Mechanisms of G protein-coupled receptors regulation

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Abstract

Within the last two decades of studies in the ever-expanding field of GPCR signaling, challenging insights were adopted. Growing evidence now asists the shift from classical linear model of signaling towards a considerably complex network of signaling pathways with many shared proteins and cross-talks. Considering the extensive and intriguing network of pathways activated by these receptors, it is apparent that multi-level system of regulation must exist to rigorously modulate the amplitude, duration and spatial aspects of the GPCR signaling. This review summarizes the principal mechanisms of GPCR regulation and gives the overview of recent advances in this field of research.

INTRODUCTION

G protein-coupled receptors (GPCRs), the largest superfamily of membrane-spanning proteins with heptahelical structure, are involved in wide variety of cell functions (Shenoy & Lefkowitz 2003). As signaling proteins they respond to various extracellular stimuli such as sensory stimuli, chemokines, local mediators, hormones and neurotransmitters (Xiao *et al.* 2010), by coupling to their intracellular partners (mostly to heterotrimeric G proteins), thus activating multiple associated signaling pathways (Cabrera-Vera *et al.* 2003). GPCRs, as dynamic structures, show a capacity to change their conformation in miliseconds or seconds according to present ligand specificity, this fact being fundamental for GPCR signaling and regulation.

In last two decades, our knowledge in GPCR signaling and regulation has become more and more complex as forth as in present, somewile, we are overloaded with information. In this review we will focus on mechanisms of GPCR signaling regulation rather than on description of a wide range of downstream signaling pathways (for extensive review about multiple GPCR signaling see (Kristiansen 2004)). To date, there is evidence that signaling specificity can be controlled at any level: at the receptor level as well as at the level of signalling components. It has been demonstrated that the enhanced or lowered activation of certain receptor type brings about changes not only in the density of this particular receptor type in cell membrane by producing its down- or up-regulation (i.e. homologous regulation – (Nathanson 1989)), but also changes of macromolecules involved in the post-receptor steps of signal transduction (Schulte & Levy 2007), and changes of receptors with synergistic or antagonistic function (i.e. heterologous regulation or cross-talk, cross-regulation) (Lee & Fraser 1993, Hur & Kim 2002, Vázquez-Prado *et al.* 2003). Besides down- or up-regulation of receptor expression, regulation at the GPCR level comprises desensitization and resensitization processes which change the availability of the receptor to respond to stimuli presentation.

REVIEW ARTICLE

GPCR SIGNALING

G protein-coupled receptors (GPCRs) are integral membrane proteins which show structural similarities in possessing seven α-helical transmembrane domains (GPCRs are also called 7-TM receptors). These receptors include binding sites for many structurally diverse endogenous ligands. Therefore they are implicated in numerous physiological functions. Although not all members of this superfamily are known and characterised to date, over 800 have been predicted from genome sequencing (Fredriksson *et al.* 2003). GPCR signaling mechanism is typically based on the classical functional model: stimuli receiver or receptor– transducer – effector, the transducer in this case being mostly presented by G rpotein (Pierce *et al.* 2002). G proteins associating with GPCRs were shown to be heterotrimeric structures composed of three subunits. The α subunit is responsible for GTP or GDP binding and for GTP hydrolysis, β and γ subunits are tightly associated forming a β/γ dimer (Milligan & Kostenis 2006). Agonist binding to the receptor induces conformational changes resulting in increased affinity for the G rpotein. The activated receptor thus serves as a guanine-nucleotide exchange factor (GEF) and enhances the release of GDP from its binding site on the α subunit allowing its immediate replacement by GTP (Pierce *et al.* 2002). In so activated heterotrimer, the affinity of α subunit for the β/γ dimer is reduced and the complex dissociates. The so released subunits act as activators or inhibitors of downstream signaling effectors on the independent manner (Cabrera-Vera *et al.* 2003) (see Figure 1). Their activated state lasts until GTP is hydrolyzed to GDP, this process being under control of RGS (regulators of G rpotein signaling) proteins. Once GTP is cleaved to GDP, the α-GDP and β/γ subunits reassociate and the activation cycle is terminated.

Classicaly, GPCRs, via G rpotein dependent signaling pathways, transmit the extracellular signal to downstream effectors (i.e. phospholipase C(PLC) or adenylate cyclase (AC)) rapidly modulating the production of intracellular signaling molecules (inositol phosphates and diacylglycerol or cyclic AMP) (Cabrera-Vera *et al.* 2003). None the less, G rpotein independent mechanisms of GPCR signaling involving direct coupling of the receptor to downstream effector proteins have been shown. Because of the frequent need of the receptor membrane anchorage, these interactions are highlighting the importance of many scaffolding proteins in GPCR signaling and regulation.

GPCR REGULATION

The exposure of GPCRs to agonist not only mediates activation or inhibition of various effectors in downstream signaling pathways, but also triggers attenuation in receptor responsiveness. Short-term loss of receptor sensitivity to the appropriate stimuli is reffered to as desensitization, whereas long-term loss of sensitivity presents a phenomenon called downregulation of receptor expression. On the other hand, the impossibility of agonist binding (blockade by antagonist, denervation, and impaired release of transmitter) can lead to the sensitization and/or to upregulation of receptor number. Desensitization and downregulation provide a negative feedback mechanism that protects against overstimulation of the receptor, while resensitization, sensitization and upregulation allow a positive feedback mechanism that ensure preservation of receptor responsiveness though decreased agonist stimulation.

DESENSITIZATION

GPCRs control their own responsiveness by activating mechanism leading to their desensitization. This process consists primarily in dissociation of the receptor/G rpotein complex which is predominantly the consequence of receptor phosphorylation mediated by two classes of protein kinases: second messenger-dependent kinases (i.e. PKA and PKC) and GPCR kinases (GRKs). The two phosphorylation processes mentioned above differ by their mechanisms of action. For both ot them, phosphorylation takes place on serine and/or threonine residues of GPCRs, but these sites are quite different for each of them. In addition, phosphorylation by GRKs only occurs at agonist-bound receptors (**homologous desensitization**), whereas second messenger-dependent kinases are recruited to phosphorylate both active and inactive receptors (**heterologous desensitization**). In the latter case, receptors that have not been ligand activated may be desensitized as a result of another receptor type activation (Claing *et al.* 2002). Moreover, homologous desensitization is rather associated to higher agonist concentrations while heterologous mechanism of desensitization is suggested to be induced by lower agonist concentrations (Hendriks-Balk *et al.* 2008, Hull *et al.* 2010).

HETEROLOGOUS DESENSITIZATION – ROLE OF PKA AND PKC IN GPCR REGULATION

One of the way by which GPCRs may be desensitized is the heterologous desensitization permitting the attenuation of signaling not only in the case of activated receptors but also in that which have not been exposed to agonist (Ferguson 2007). Indeed, second messengers (e.g. AC, PLC) produced by agonist stimulation of one GPCR may enhance activation of protein kinases phosphorylating other GPCRs (see Figure 1). The best studied examples of these second messenger-dependent kinases are proteinkinase A (PKA) and proteinkinase C (PKC). Phosphorylation by PKA and PKC occurs on serine and/or threonine residues contained in the third intracellular loop and/or the C-terminus of the receptor (Hendriks-Balk *et al.* 2008). Moreover, the phosphory-

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lated sequences are localized near the G rpotein contact sites whence it follows that phosphorylation is in direct competition with G rpotein coupling (Kristiansen 2004).

Contrary to phosphorylation by GRKs, heterologous mechanism of desensitization does not promote β-arrestin binding (Moore *et al.* 2007) but is thought to be responsible of GPCRs internalization via lipid rafts and/or caveolae (Rapacciuolo *et al.* 2003).

Another type of heterologous regulation can be heterologous down- or up-regulation comprising changes in gene expression and/or translation processes (see related paragraphs below), often caused by cytoplasmic receptors (e.g. (Kasahara *et al.* 2011)).

HOMOLOGOUS DESENSITIZATION – ROLE OF GRKS IN GPCR REGULATION

Another process contributing to attenuation of GPCR signaling is the homologous desensitization.

Phosphorylation of the receptor is there ensured by G rpotein coupled receptor kinases (GRKs), key modulators of GPCR signaling. The GRK protein family consists of seven mammalian serine-threonine protein kinases (Yang & Xia 2006) phosphorylating and so regulating exclusively the agonist-occupied receptors (Premont *et al.* 1995). The GRKs family membres can be subgrouped according to sequence homology and functional similarities to three subfamilies. Members of the first subfamily, GRK1 (rhodopsin kinase) and GRK7 (cone opsin kinase,) are only found in retinal cells. Members of two other sub-groups are non-visual: GRK2 (β-ARK1) and GRK3 (β-ARK2)composing the subfamily with better known characteristics, and GRK 4, GRK5 and GRK6, whose roles are studied more profoundly today.

GRKs consist of more than 500 amino acid residues. As well as in the case of heterologous desensitization, GPCR phosphorylation by GRKs occurs at residues contained either in the carboxy-terminal tail (rhodopsin, β_2-AR) or the third intracellular loop of the receptor (e.g. M_2 muscarinic acetylcholine receptor), but the serine and/or threonine residues concerned are different from those phosphorylated by second messenger-dependent protein kinases (Hendriks-Balk *et al.* 2008). Both visual and non-visual GRKs share a similar structure, in particular at the highly conserved central catalytic domain containing D-L-G sequences, and at the level of N-terminal region. Because of considerable homology of 185-amino acids region, this latter is thought to be implicated in receptor recognition and, in addition, it embodies an RGS domain providing a phosphorylation-independent mechanism of receptor attenuation (Yang & Xia 2006, Brinks & Eckhart 2010).

In unstimulated cells are kinases predominantly localized to the cytosol and become associated with the plasma membrane after GPCR activation. GRK4, GRK5 and GRK6 exhibit substantial membrane localisation in the absence of agonist stimulation (Premont *et al.* 1995). The basis of these properties could be hypothesized from the structure-functional relationship of GRKs at C-terminus, which has been demonstrated to be the key structure for membrane targeting. GRK5 and GRK6 are not able to interact with βγ subunits of G proteins via a carboxy-terminal plecstrin homology domain as GRK2 and GRK3 do, but associate with the membrane by protein palmitoylation (GRK4 and GRK6) or electrostatic interaction between C-terminal aminoacids and membrane phospholipids (GRK5). For GRK1 the membrane association is facilitated by post-translational farnesylation of its carboxy-terminal CAAX motif. Moreover, β-ARKs are able to interact with PIP_2 (Touhara *et al.* 1995).

Contrary to heterologous desensitization, phosphorylation by GRKs promotes β-arrestin binding (Moore *et al.* 2007) and GRKs thus play an important role not only in GPCRs desensitization but also in receptor internalization (see Figure 1).

ARRESTINS – MORE THAN KEY REGULATORS OF GPCR DESENSITIZATION

The arrestin family comprises four members. Visual arrestins, arrestin 1 and arrestin 4, are expressed almost exlusively in the retina. Non-visual arrestins, arrestin 2 (β-arrestin1) and arrestin 3 (β-arrestin2) are expressed ubiquitously in most tissues (Patel *et al.* 2009). As the visual information has some specific properties that are not common to the GPCR regulation process, here, we will focus on β-arrestins only.

In unstimulated cells, despite their recruitment as well on plasma membrane as in the nucleus, β-arrestins are localized in the cytoplasm. These proteins are long ago known by their capacity to desensitize the activated receptor. On agonist stimulation of GPCR, they translocate to the cell membrane and bind to the cytoplasmic side of the activated receptor (Gurevich & Gurevich 2006). The affinity of the receptor for arrestin proteins is increased by the receptor phosphorylation mediated by GRKs (Moore *et al.* 2007) (see Figure 1). The binding of arrestin prevents further receptor/G rpotein interaction thereby reducing or preventing receptor signaling (Claing *et al.* 2002). Indeed, GRK phosphorylation alone, without presence of arrestins, has a weak effect on the uncoupling of the receptor/G rpotein complex.

Besides its role in homologous desensitization, β-arrestin has been shown to take an important part in receptor internalization and its subsequent fate in subcellular trafficking (see the related paragraph below). Moreover, accumulating evidence reflects the capacity of β-arrestins to interact with several cytoplasmic proteins via their ability to function as scaffolders, thereby linking GPCRs to various signaling pathways entirely independent of G rpotein activation such as MAPK cascades (Gurevich & Gurevich 2006, Ma & Pei 2007, Patel

et al. 2009). Furthermore recent studies have also indicated that in response to activation of certain GPCRs, β-arrestins associate with transcription cofactors (in the nucleus) or their regulators (in the cytoplasm) and thus play important roles in cell growth, apoptosis and immune functions (Ma & Pei 2007). Finaly, increasing evidence demonstrates that non visual arrestins may also influence endocytosis of non 7TMRs and thus regulate signaling via receptors of different types than the GPCRs (example of IGF-1R. see (Shenoy & Lefkowitz 2011).

PHOSPHORYLATION-INDEPENDENT MECHANISMS OF GPCR REGULATION

Besides the phosphorylation mediated desensitization, recent studies show that phosphorylation-independent mechanisms may also take part on the GPCR signalling attenuation (Ferguson 2007). Indeed, the N-terminal region of all GRKs embodies an RGS domain which has been shown to participate on phosphorylation-independent regulation of GPCRs. This mechanism of receptor regulation was demonstrated above all for GRK2 and GRK3 (Carman *et al.* 1999).

INTERNALIZATION AND POSTENDOCYTIC SORTING OF GPCRS

Long-term desensitization (minutes to several hours) of GPCR leads to the receptor internalization also termed receptor sequestration or endocytosis. The endocytic process is important not only in attenuation of GPCR signaling in persisting agonist stimulation but also plays a major role in resensitization and downregulation of the receptor (Luttrell & Lefkowitz 2002).

Internalization

The internalization of the receptor is an agonist-dependent process which promotes the removal of agonist-activated cell surface receptors from the plasma membrane to a membrane-associated intracellular compartment. This process is mediated by serine and threonine phosphorylation and arrestin binding (Marchese *et al.* 2008). From the biochemical view, the GPCR internalization is charachterized as a decrease of receptor binding sites on the plasma membranes without changes in the total number of receptors. The process of internalization may occur via different pathways including clathrin-coated pits (CCPs) and lipid rafts or caveolae (Hendriks-Balk *et al.* 2008) (see Figure 1). Herein, for many GPCRs, β-arrestins re-appear as key reagulators acting as adaptor-like proteins with the capacity to link activated receptors to the components of the internalization machinery – clathrin and AP2 (Gurevich & Gurevich 2006) (see Figure 1). Once GPCRs are concentrated in CCPs, β-arrestins also facilitate the subsequent intracellular trafficking of the cargo by recruitment of dynamin, a large GTPase necessary for the fission of vesicles from the plasma membrane (Jean-Alphonse & Hanyaloglu 2011). Besides this most frequent and most studied arrestin and clathrin-dependent mechanism of endocytosis, other mechanisms have been reported including arrestin, clathrin and/or dynamin dependance (for related works see (Prossnitz 2004)).

Trafficking

Following internalization, GPCRs undergo different trafficking fate. They are either rapidly targeted to lysosomes for enzymatic degradatioin (process leading to the downregulation of receptors), recycled back to the plasma membrane (process termed as resensitization) or retained in endosomes by which means the processes of degradation or recycling are much slower (Jean-Alphonse & Hanyaloglu 2011). Accumulating evidence now shows that β-arrestins play an important regulatory role even in various levels of receptor trafficking. Indeed, β-arrestins operate as sorting agents deciding between the degradative and the recycling fate of the internalized receptors. The final sort of each receptor is dependent on the strenghth of its interaction with β-arrestin during the processes of internalization and/or trafficking. One group of receptors, class A receptors, interacts preferentially with β-arrestin2. This interaction is weak and transient which results in rapid dissociation of the internalized receptor-β-arrestin complex in endosomes. This leads to a rapid recycling of the receptor to the plasma membrane. Class B receptors bind to both β-arrestins with near equal affinity. This interaction is stronger and induces a long-lasting association. The internalized receptor-β-arrestin complexes thus traffic together resulting in retarded recycling and prefered lysosomal degradation (Luttrell & Lefkowitz 2002, Patel *et al.* 2009).

Herein is also interesting to mentione that the endocytic activity of arrestins as well as their effect on GPCR trafficking is controlled by posttranslational modifications such as phosphorylation or ubiquitination (for further information see (Shenoy & Lefkowitz 2003, Wolfe & Trejo 2007)). In addition, recent studies have also revealed a possible regulatory role for S-nitrosylation of core mediators of GPCR trafficking (see paragraph 4.2. in (Jean-Alphonse & Hanyaloglu 2011) and (Lima *et al.* 2010, Daaka 2011).

Recent studies have also exposed some other types of sorting agents allowing degradation or recycling of internalized receptors. Indeed, short linear peptide sequences including tyrosine- and dileucine-based motifs and PDZ ligands mediate an endosomal sorting of GPCRs while ubiquitination might have an essential role in their lysosomal sorting (Marchese *et al.* 2008) (see Figure 1).

Resensitization

Resensitization process includes endocytosis of desensitized receptor, dephosphorylation of receptor in endocytic vesicles, and receptor recycling back to the

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plasma membrane. This process of receptor recycling prevents intracellular receptor accumulation and degradation (Kristiansen 2004) and allows initiation of further rounds of signaling. As mentioned previously, short linear peptide sequences including tyrosine- and dileucine-based motifs and PDZ ligands play a key role in GPCRs recycling rate. To sum up the case of β-arrestins, GPCRs of group A resensitize faster (are recycled back to the plasma membrane more quickly) than GPCRs of group B due to the weaker association of the receptor-β-arrestin complex.

Downregulation

Downregulation occurs as a result of long- term or repeated exposure to agonist (hours or days). Contrary to the rapid receptor internalization corresponding to intracellular redistribution of receptors, downregulation is slower and characterised by a decrease in the total number of receptors present in cells or tissues (Tsao *et al.* 2001). Moreover, the recycling of down-regulated receptors is not possible as it is in the case of receptors which are just internalized. The process of downregulation first comprises the enhancement of

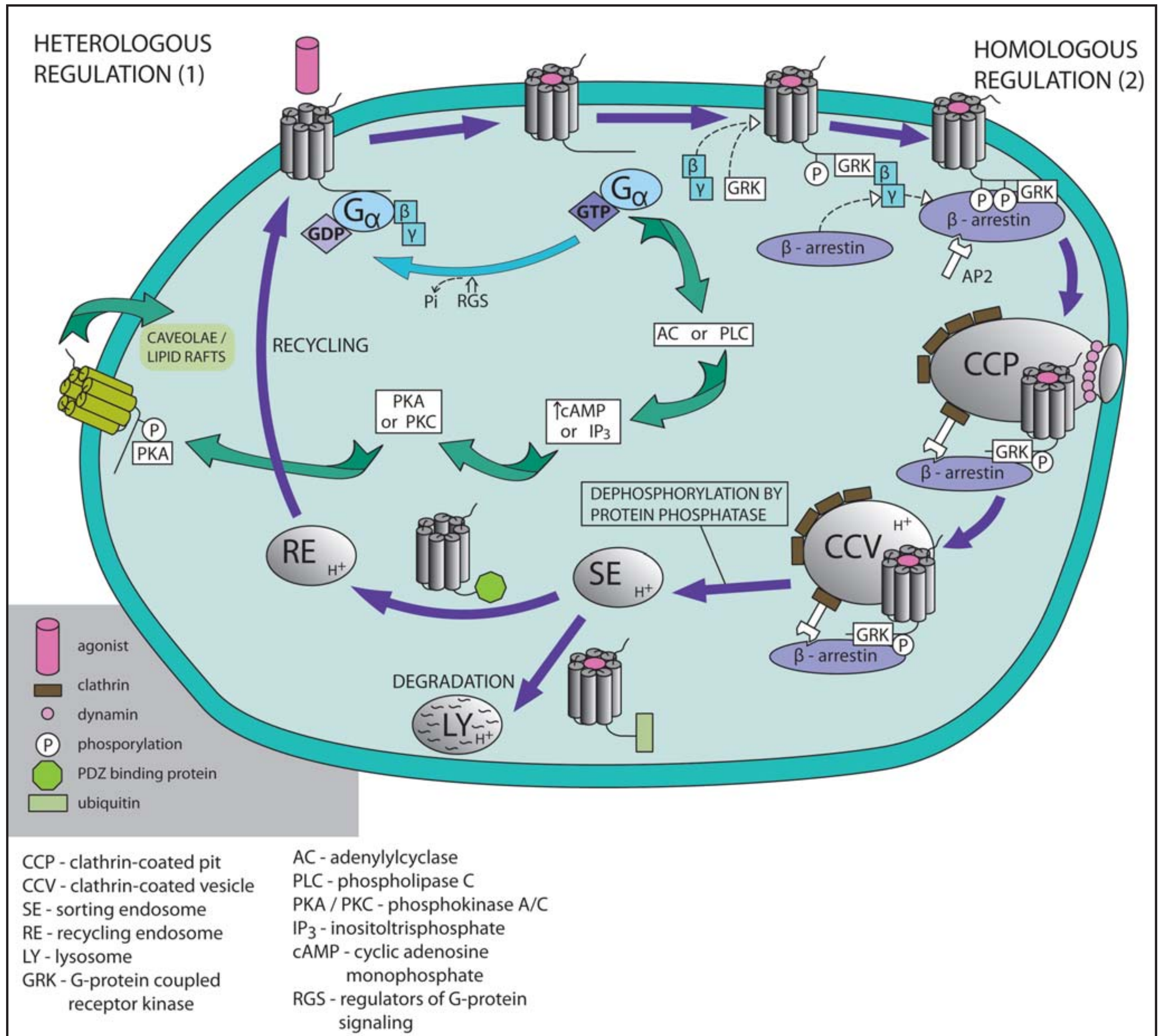

Fig. 1. Following agonist binding, a transient complex of agonist, activated receptor and G protein is formed. GDP is rapidly replaced by GTP and G protein dissociates into α subunit and β/γ dimer which have a different subseguent role in the regulatory process. 1) Gα activates several effectors such as AC or PLC which leads to the activation of second-messenger dependent kinases (PKA and PKC respectively). These kinases can then phosphorylate other receptors than the activated ones thereby producing their desensitization. Receptor internalization in this case seems to occur via caveolae or lipid rafts. These processes are included in so-called **heterologous regulation**. 2) Contrarywise, β/γ dimer recruits GRKs which specifically phosphorylate the agonist-occupied receptor and recruit by themselves β-arrestins. β-arrestin binding promotes recruitment of adaptors such as clathrin, AP2 and dynamin, which targets receptor to CCP for internalization. The internalized receptor in sorting endosome is then trafficked towards the degradatory compartment (lysosome) or the recycling pathway according to the sorting signal carried by the receptor. These processes fall within the so-called **homologous regulation**.

proteosomal and/or lysosomal degradation of internalized GPCRs. The degradatory processes are enhanced by agonist-induced ubiquitination of GPCRs. Second, the mechanism of downregulation consists also in the reduction of GPCRs synthesis. This may be mediated at one or more molecular levels (Schmidt & Meyer 1994):

- changes at the level of transcription – i.e. decrease in the rate of receptor gene transcription
- changes at the posttranscriptional level – i.e. changes in mRNA stability
- changes at the posttranslational level – i.e. shortening of the receptor protein half-life.

These molecular mechanisms can be mediated by these pathways: the first is the direct interaction of regulatory molecule with target gene structure. For β-AR there are at least two regulatory molecules which are able to modulate the β-adrenergic receptor protein synthesis – CREB – cAMP response element binding protein or CREM – cAMP response element binding modulator . These molecules interact with CRE (cAMP response element) located in the promoter region of the β-adrenergic receptor gene (Vallejo 1994) and are able to effectively regulate GPCRs (Huang *et al.* 2010).

The second (and hypothetical) mechanism is indirect pathway via "third messengers" (Hughes & Dragunow 1995). Third messengers are proteins synthesized immediately after the activation of receptor which can modulate target genes. Because of very fast synthesis their own genes are called *immediate early genes*.

SENSITIZATION AND UP-REGULATION

On the other hand there are situations when the receptor is not enough stimulated (denervation, receptor blockade by antagonist). In such situations the receptor can be sensitized or can be up-regulated. The mechanisms of sensitization can include a) increase in second messenger level, b) increase in the concentration of enzymes catalyzing the second messenger synthesis or c) changes in sensitivity of receptor to agonist. The up-regulation has the similar nature as the down-regulation (i.e. changes at the level of transcription, or changes at the posttranscriptional level , or changes at the posttranslational level) (Myslivecek & Trojan 2000).

RGS IN GPCR REGULATION

In recent years, in the field of GPCR studies, we pay many attention to an additional mechanism of signaling regulation, to the family of proteins called regulators of G protein signaling or RGS. In the second half of 1990´s, this new class of proteins emerged (Hollinger & Hepler 2002) and 37 genes encoding proteins with RGS or RGS-like domain have since been identified within the human genome (Willars 2006). This family members share a characteristic RGS-homology domain of 120–130 amino acid residues and are classified into six distinct subfamilies (see (Hendriks-Balk *et al.* 2008). RGS proteins were first described as GTPase activating proteins (GAPs) by their capacity to enhance Gα mediated GTP hydrolysis and thus terminate the G protein activation cycle (De Vries *et al.* 2000, Ross & Wilkie 2000) (see Figure 1). Further studies have exposed that RGS proteins also may regulate GPCR signaling via mechanisms independent of the GAP activity by modulating either protein–protein interactions (i.e. the effector antagonism of RGS4 in the case of G rpotein-PLC interaction), subcellular localization of signaling molecules or protein translation (Bansal *et al.* 2007, Sjögren *et al.* 2010). In the sense of regulation through protein-protein interaction, increasing evidence also supports the notion that many RGS proteins directly bind to certain GPCRs to modulate the signaling (for an extensive review of these interactions see (Abramow-Newerly *et al.* 2006, Neitzel & Hepler 2006, Bansal *et al.* 2007). The existence of direct RGS–GPCR interaction has also been sustained by the observation that co-expression of both proteins results in recruitement of RGS to the membrane (Roy *et al.* 2003). In the case of small RGS, selectivity for the receptor is thought to be carried by N-terminal region, while the larger RGS might interact with the receptor directly throught one of their protein interaction domains (e.g. PDZ domain). In addition, series of reports show that RGS, especially the larger one, act as integrators of GPCR signaling by virtue of their capacity to form multiprotein complexes (Hollinger & Hepler 2002). These complexes are multifunctional signaling cores which manifestly recruit many scaffolding proteins. Moreover, certain scaffolds serve as regulators of RGS function such as spinophilin (see spinophilin/neurabin balance (Wang *et al.* 2007)).

This paragraph only summarizes a few of RGS proteins functions in cells. As it rises from the title, we centred our interest especially on the field of RGS protein-GPCR interactions. Nonetheless these proteins enter in contact with many other proteins which give them a much wider biological role.

CONCLUSIONS

Signaling via GPCRs, the largest and the most omnifaceted and adaptative family of membrane receptors, plays an essential role in many physiological functions. It is a highly controlled process which comprises multiple steps of regulation at each level of signaling. On the GPCR level, three processes lead to reduction and/or attenuation of signaling: desensitization, internalization and down-regulation, while resensitization, sensitization and up-regulation increase the potential of agonist stimulation and thus re-enables signaling. Besides these discrete mechanisms of regulation, complex receptor trafficking has been recently appreciated as an important regulatory mechanism of GPCR signaling. In addi-

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tion, receptor responsiveness to extracellular stimuli is also controlled by interactions with many proteins, especially the RGS. In fact, newer models postulate that GPCRs function as multifunctional platforms where receptors and G rpoteins are closely colocalised with other proteins involved in the specific signal transduction, as well as with regulatory proteins, scaffolding proteins and adaptors. Moreover, GPCR signaling is no longer thought to be the exceptional quality of homologous regulation but heterologous regulation can also participate to change the level of receptor signaling.

PERSPECTIVES

Regarding the vaste implication of GPCR signaling in many disorders such as cardiovascular diseases, immune or nervous system disfunction etc., these receptors as well as the downstream signaling molecules are the most common target of therapeutic agents. In this sense, for drug development, there is a new potential to study how to disrupt interactions of adaptors and scaffodling proteins with core mediators of GPCR signaling. Therewithal GPCR trafficking assays might also be a valuable trend in targeting the drugs for therapeutic interventions. In any case, these findings in drug development might be facilitated if new advances in computer simulation and modeling of protein-protein interactions as well as site directed mutagenesis are exploited.

Finaly, new perspectives of research in the field of GPCR regulation machinery are opened up by a potential regulatory mechanism of GPCRs oligomerization for which is still uncertain if it occurs as generally relevant phenomenon for all types of GPCRs or not (Milligan 2007, Maurice *et al.* 2011)

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DEFINITION OF SELECTED TERMS:

G proteins (**guanine nucleotide-binding proteins) –** proteins that are involved in transmission of various signaling factors. They belong to the group of GTPases. The large G proteins are heterotrimeric structures composed of α, β and γ subunits and are activated by GPCRs. The small G proteins (20–25kDa) are monomeric and belong to the Ras superfamily of small GTPases. Both types bind GTP (active state of G protein) or GDP (inactive state).

GTPase – hydrolase enzyme that can bind and hydrolyze the GTP on GDP. Regulatory GTPases such as G proteins have an intrinsic GTPase activity which enables them to switch the signal transduction on and off.

GEF (guanine-nucleotide exchange factor) – proteins that activate GTPases by facilitating the dissociation of GDP and its replacement by GTP.

GAPs (GTPase activating proteins) – regulatory proteins that can bind to activated GTP-binding proteins (e.g. G proteins) and increase their rate of GTP hydrolysis thereby terminating their signaling.

RGS (regulators of G rpotein signaling) – multifunctional proteins that rapidly inactivate G proteins by promoting the GTP hydrolysis (i.e. they act as GAPs). This regulatory role is encoded in RGS homology domain. Some RGS proteins contain however additional domains with further functionality.

PLC (phospholipase C) – enzyme that cleave phospholipids before the phosphate group. Phosphatidylinositol 4,5-bisphosphate (PIP_2) is thus cleaved into two important second messenger molecules: diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). IP_3 increases the concentration of cytosolic calcium, calcium and DAG together activate protein kinase C (PKC) which enter the regulation of many signal transduction processes.

AC (adenylate cyclase) – enzyme that catalyses the conversion of AMP to cAMP when activated by G protein (G_S).

cAMP (cyclic adenosine monophosphate) – important second messenger involved in many signal transduction pathways capable to activate protein kinase A (PKA), for example.

PKA/PKC (protein kinase A/C) – enzymes that are involved in regulation of other proteins through their capacity to phosphorylate serine and/or threonine residues on these proteins. They are activated by second messengers such as cAMP or DAG/Ca^{2+}. Besides their other roles in cells, they are implicated in heterologous desensitization of GPCRs.

GRK (GPCR kinase) – protein kinases that are involved in homologous desensitization (regulation) of GPCRs. They phosphorylate serine and/or threonine residues of receptors after the release of the activated G proteins. The phosphorylated residues then bind arrestins thereby preventing reassociation of G proteins with their receptors and activating the process called receptor internalization or endocytosis.

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β-arrestins – small proteins important in regulation of signal transduction by their implication in processes of receptor desensitization, internalization and subsequent trafficking. In addition to their interaction with GPCRs, they are also able to link other receptors and signaling molecules.

Scaffolding proteins or scaffolders – proteins that are able to interact with multiple members of signaling complexes thereby enhancing the efficiency and specificity of cellular signaling pathways.

Clathrin-coated pit (CCP) – are small structures that recruit clathrin, adaptors and other regulatory proteins to the intracellular layer of the membrane in order to allow its deformation. This process leads to the progressive enclosure of the invaginated membrane and to the formation of clathrin coated vesicle (CCV).

Clathrin – protein that is associated with clathrin coated vesicles (CCV) involved in selectively sorting cargo both in endocytosis and biosynthetic pathways.

AP-2 – multimeric protein that works as clathrin adaptor protein and facilitate the internalization of the cargo in clathrin mediated endocytosis.

Dynamin – a GTPase involved in endocytosis throught its capacity to form a spiral around the connection between the nascent vesicle and the donor membrane thereby causing the scission of the invaginating vesicle.

Caveolae – the special type of lipid rafts involved in clathrin-independent endocytosis. This structure contains the protein caveolin which causes the local morphological changes of the plasma membrane resulting in formation of flask-shaped, cholesterol-rich, invaginations.

Lysosome – cellular organelle that contains hydrolase enzymes implicated in digestion of debris and waste materials

Endosome – cellular organelle that contains materials ingested by endocytosis. Different states of endosomes are known. The endocytic vesicle first enter in fusion with early endosome. Early endosomes include sorting endosomes and recycling endosomes. In sorting endosomes occurs the uncoupling of the receptor and its ligand. Free receptors designated to recycling are transported to the recycling endosomes. Others, as well as free ligands, stay in early endosomes which become more and more acidic and mature into late endosomes before fusing with lysosomes. The sorting between the degradative and recycling pathways occurs via sorting agents (ubiquitin, arrestin, PDZ binding protein etc...)

Ubiquitin – a 76 amino-acid residues regulatory protein that conjugates with lysine residues of target proteins. The resulting ubiquitin tag directs proteins to degradative pathways.

PDZ domains – protein-interaction domains of 80–90 amino-acid residues that is often contained in signaling proteins and scaffolders. The name results from combining the first letters of founding members of this family: post synaptic density protein (Psd95), Drosophila disc large tumor suppressor (Dlg1), and zonula occludens-1 protein (ZO-1)