# G-protein cycle and its regulation by rgs proteins

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G Proteins, also known asguanine nucleotide-binding proteins, are a family of membrane resident “ go-between” proteins that are important molecular switches in the mediation of GPCR signalling [1] . In their inactive state, G-proteins exist as heterotrimeric complexes composed of α, β and γ-subunits. Upon its stimulation, a GPCR will catalyse the GDPïƒ GTP exchange at Gα leading to the dissociation of the trimer complex as a Gα-subunit and the Gβγ-dimer, both able to interact with a number of effector systems responsible for cellular responses. Upon hydrolysis of GTP to GDP+P by Gα, the G-protein mediated signalling is terminated, whereby a group of proteins, the regulators of G-protein signalling (RGS) appear to play substantial role [1, 2] .

Every organ system utilises G-protein mediated signal transduction evoking such diverse outcomes as neurotransmission, immunity, cardiovascular function and hormone secretion [3] . Consequently, GPCRs present a variety of opportunities as therapeutic targets for treating cancer, cardiac dysfunction, central nervous system disorders and pain. In fact, drugs targeting members of this protein superfamily account for 40% of all prescription pharmaceuticals on the market [2] .

GPCRs constitute the largest and most diversefamily of heptahelical transmembranereceptorsthat receive a signal (e. g. small peptides, lipid analogues, amino-acid derivatives, and sensory stimuli such as light, taste and odour [2] )from outside thecelland transmit this signal to the cell interior via interactions with G-proteins leading to activation of downstream effector

systems [4] . In mammals 21 Gα-subunits, six Gβ-subunits and twelve Gγ-subunits have been described [5] . Depending on their Gα similarity, G-proteins are grouped into four main classes: $G\alpha_{s}$ , $G\alpha_{i/o}$ , $G\alpha_{q/11}$ and $G\alpha_{12/13}$ which show selectivity with respect to both, receptors and effectors due to the presence of recognition domains complementary to G-protein binding domains in receptors/effectors [6] . The main targets for G-proteins include *adenylyl cyclase* , *phospholipase isoforms* , *Rho A/Rho kinases (* a system that controls mainly signalling pathways involved in cell growth/proliferation), and the *mitogen activated protein kinase (* involved in the control of many cell functions such as cell division), and ion channels [7, 8] .

In its inactivated state the complex is freely diffusible in the plane of the cell membrane due to fatty acid chain anchors on each subunit [7] . Stimulation of GPCRs by agonists leads to conformational changes in the receptor resulting in the acquirement of high affinity to the G(αβγ) complex. Due to their subsequent association, a GDP-> GTP exchange in the α-subunit will occur leading to dissociation of the G-protein complex from the receptor in form of a Gα(GTP)-subunit and a Gβγ-dimer. Prior the activation of the G-protein the Gβγ-dimer is bound to a hydrophobic pocket present in Gα-GDP. GTP binding to Gα removes the hydrophobic pocket and reduces the affinity of Gα for Gβγ [9] . Both have a signalling function and can interact with various downstream effector systems [7, 9] . The duration of G protein-mediated effector activation is dependent on the intrinsic GTPase activity of the Gα-subunit. GTP-

hydrolysis results in dissociation of Gα(GDP) from the effector to reunite with Gβγ completing the cycle [7] .

Several studies have shown that the kinetics of G-protein signalling are regulated by RGS proteins that can not only act as GTPase activating proteins (GAPs) on Gα-subunits hereby accelerating GTP-hydrolysis, but also as scaffolds to help assemble signalling complexes and providing a critical mechanism of regulation of cellular responses [10] .

Over 30 RGS/RGS-like domain containing proteins have been described and classified into nine distinct subfamilies based on primary sequence homology and presence of additional domains, including the A/RZ (prototype RGSZ), the B/R4 (prototype RGS4), the C/R7 (prototype RGS7), the D/R12 (prototype RGS12), the E/RA (prototype Axin), the F/GEF, G/GRK, H/SNX and I/D-AKAP2 subfamilies [3, 10, 11] . They differ widely in their overall size and amino acid identity, and many family members possess a remarkable variety of structural domains and motifs that regulate their actions and/or enable them to interact with other binging partners with diverse cellular roles.

RGS proteins have a highly conserved RGS domain of 120 amino acids [3, 11] which allows for selective binding to the transition state of Gα(GTP-> GDP+P) [8] , accelerating the GTP-hydrolysis up to a 1000-fold [5, 10] by stabilising this transition. Some studies have shown that RGS proteins can also act as effector antagonists by binding tightly to Gα(GTP), hereby blocking effector activation [5, 11] .

It has been suggested that simple RGS proteins (those of A/RZ and B/R4) have an almost exclusively negative regulatory function acting as modulators of G-protein signalling as for instance shown by the function of RGS4, an effective GAP protein for $G\alpha_q$ family members. In mammalian cells RGS4 doesn't block the receptor and $G\alpha_{q/11}$ -directed inositol lipid/$Ca^{2+}$ signalling completely but elicit rhythmic $Ca^{2+}$ oscillations in mammalian cells [10, 11] . On the contrary, the larger RGS proteins can link active Gαs to other signalling pathways and therefore serve as multifunctional integrators. Integration can occur via activation of kinases, recruitment of cellular scaffolds/associated proteins or by direct receptor interactions [11] . Two of the R12 family members (RGS12 and RGS14) were shown to coordinate components of the Ras/Raf/mitogen-activated protein kinase signalling pathway [8, 10] .

RGS proteins display specificity and selectivity in their interactions not only with G-proteins, but also GCPRs, ion channels and other signalling events [3, 5] which may be accomplished by firstly, differences in GAP activity towards different types of Gα-subunits (e. g. RGS19 was shown to interact strongly with $G\alpha_{i1,}$ $G\alpha_{i3}$ and $G\alpha_o$ , weakly $G\alpha_{i2}$ but not with $G\alpha_s$ and $G\alpha_q$ [5] ; secondly, the ability to interact with specific GPCRs or with effectors within the GPCR signalling axis directly, due to presence of characteristic structural domains and motifs (e. g. RGS2 and RGS4 bind selectively the 3 rd intracellular loop of M2 and M5 muscarinic receptors [5] ); thirdly, by formation of an RGS/G-protein complex that prevents the G-protein from binding its receptor or the downstream effectors; and lastly, by co-expression of the

RGS-proteins with its target protein(s) in order for selective interactions to take place. An example is illustrated by the expression of the alternative spliced RGS9-1 and RGS9-2 in entirely different tissues, thus having different functions and selectivity for different targets. While RGS9-1 is expressed in the photoreceptor cell layers of the retina and is involved in the phototransduction pathway by regulation of photoreceptors, RGS9-2 is predominantly found in the brain and shows selectivity for the regulation of dopamine D2 and opioid μ receptor signalling pathways [5] .

RGS proteins play and essential regulatory role in G-protein mediated signal transduction, being able to regulate a great number of GCPR signalling events with great specificity and accuracy. By fully understanding the mechanisms and the significance of their expression, role and targets it can lead science to advances in the development of novel therapeutic drugs against disorders involving G-protein mediated signalling.

# References

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