer for effector stimulation, it is not known which  $\alpha\beta\gamma$

combinations exist *in vivo*, likewise the factors governing their selective assembly are also not known. Although *in vitro* most  $\alpha$  subunits can associate with most  $\beta\gamma$  dimers, specificity of *in vivo*  $\alpha\beta\gamma$  dimer assembly may be controlled by cell-type specific or temporal regulation of expression.

Pharmacological agonists and antagonists are used to define  $G\alpha$  protein function. They include both the hydrolysis resistant GTP analogs, GTP- $\gamma$ -S and GDP- $\beta$ -S, that hold the  $G\alpha$  subunit in active and inactive conformations, respectively, and various bacterial toxins. CTX (produced by *Vibrio cholerae*) is responsible for the infectious gastro-enteritis known as cholera. CTX irreversibly activates  $G\alpha_s$  by inhibiting its intrinsic GTPase activity. PTX (produced by *Bordetella pertussis*) irreversibly inactivates most members of the  $G\alpha_i$  family by uncoupling them from their cognate receptors.

PTX is responsible for the highly contagious respiratory tract infection known as whooping cough. *Pasteurella multocida* toxin (PMT) offers the possibility to discriminate between  $G\alpha_q$  and  $G\alpha_{11}$  proteins, since it stimulates inositol phosphate formation in a strictly  $G\alpha_q$ -dependent manner. It should be noted however that PMT stimulates a variety of additional cellular signaling events, which are independent of  $G\alpha_q$  protein function, thus limiting its use to dissect cellular signaling pathways. Recently, YM-254890 has been described as a novel, specific, and cell permeable inhibitor of  $G\alpha_{q/11}$  proteins. YM-254890 blocks the exchange of GDP for GTP on  $G\alpha_{q/11}$  but not on  $G\alpha_i$  or  $G\alpha_{15}$  subunits. It is a cyclic depsipeptide isolated from the culture broth of *Chromobacterium* sp. QS3666.

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|  | G $\alpha_s$   | G $\alpha_{i/o}$  | G $\alpha_{q/11}$   | G $\alpha_{12/13}$   | $\beta\gamma$ dimers  |
|--|--|---|---|--|---|
| <b>FAMILY MEMBERS AND STRUCTURAL INFORMATION<sup>a</sup></b> | G $\alpha_{s(s)}$ <sup>b</sup> : 380 aa<br>G $\alpha_{s(L)}$ <sup>b</sup> : 394 aa<br>G $\alpha_{s(XL)}$ : 485 aa<br>G $\alpha_{olf}$ : 380 aa   | G $\alpha_{o(1)}$ : 354 aa<br>G $\alpha_{o(2)}$ : 354 aa<br>G $\alpha_{i1-13}$ : 354 aa<br>G $\alpha_z$ : 381 aa<br>G $\alpha_{t1/2}$ : 350 aa<br>G $\alpha_{gust}$ : 353 aa  | G $\alpha_q$ : 359 aa<br>G $\alpha_{11}$ : 359 aa<br>G $\alpha_{14}$ : 359 aa<br>G $\alpha_{15}$ : 359 aa<br>G $\alpha_{16}$ : 359 aa   | G $\alpha_{12}$ : 359 aa<br>G $\alpha_{13}$ : 359 aa   | $\beta_{1-5}$ : 340-353 aa<br>$\gamma_{1-13}$ : 68-75 aa  |
| <b>EFFECTORS AND EFFECT</b>                                  | G $\alpha_{s(s)}$ <sup>b</sup> : adenylyl cyclases $\uparrow$ , MaxIK channel $\uparrow$ , Src tyrosine kinases (c-Src, Hck) $\uparrow$ , GTPase of tubulin $\uparrow$<br>G $\alpha_{s(XL)}$ : adenylyl cyclases $\uparrow$ , G $\alpha_{olf}$ : adenylyl cyclase $\uparrow$ | G $\alpha_i$ : adenylyl cyclase $\downarrow$ , Rap1 GAPII-dependent ERK/MAPkinase activation $\uparrow$ , Ca <sup>2+</sup> channels $\downarrow$ , K <sup>+</sup> channels $\uparrow$ , GTPase of tubulin $\uparrow$ , Src tyrosine kinases (c-Src, Hck) $\uparrow$<br>G $\alpha_o$ : adenylyl cyclases $\downarrow$ , Ca <sup>2+</sup> channels $\downarrow$ , K <sup>+</sup> channels $\uparrow$ , G $\alpha_z$ : adenylyl cyclases $\downarrow$ , Ca <sup>2+</sup> channels $\downarrow$ , K <sup>+</sup> channels $\uparrow$ , Rap1 GAP GRIN1-mediated activation of Cdc42 $\uparrow$ (G $\alpha_{i,o,z}$ )<br>G $\alpha_t$ : cGMP-PDE $\uparrow$ | Phospholipase C $\beta$ isoforms $\uparrow$<br>p63-RhoGEF $\uparrow$ (G $\alpha_{q/11}$ )<br>Bruton's tyrosine kinase $\uparrow$ (G $\alpha_q$ )<br>K <sup>+</sup> channels (G $\alpha_q$ ) | Phospholipase D $\uparrow$<br>Phospholipase C $\epsilon$ $\uparrow$<br>NHE-1 $\uparrow$<br>iNOS $\uparrow$<br>E-cadherin-mediated cell adhesion: $\uparrow$<br>p115RhoGEF $\uparrow$<br>PDZ-RhoGEF $\uparrow$<br>Leukemia-associated RhoGEF (LARG) $\uparrow$<br>radixin $\uparrow$<br>protein phosphatase 5 $\uparrow$<br>AKAP110-mediated activation of PKA $\uparrow$<br>HSP90 $\uparrow$ | PLC $\beta$ s $\uparrow$<br>Adenylyl cyclases I $\downarrow$<br>Adenylyl cyclases I, II, IV, VII $\uparrow$<br>PI 3 kinases <sup>c</sup> $\uparrow$<br>K <sup>+</sup> channels (GIRK1,2,4) $\uparrow$<br>Ca <sup>2+</sup> (N-, P/Q-, R-type) channels $\downarrow$<br>P-Rex1 (guanine nucleotide exchange factor for the small GTPase Rac) $\uparrow$<br>c-Jun N-terminal kinase (JNK) $\uparrow$<br>Src kinases $\uparrow$<br>Tubulin GTPase activity $\uparrow$<br>G protein-coupled receptor kinase recruitment to membrane $\uparrow$<br>Protein kinase D $\uparrow$<br>Bruton's tyrosine kinase $\uparrow$<br>p114-RhoGEF $\uparrow$ |
| <b>EXPRESSION</b>  | G $\alpha_s$ : ubiquitous<br>G $\alpha_{olf}$ : olfactory epithelium, certain CNS ganglia  | G $\alpha_{o(1/2)}$ <sup>b</sup> : neurons, neuroendocrine cells, astroglia, heart<br>G $\alpha_{i1-13}$ : neurons and many others<br>G $\alpha_z$ : platelets, neurons, adrenal chromaffin cells, neurosecretory cells<br>G $\alpha_{t1}$ : rod outer segments, taste buds<br>G $\alpha_{t2}$ : cone outer segments<br>G $\alpha_{gust}$ : sweet and/or bitter taste buds, chemoreceptor cells in the airways  | G $\alpha_{q/11}$ : ubiquitous<br>G $\alpha_{15/16}$ : hematopoietic cells  | Ubiquitous   | $\beta_1\gamma_1$ : retinal rod cells<br>$\beta_3\gamma_8$ : retinal cone cells<br>$\beta_5$ : neurons and neuroendocrine organs<br>$\beta_5(L)$ : retina<br>but most cell types express multiple $\beta$ and $\gamma$ subtypes   |
| <b>PHARMACOLOGICAL MODULATION (TOXIN SITE OF ACTION)</b>     | G $\alpha_s$ (generic): CTX (Arg <sup>201</sup> )<br>G $\alpha_{olf}$ : CTX (Arg <sup>188</sup> )<br><b>(C8052)</b>  | G $\alpha_{o(1/2)}$ <sup>b</sup> : PTX (Cys <sup>351</sup> ) <b>(P7208)</b><br>G $\alpha_{i1-13}$ : PTX (Cys <sup>351</sup> )<br>G $\alpha_z$ : Not found<br>G $\alpha_{t1/2}$ : PTX (Cys <sup>347</sup> ) CTX (Arg <sup>174</sup> ) <b>(C8052)</b><br>G $\alpha_{gust}$ : PTX (Cys <sup>350</sup> )  | G $\alpha_{q/11}$ : YM-254890<br>G $\alpha_q$ : (PMT)<br>G $\alpha_{14}$ : Not found<br>G $\alpha_{15}$ : Not found<br>G $\alpha_{16}$ : Not found  | G $\alpha_{12}$ : Not known<br>G $\alpha_{13}$ : Not known   | $\beta\gamma$ dimers: Not known   |
| <b>DISEASE RELEVANCE</b>                                     | G $\alpha_{s(XL)}$ : brachydactyly, trauma-related bleeding tendency, neurological problems<br>G $\alpha_s$ : McCune-Albright syndrome, pseudohypoparathyroidism type Ia/b, testotoxicosis, adenomas of pituitary and thyroid, cholera                                       | G $\alpha_i$ : pertussis, adrenal and ovarian adenomas<br>G $\alpha_t$ : congenital cone dysfunction, night blindness   | G $\alpha_{q/11}$ : dermal hyperpigmentation and melanocytosis?   | Not known  | G $\beta_3$ : atherosclerosis, essential hypertension, metabolic syndrome   |
| <b>Abbreviations:</b>  | <b>CTX:</b> Cholera toxin  | <b>PMT:</b> Pasteurella multocida toxin   | <b>PTX:</b> Pertussis toxin   |  |   |

## FOOTNOTES

**a** G $\alpha$  subunit nomenclature: G $\alpha_s$  and G $\alpha_i$  are so named for stimulation and inhibition, respectively of adenylyl cyclases: G $\alpha_o$  is so named for other, identified as a PTX-sensitive non G $\beta$  protein with unknown function. **b** Two splice variants of G $\alpha$  genes. **c** Blocked by wortmannin (**W1628**) and LY-294002 (**L9908**).