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Feedback Regulation of G Protein-Coupled Receptor Signaling by GRKs and Arrestins

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Abstract

GPCR are ubiquitous in mammalian cells and present intricate mechanisms for cellular signaling and communication. Mechanistically, GPCR signaling was identified to occur vectorially through heterotrimeric G proteins that are negatively regulated by GRK and arrestin effectors. Emerging evidence highlights additional roles for GRK and Arrestin partners, and establishes the existence of interconnected feedback pathways that collectively define GPCR signaling. GPCR influence cellular dynamics and can mediate pathologic development, such as cancer and cardiovascular remodeling. Hence, a better understanding of their overall signal regulation is of great translational interest and research continues to exploit the pharmacologic potential for modulating their activity.

1. Introduction

Cellular differentiation is a hallmark of multicellular organisms that is essential for specialization at the cell, tissue, and organ level. Specialization promotes organismal development and survival by permitting cells to differentiate into cellular lineages that possess unique properties, such as sensation, secretion, absorption, or conductivity [1]. Multicellular organisms coordinate these activities to sustain homeostasis. Cell surface receptors and signaling molecules represent a pivotal mechanism for overcoming this challenge by facilitating cellular communication [2, 3]. The Seven Transmembrane Span Receptors (7TMR) / G Protein-Coupled Receptor (GPCR) family represents the largest superfamily of cell surface receptors, with over 800 distinct receptors encoded within the human genome [4]. This review highlights the biologic role and regulation of 7TMR/GPCR and their interacting proteins, while reviewing their structure, classification, and mechanism of action.

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2. 7TMR/GPCR Signaling Overview

2.1 Diversity

7TMR/GPCR are activated by diverse extracellular signals, such as proteins, peptides, lipids, small molecules, ions, and photons. These receptors transduce extracellular cues into intracellular signals to facilitate cell responses. 7TMR/GPCR and their diversity underpin their ability to regulate highly varied physiological functions responsible for sensation, adaptation, and survival [4–7]. In the context of sensation, ocular processing is initiated by four visual 7TMR/GPCR, gustation uses several sweet, bitter and umami (glutamate) GPCR; and olfactory sensation is initiated by several hundred 7TMR/GPCR [7–10]. In addition, over 150 distinct GPCR and their cognate ligands regulate critical biophysical processes, such as endocrine physiology, immunomodulation, and neurotransmission/neuromodulation. Despite extensive research over the past 3 decades, over 100 “orphan” receptors remain that have either no known endogenous ligand or unclear physiological functions [11].

2.2 Structure and classification

The 7TMR/GPCR family name reflects its member’s structure and function. This receptor family is structurally defined by a seven alpha-helix transmembrane span topology [4, 7, 12]. The amino terminus and three transmembrane connecting loops are positioned extracellularly, while the carboxy terminus and three additional transmembrane connecting loops are located intracellularly. In addition to structural homology, this receptor family largely shares a common mechanism of action: GPCR activation initiates signal transduction across the cell membrane, which activates an intracellular heterotrimeric GTP-binding proteins (G proteins) [5].

The 7TMR structural superfamily is divided into three canonical receptor families and other non-canonical families [13]. These families are defined by conserved sequence elements located primarily in the receptor transmembrane region. Overall, these classifications are subdivided into canonical (family A-C) and non-canonical groupings. Unlike family A-C receptors, non-canonical 7TMR appear to primarily utilize mechanisms other than G proteins for signal propagation. However, numerous recent reports argue that specific non-canonical 7TMR can indeed activate G proteins and be regulated like GPCRs, in addition to their non-canonical activities [14]. Family A represents the largest canonical subfamily and is defined by homology with the visual receptor rhodopsin and the β_2 -adrenergic receptor (β_2 AR). Family B is defined by sequence homology to secretin and glucagon receptors. Expression of family B receptors is localized largely to gastrointestinal and neuronal tissue. The smallest canonical 7TMR subfamily, family C receptors are neuromodulatory metabotropic glutamate and GABA_B receptors. This family is expressed in both the peripheral and central nervous systems and is responsible for processing diverse signals, such as pain, anxiety, and memory formation. Non-canonical receptors include those of the Frizzled family that recognize Wnt ligands, or the Hedgehog co-receptor Smoothened. These receptors exert their effect through G protein independent mechanisms [13].

2.3 G protein signaling mechanism

G protein activation is dependent primarily upon GPCR stimulation. GPCRs remain mostly inactive on the cell surface until bound by a cognate activating agonist and initiate G protein activation. A GPCR's corresponding G protein generally remains inactive until GPCR activation. In their inactive conformation, G proteins exist in a heterotrimeric state: the constituent α -subunit, bound to GDP, is tightly associated with the $\beta\gamma$ -subunit dimer. GPCRs act as guanine nucleotide exchange factors (GEF) for G proteins when agonist-activated [15–17]. Consequently, GPCR activation stimulates the exchange of GDP for GTP on the α -subunit. GDP/GTP exchange on the α -subunit stimulates the dissociation of activated α •GTP from the $\beta\gamma$ -subunits. Activated α •GTP then binds to downstream signaling effectors, such as adenylyl cyclase or phospholipase C, to alter their activity to increase levels of intracellular second messengers, such as cyclic AMP (cAMP) or inositol tris-phosphate (IP₃) [17, 18]. In addition to α •GTP, unbound $\beta\gamma$ -subunits bind to and activate their own effectors, including G protein-regulated Inward Rectifying K (GIRK) channels and protein kinases, such as GRK2 and PAK [19–24].

For deactivation, the α -subunit encodes an intrinsic GTPase that hydrolyzes GTP to GDP to inactivate its ability to propagate the signal cascade [15, 16]. Additional GTPase-activating proteins accelerate this deactivation, such as regulator of G protein signaling (RGS) family accessory proteins [25, 26]. When GTP bound to an α -subunit is hydrolyzed to GDP, the α •GDP dissociates from its effectors and re-associates with $\beta\gamma$ -subunits, thereby reassembling an inactive G protein heterotrimer. The dissociation of the α -subunit and $\beta\gamma$ -subunit from their effectors effectively ends the propagation of the signal cascade by deactivation of the effector [15–17].

G protein mediated GPCR signaling is a highly amplified system. Upon activation, GPCR's guanine nucleotides exchange activity last long enough to induce activation of numerous individual G proteins. Activated G proteins provide further signal amplification owing to α -subunit's ability to produce many second messenger molecules (e.g., cAMP, IP₃) during the time it remains bound to an effector enzyme. Consequently, small amounts of a hormone released from a distant part of a multicellular organism can profoundly influence the function of a distant target cell.

2.4 G protein classification

G proteins are classified based upon their α subunit structure and function, and fall into four subclasses. Each G protein subclass has distinct downstream effectors and produces unique signaling effects on the cell. First, the stimulatory G proteins (G_s) stimulate adenylyl cyclase to convert ATP into cyclic AMP (cAMP). Elevated cAMP results in the activation of cAMP-regulated proteins, such as, specific kinases (protein kinase A, PKA), cyclic nucleotide-gated channels, and the Epac GEF for Rap small GTP-binding proteins [5, 15, 16, 27]. Second, the inhibitory heterotrimeric G_i proteins mechanistically oppose G_s activity by inhibiting adenylyl cyclase, which reduces intracellular cAMP levels [15, 16, 28]. Furthermore, the $\beta\gamma$ -subunits of G_i proteins can exert cellular effects by regulating GIRK channels and other effectors. Transducins (G_t) represent specific G_i family members and are stimulated by light-activated rhodopsin to regulate retinal cGMP phosphodiesterase [29].

Similarly, gustducin (G_{gust}) is a G_i family member stimulated by tastant receptors in taste buds. Third, G_q proteins activate phospholipase C- β enzymes upon stimulation by cognate GPCRs. Phospholipase C- β hydrolyzes plasma membrane phosphatidyl inositol lipids, releasing IP_3 and diacyl glycerol that act to increase intracellular calcium and protein kinase C activity [28]. Fourth, the G_{12} proteins activate GEFs for the RhoA small GTP-binding protein, activating it to regulate the cytoskeleton. [28, 30, 31].

2.5 GPCR functional classification

In addition to classifications based upon sequence similarity (section 2.2), GPCRs can be categorized by their cognate G protein and the downstream pathways they regulate [32]. Interestingly, while some GPCRs appear to exclusively couple with one class of G protein (e.g., “ G_s -coupled receptor” or “ G_q -coupled receptor”), other GPCR can activate G proteins from two or more classes. Not all G proteins within a class are equivalent: receptors can have a preference for specific subtypes of G proteins within a class. In particular, evidence using mice deficient in individual G_i family members has made it clear that a receptor may couple to only one member of the G_i subfamily even when other members are available. This signaling diversity is further increased by the fact that there are five β -subunits and twelve γ -subunits that can theoretically assemble (with the α -subunits) several hundred combinations, and both receptors and $\beta\gamma$ -subunit-activated effectors can prefer specific $\alpha+\beta\gamma$ combinations. Despite this complexity, the general principles of G protein mediated signaling hold true [28, 33].

GPCRs can also be categorized according to their ligands [32, 34]. Receptors that bind the same ligand are generally also similar based upon other classification systems. For instance, receptors that bind the same ligand are usually very highly related and form distinct subgroups by homology clustering. For example, receptors for glycoprotein hormones (e.g., LH, FSH) are structurally similar to each other, as are receptors for the biogenic amines (e.g., serotonin, dopamine, adrenaline). While many unique ligand – receptor pairs exist, some endogenous ligands can bind with high affinity to multiple receptor subtypes (e.g., 12 GPCRs activated by serotonin, 5 by acetylcholine). Overall, this complexity of GPCRs and ligands increases the specificity of desired target organ effects.

3. 7TMR/GPCR Dynamics

As noted, receptors are generally inactive in the absence of their activating agonist. Once a specific 7TMR/GPCR is stimulated, it activates downstream intracellular signaling pathways in response to agonist binding. However, this model vastly oversimplifies the dynamics of 7TMR/GPCR mediated signaling. This section seeks to define the mechanisms that regulate the signaling process.

3.1 Receptor desensitization

7TMR/GPCR-regulated systems exhibit graded responses upon activation. The amount of ligand signal defines the downstream signal; more agonist promotes more response. Because GPCR systems are highly amplified a small amount of ligand occupying a fraction of receptors, may induce a maximal response. However, each receptor system displays a

memory of prior activation that influences its ability to be stimulated in the future. The fraction of receptors that must be activated to achieve a full effect varies based on how active the system has been recently. Cells with receptors that have been active recently respond by becoming less sensitive to further stimulation, while cells that have not been stimulated for some time become more sensitive to future stimulation. This phenomenon is termed desensitization [22, 35–37]. Desensitization serves to prevent overstimulation of target cells in the face of high agonist concentrations, while the reverse process of resensitization allows cells to still recognize and respond to low amounts of agonist [38–40]. Overall, these interlinked processes allow a cell to maintain sensitivity to a ligand in conditions of either high and low signaling tone.

The ability to alter GPCR sensitivity is critical for cellular responses such as chemotaxis, wherein a cell responds to a gradient of chemoattractant agonist. Cells must interpret differences in agonist concentration that vary by a few percent difference from one side of the cell to other to effectively migrate towards the source of a ligand, such as a site of infection. Receptor desensitization mechanisms allow cells to decipher the cues of a shallow ligand gradient through differential signaling sensitivity to define the ‘front’ versus the ‘rear’ of a migrating cell [41]. Immune cells utilize such a mechanism in order to migrate towards injured tissues following gradients of tissue-released GPCR ligand chemokines [42].

Overstimulated receptors require higher levels of agonist to produce equal downstream effects. This is clinically exemplified in heart failure, wherein poor contractility of the heart drives catecholamine release to act on cardiac adrenergic receptors to increase contractility. As heart receptors adapt to the high levels of stimulation, this positive feedback overdrive eventually becomes ineffective and actually counterproductive, as it promotes further pathological remodeling of the heart [28, 43, 44].

3.2 Receptor sequestration

Receptor levels on the cell surface are dynamic and change in response to agonist stimulation. Receptors are removed from the cell surface upon activation, and return to the cell surface after agonist levels subside. Short-term endocytotic removal of receptors from the cell surface is termed sequestration [45]. Different receptor types utilize distinct pathways for removal from the cell surface, such as clathrin-coated pits or caveolae-mediated endocytosis. Once internalized, receptors traffic in intracellular vesicles through a series of intracellular vesicular compartments that eventually lead to release of the receptor back onto the cell surface in an activatable state [46, 47].

3.3 Receptor downregulation

Long-term over-activation of a GPCR can lead to a substantial reduction in cell surface receptor levels. This is termed down-regulation and can result in substantial reductions in receptors present at the cell surface. Mechanistically, down-regulation is initially comparable to receptor sequestration. However, unlike sequestration, down-regulation targets internalized receptors for lysosomal degradation. Thus, recovery of normal receptor level at the cell surface requires new receptor synthesis [48, 49].

3.4 Constitutive activity

Receptors possess varying degrees of agonist-independent activity, termed constitutive activity, for activating downstream signaling pathways [50]. Constitutive activity levels are receptor specific and depend on receptor expression level. Receptors with low constitutive activity exhibit the highest degree of ligand-stimulated activity, whereas agonist activation of receptors with high constitutive activity may only promote a two-fold increase in signal upon agonist stimulation, due to their high basal level of activity. Constitutive activity is hypothesized to occur as a consequence of the molecular dynamics of the receptor protein: receptors constantly transition among multiple conformations, and a subset of these conformations are capable of inducing G protein activation, thus producing constitutive activity. A receptor with high constitutive activity thus has more low-energy states that favor G protein activation than a receptor with low constitutive activity. [51–54].

3.5 Agonist and antagonist dynamics

GPCR conformational states determine their ability to activate coupled G proteins. Agonists stabilize active GPCR conformations to induce signaling, while antagonists stabilize inactive conformational states to prevent signaling. Agonists are classified based upon the degree to which they activate GPCRs. A *full agonist* promotes maximal GPCR activation (maximal as compared to the native ligand), while a *partial agonist* activates GPCR activity to a lesser degree even at the highest concentrations of that ligand. In contrast, an *antagonist* inhibits activation by either occupying the binding site for the natural ligand (orthosteric site) or another site (allosteric site) that precludes subsequent agonist mediated GPCR activation [55, 56]. Furthermore, agonists can alter receptor constitutive activity. *Inverse or negative agonists* silence the activity of constitutively active receptors by suppressing their basal constitutive activity, through preventing spontaneous transitions to active states. In contrast, *neutral antagonists* bind a receptor without altering the ability to transition among inactive and active states, thus permitting basal activity to continue [57, 58]. Overall, molecules that bind to a receptor can affect GPCR function along a continuum, from full activation through complete inactivation.

4. Signal Termination: GRK, Arrestin, and G protein switching

Termination of GPCR signaling is mediated by multiple mechanisms. Signaling at the receptor can be terminated by degrading the ligand, as is the case for most peptide agonists, or by removing the ligand from the vicinity of the receptor, as through reuptake of the biogenic amine dopamine from synapses into presynaptic neurons for re-storage in vesicles for later release. The intrinsic GTPase activity of G proteins and its acceleration by GTPase-activating proteins of the RGS protein family (section 2.3) limits the lifetime of downstream signaling once an agonist is no longer present at sufficient concentration at the receptor to drive new G protein activation. But in addition to these general modes of regulation, GPCRs themselves are subject to direct regulation by a two part mechanism consisting of G protein-coupled receptor kinases (GRKs) and arrestins. These two classes of cellular regulatory proteins cooperatively function to terminate GPCR signaling, as well as by mechanisms that alter receptor coupling to G proteins.

4.1 Regulated GPCR-G protein coupling and G protein switching

GPCR affinity for specific G protein subtypes is influenced by several factors, including receptor structure and posttranslational modification. Individual GPCRs may dynamically couple amongst several G protein subtypes. Indeed, receptors may experience a phenomena termed “G protein switching”, wherein a GPCR’s affinity for a particular G protein subtype alters in response to cellular dynamics, such as receptor posttranslational modifications. The β_2 AR demonstrates G protein switching, as it dynamically couples with both G_s and G_i proteins [33, 59]. β_2 AR G protein switching occurs as a negative feedback regulatory mechanism mediated by PKA phosphorylation of the receptor. Upon initial stimulation, β_2 AR activates the G_s protein to increase adenylate cyclase signaling and PKA activity to propagate the cellular response. In addition to eliciting second messenger responses, PKA phosphorylates site-specific residues on β_2 AR [60]. PKA phosphorylation of β_2 AR alters the receptor coupling from G_s to G_i , and activated G_i subsequently deactivates G_s protein and results in a decrease in cAMP production and PKA activity [33, 61]. Effectively, PKA phosphorylation of β_2 AR and G protein switching mediates a negative feedback loop with respect to the initial G_s signal activation.

4.2 GRK signal termination

GPCR kinases (GRKs) are protein serine/threonine kinases of the protein kinase A/G/C (AGC) subfamily. This kinase family initiates GPCR signal termination by phosphorylating ligand-bound GPCR. These kinases recognize the active conformation of GPCR and phosphorylate serine and threonine residues within the carboxy terminal tail and third intracellular loop of the receptor. GPCR phosphorylation itself generally does not decouple the activated GPCR from its cognate G protein. Instead, this phosphorylation triggers the second step of signal termination, by marking activated receptors for binding of arrestin proteins to the phospho-receptor [36, 62–64].

The GRK family consists of seven members that are categorized into three subfamilies. GRK1 subfamily consists of GRK1 (rhodopsin kinase) and GRK7 (cone kinase); the GRK2 subfamily is composed of GRK2 and GRK3; and the GRK4 subfamily is formed by GRK4, GRK5 and GRK6 [45, 63]. The GRK1 kinases are predominately localized to retinal tissue and influences visual acquisition by regulating visual GPCR. The GRK2 subfamily, in contrast, is ubiquitously expressed and possess a unique $\beta\gamma$ -subunit binding domain. The $\beta\gamma$ -subunit binding domain of this subfamily permits translocation of these GRKs to the inner leaflet of the plasma membrane upon interaction with the $\beta\gamma$ -subunit released by a receptor-activated G protein. In the GRK4 subfamily, GRK5 and GRK6 are expressed in many tissues, while GRK4 expression is primarily localized to the testes. Within the GRK family, GRKs 2, 3, 5, and 6 are the most widely expressed isoforms that are responsible for the bulk of receptor phosphorylation throughout the body.

A large mismatch exists between the number of GPCRs and the number of GRKs expressed within an organism [36, 62–64]. Inherit within this mismatch, GRKs are relatively promiscuous in recognizing receptor. While there appear to be some GRK preferences among receptor types, specificity is largely driven by the context of GPCR activation and by relative GRK concentrations in a cell of interest, rather than by recognition of any consensus

phosphorylation site [65]. GRKs exhibit some preference for phosphorylation sites on receptors, but many studies have shown that GRKs will find other sites to phosphorylate on a receptor if their preferred sites are unavailable, such as after receptor mutagenesis.

4.3 Arrestin signal termination

Arrestins constitute a scaffold protein family that initiate the second part of GPCR signal termination [38, 45]. Arrestins recognize active GPCR and their binding affinity is augmented by GRK mediated phosphorylation. Arrestins have varying affinities for different phosphorylation patterns. Highly phosphorylated serine/threonine clusters induce stronger arrestin-GPCR binding. The arrestin family consists of four proteins, arrestin1-4. Arrestin1 and arrestin4 are unique for their involvement in visual processing. These proteins are expressed in the rods and cones of retinal tissue, wherein they bind to phospho-rhodopsin and phospho-iodopsin to regulate GPCR mediated visual perception [63, 66, 67]. Unlike the visual arrestins, Arrestin2 (aka β arrestin1) and arrestin3 (aka β arrestin2) are expressed in most tissue types [63].

Arrestins promote GPCR signal termination by two distinct, but complementary mechanisms: receptor desensitization and receptor sequestration. These scaffold proteins mediate receptor desensitization by directly inhibiting G protein - GPCR coupling [45]. Mechanistically, arrestin-bound GPCRs are sterically blocked from interacting with their cognate G protein [38]. This occurs almost immediately after receptor activation, since G proteins, GRKs, and arrestins compete for binding to the receptor in its active state. Thus the relative abundance of these three classes of proteins in a cell determine much of the kinetics of receptor signaling. Though GRK-mediated receptor phosphorylation may partially accomplish G protein decoupling, the binding of arrestins to a GRK-phosphorylated GPCR significantly augments this signal blockage mechanism [35, 38, 46]. Efficient arrestin binding results in homologous desensitization, or the turning off of those specific receptors that have been recently stimulated.

Arrestin-mediated receptor trafficking from the cell surface, or sequestration, occurs minutes to hours after agonist stimulation. Arrestins act as adaptors to associate bound receptors with cellular endocytic machinery proteins to mediate GPCR internalization into vesicles as specific cargo. Arrestin binding to phosphorylated GPCRs promotes arrestin interaction of adaptin and clathrin, two proteins responsible for mediating receptor internalization [68, 69]. GPCR internalization sequesters the activated receptors from further access to agonists outside the cell and effectively reduces the number of receptors present on the cell surface for ligand stimulation.

After mediating GPCR internalization, arrestins may either dissociate or remain bound to a receptor as it begins to transit through intracellular vesicular compartments, depending on arrestin affinity for the phospho-receptor [70]. Early dissociation of arrestin from a weak-binding GPCR keeps the receptor on a quick itinerary leading to receptor recycling, wherein the endocytosed GPCR quickly returns to the cell surface to engage in further ligand binding. Failure of the arrestin-GPCR complex to dissociate early marks that receptor for further processing through intracellular compartments that result in either receptor degradation or delayed receptor recycling [22, 49]. Among other factors, the conformational

state of the receptor and the pattern of GRK-mediated phosphorylation collectively influence arrestin-GPCR binding dynamics.

GRK/arrestin signal regulation is stoichiometric, with one arrestin molecule able to bind to and regulate only one GPCR. Low receptor ligand occupancy can induce full G protein signaling for an extended period with low desensitization, since few receptors can activate many G proteins through amplification, but also leads to only a few receptors at a time being removed from a functional state on the cell surface. The endocrine system demonstrates this phenomenon, where agonists released into the systemic circulation are highly diluted within the vasculature that carries them to their target tissues where they can have sustained effects. In contrast, high receptor ligand occupancy will induce an intense burst of G protein activity, followed by profound GRK/arrestin mediated desensitization. This type of regulation is most evident at neuronal synapses, where a neurotransmitter agonist is released in large quantities into synapses, and signaling occurs in bursts rather than constantly.

4.4 Arrestin GPCR feedback regulation

Arrestins additionally regulate GPCR activity through non-adaptor based feedback regulatory mechanisms. The β_2 AR system is regulated by arrestin through feedback regulation. β_2 AR activation yields an increase in PKA activity secondary to cAMP production. Phosphodiesterase (PDE) opposes this activity by degrading cAMP. Upon β_2 AR activation, arrestin scaffolds with PDE4D and promotes its translocation to the plasma membrane [71]. PDE4D translocation and activation negatively regulates the β_2 AR cascade by degrading the substrate necessary for PKA activation. Interestingly, observations demonstrate that these events additionally inhibit PKA mediated phosphorylation of active β_2 AR [71]. Hindrance of PKA-mediated β_2 AR phosphorylation inhibits G protein switching [33, 61]. Arrestins additionally partake in G_q -coupled M1 muscarinic receptor feedback regulation [72]. DAG and IP3 are formed upon G_q stimulation. Diacylglycerol kinases (DGKs) terminate G_q signaling by phosphorylating the second messenger DAG. Similar to PDE4D interactions in the β_2 AR- G_s system, arrestins scaffold with and activate DGKs to degrade DAG. Effectively, arrestin promotes G_q negative feedback regulation by activation removal of the second messenger DAG.

5. GRK and Arrestin-Mediated Signaling

Arrestins and GRKs also function in GPCR signal termination-independent processes. They serve as molecular scaffolds that can alter the activity of partners including protein kinases, transcription factors, and other cellular signal modulators. GRKs can regulate the activity of many cytoplasmic and nuclear non-GPCR substrates through both scaffold- and kinase activity-dependent functions.

5.1 Non-canonical GRK activity

Accumulating evidence highlights a non-canonical GRK function in regulating cellular processes independent of GPCR signal termination. Non-canonical GRK activity extends to regulating non-GPCR substrates, cytoskeletal dynamics, protein catalytic activity and other diverse functions. Non-GPCR GRK substrates are documented in several cellular

compartments, including the plasma membrane, cytoplasm, and nucleus. This kinase family regulates non-GPCR substrates in both kinase-dependent and -independent fashion [36, 62, 63].

GRK2 and GRK3 have two distinct receptor-independent functions in regulating G proteins. As noted previously, GRK2 family members bind to G protein $\beta\gamma$ -subunits released by receptor-activated G proteins [36, 63]. Since $\beta\gamma$ -subunits are plasma membrane anchored, this recruits GRK2 to the plasma membrane in the vicinity of activated receptors. Although $\beta\gamma$ -subunit binding does not directly alter GRK2 kinase catalytic activity, GRK2 can in fact be considered a $\beta\gamma$ -subunit effector because this efficient localization greatly enhances the effectiveness of the kinase. In addition, GRK2 competes for G protein $\beta\gamma$ -subunits with other $\beta\gamma$ -stimulated effectors, and this domain has been used widely as a $\beta\gamma$ -subunit scavenger to assess $G\alpha$ versus $G\beta\gamma$ functions [20, 73]. Disease states that increase or decrease GRK2 levels thus alter $G\beta\gamma$ signaling tone. The second G protein function is that GRK2 and GRK3 also bind to activated G_q α -subunits through their RGS-like domain. Again, activated G_q does not appear to alter GRK2 function, but GRK2 does compete with PLC- β and other effectors for activated G_q subunits.

GRK-mediated phosphorylation regulates non-GPCR receptor activation. For instance, the platelet-derived growth factor receptor β (PDGFR β) is a single-transmembrane span tyrosine kinase receptor responsible for mediating cellular proliferation and survival signals upon ligand stimulation. GRK2 negatively regulates PDGFR β kinase activity by phosphorylating specific serine residues, thus decreasing PDGFR β -mediated phosphorylation of its substrates [74, 75]. Similarly GRK5/6 positively regulate the tyrosine kinase receptor, insulin-like growth factor-1 receptor (IGF-1R). Indeed, IGF1-mediated ERK and AKT activation is diminished in cells when either GRK5/6 expression is decreased [76].

GRKs regulate cytoskeletal dynamics through GPCR-dependent and - independent mechanisms. The ezrin–radixin–moesin (ERM) protein family constitutes three GRK-regulated proteins that mediate cytoskeletal rearrangement [77, 78]. ERM proteins localize to the cytoplasm and promote cytoskeletal rearrangement by facilitating crosstalk between plasma membrane-bound proteins and F-actin [79]. GRK-mediated phosphorylation on specific ERM residues conformationally activates ERM to yield F-actin polymerization. GRK mediated ERM activation promotes cytoskeleton remodeling and cell adhesions [62]. Interestingly, GRK2 mediates muscarinic M1 receptor membrane ruffling through ezrin phosphorylation [62, 78]. Furthermore, GRK5 mediated moesin phosphorylation promotes cellular invasion and migration [77]. Additionally, GRKs influence microtubule (MT) processing, which functions in cytoskeletal polarization and migration [80]. MT dynamics are regulated by post-translational modifications, such as acetylation and phosphorylation [81]. Indeed, GRKs phosphorylate tubulin, the basic monomer of MT [82]. GRKs phosphorylate and activate histone deacetylase 6 (HDAC6) to deacetylate α -tubulin, an MT subunit. GRK mediated HDAC6 deacetylation promotes MT polymerization [80].

This kinase family additionally regulates nuclear proteins involved in diverse functions, such as gene transcription and epigenetics. Exeplar transcription factor NF- κ B undergoes GRK5-mediated regulation. I κ B family members negatively regulate NF- κ B by binding and

sequestering NF- κ B in the cytoplasm. I κ B kinase (IKK) phosphorylation of I κ B family members promotes NF- κ B dissociation and subsequent I κ B family member degradation. GRK5 additionally mediates I κ B family member degradation and NF- κ B activation by phosphorylating this protein family upon TNF α stimulation [83]. Interestingly, additional reports argue that GRK5 can oppose NF- κ B activity through mediating I κ B α nuclear accumulation [84]. Furthermore, GRK5 augments nuclear factor of activated T cells (NFAT) transcriptional activity in an apparent kinase independent manner, potentially by some molecular scaffold activity [85].

5.2 Arrestin-mediated signaling

Arrestin-mediated GPCR signal termination constitutes their defining function. Available evidence, however, highlights that arrestins additionally mediate signal transduction, cellular organization, cytoskeletal dynamics, and other cellular processes through molecular scaffold activity [45]. Molecular scaffolds regulate the subcellular spatiotemporal distribution of proteins, promote complex formation by bridging interacting components, and reduce off target effects by increasing reaction fidelity. Proteomic screens identified novel arrestin interactors in diverse pathways, such as signal transduction, motor processing, metabolomics, and cytoskeletal arrangement [22]. This scaffold ability increases mechanistic impact of arrestins on cell physiology in both GPCR-dependent and -independent states [22, 38].

Arrestins extend the GPCR signaling pathway beyond G proteins by scaffolding with kinases. GPCR/arrestin-mediated scaffolding occurs secondary to GPCR agonist activation [86]. For instance, arrestin-bound GPCR promotes tyrosine kinase Src recruitment . Arrestin-bound GPCR enhances the catalytic activity of Src to further diversify cellular effects, including promoting further GPCR signaling. Src-bound arrestin potentiates GPCR signaling by targeting GRKs for degradation, thus inhibiting the initial step of GPCR signal termination [70]. Furthermore, the Src-arrestin complex extends GPCR signaling pathways by promoting G protein-independent signaling. This complex trans-activates other pathways, such as the pro-mitogenic EGF receptor that can activate ERK to initiate pro-mitogenic transcriptional activity [23, 87].

Arrestins exert a contextual influence on kinases. Their scaffolding ability can either directly or indirectly alter kinase activity. Src-independent arrestin ERK activation demonstrates direct arrestin mediated regulation. Arrestin2 and 3 each complex with ERK independent of Src [22]. However, Arrestin2 and 3 maintain opposing functions. Arrestin2 inhibits ERK activation, while arrestin3 augments the kinase activity [22]. In contrast, arrestin indirectly regulates the transcription factor NF- κ B by directly altering the stability of a regulatory scaffold protein. Arrestin indirectly promotes NF- κ B inhibition by stabilizing I κ B α . Arrestin-I κ B α scaffolding prevents IKK mediated phosphorylation and subsequent NF- κ B release. Consequently, arrestin indirectly regulates NF- κ B by stabilizing the negative regulator protein responsible for its cytoplasmic localization [88].

Scaffold proteins regulate the spatiotemporal distribution of cellular components ERK activation post GPCR activation supports this conclusion. GPCR stimulation promotes ERK activation via G protein mediated mechanisms. Activation by this pathway results in nuclear

translocation of ERK and subsequent activation of downstream transcription factors. Arrestin mediates an alternative pathway: ERK can form a complex with GPCR-bound arrestin via a G protein-independent mechanism. ERK recruitment to the plasma membrane results in its presence in clathrin-coated pits together with GPCR and arrestin. Vesicle-bound ERK is sequestered in the cytoplasm where it activates a separate set of effectors distinct from the subset of G protein-activated ERK substrates [22, 89, 90].

5.3 GPCR biased signaling

GPCR ligand activation induces receptor conformational changes, which stimulate downstream effector molecules. The G protein and arrestin signal pathways present two exclusive effector molecules influenced by GPCR structural dynamics. Active GPCR conformations are dynamic and exist in several structural forms, with partially non-redundant functions. Indeed, certain GPCR conformations specifically promote either a G protein or arrestin signaling pathway [91]. Ligand-receptor dynamics reflect this conformational complexity. Balanced ligands permit all potential active GPCR conformations, while biased ligands only permit GPCR to assume a subset of potential active structures [34, 92, 93]. In contrast to balanced ligands, biased ligands induce conformations that permit the specific activation of either G protein or arrestin based signaling [34, 92, 93]. For example, carvedilol, an FDA approved beta-blocker, demonstrates biased activity in the cardiovascular system. This therapeutic induces GPCR conformations that selectively activate arrestin pathway, without stimulating G protein activity [94].

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Perspective

GPCR-mediated signal transduction presents an intricate mechanism for controlling cellular activities and facilitating communication. Diverse processes ranging from cardiovascular remodeling to endocrine signaling utilize GPCR activity for eliciting cellular responses. Pharmacotherapeutics target GPCR pathways for clinical interventions due to their applicability in controlling clinically-relevant pathways. Indeed, over 50% of pharmacologic interventions target GPCR. However, GPCR's intricate and diversified mechanisms of activity contribute towards unwanted side effects. Consequently, fundamental advances in GPCR biology directly influence novel pharmacologic development void of deleterious effects. Indeed, gained knowledge in GPCR structure and signal transduction augments the opportunity for smart drug development seeking to produce receptor signal-specific therapies without off-target effects. Identifying GPCR conformation states that selectively activate either arrestin or G protein signaling networks presents an additional control to prevent drug deleterious side effects. While GPCR present a heavily targeted family, less than 10% of GPCR currently are targeted for interventions. This offers the opportunity to identify and target new GPCRs. Overall, the advancement of GPCR research presents profound clinical applications inherit within their influence of cellular dynamics. Recognizing GPCR regulatory feed-back loops expands opportunities to harness specific signals for therapeutic interventions.

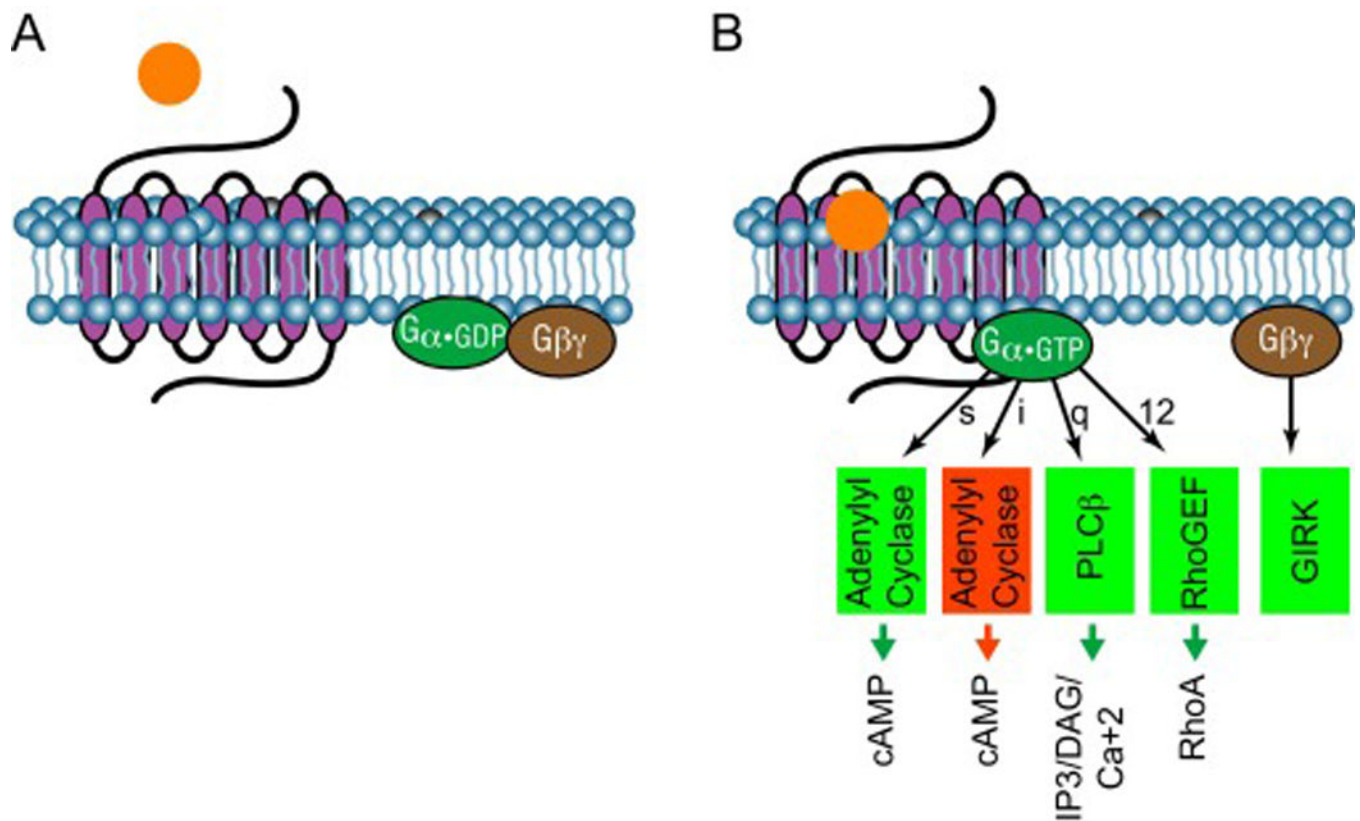


Figure 1. Classical 7TMR/GPCR signaling

A) Inactive G proteins exist in a heterotrimeric state: the constituent α -subunit, bound to GDP, is associated with a $\beta\gamma$ -subunit dimer. B) GPCR activation promotes GDP/GTP exchange on the α -subunit, which stimulates the dissociation of activated $\alpha \cdot GTP$ from the $\beta\gamma$ -subunits. $G_s\alpha \cdot GTP$ and $G_q\alpha \cdot GTP$ respectively activate adenylyl cyclase and phospholipase C, which increase intracellular second messengers cyclic AMP (cAMP) and inositol tris-phosphate (IP₃). $G_i\alpha \cdot GTP$ inhibits adenylyl cyclase thereby decreasing cAMP accumulation.

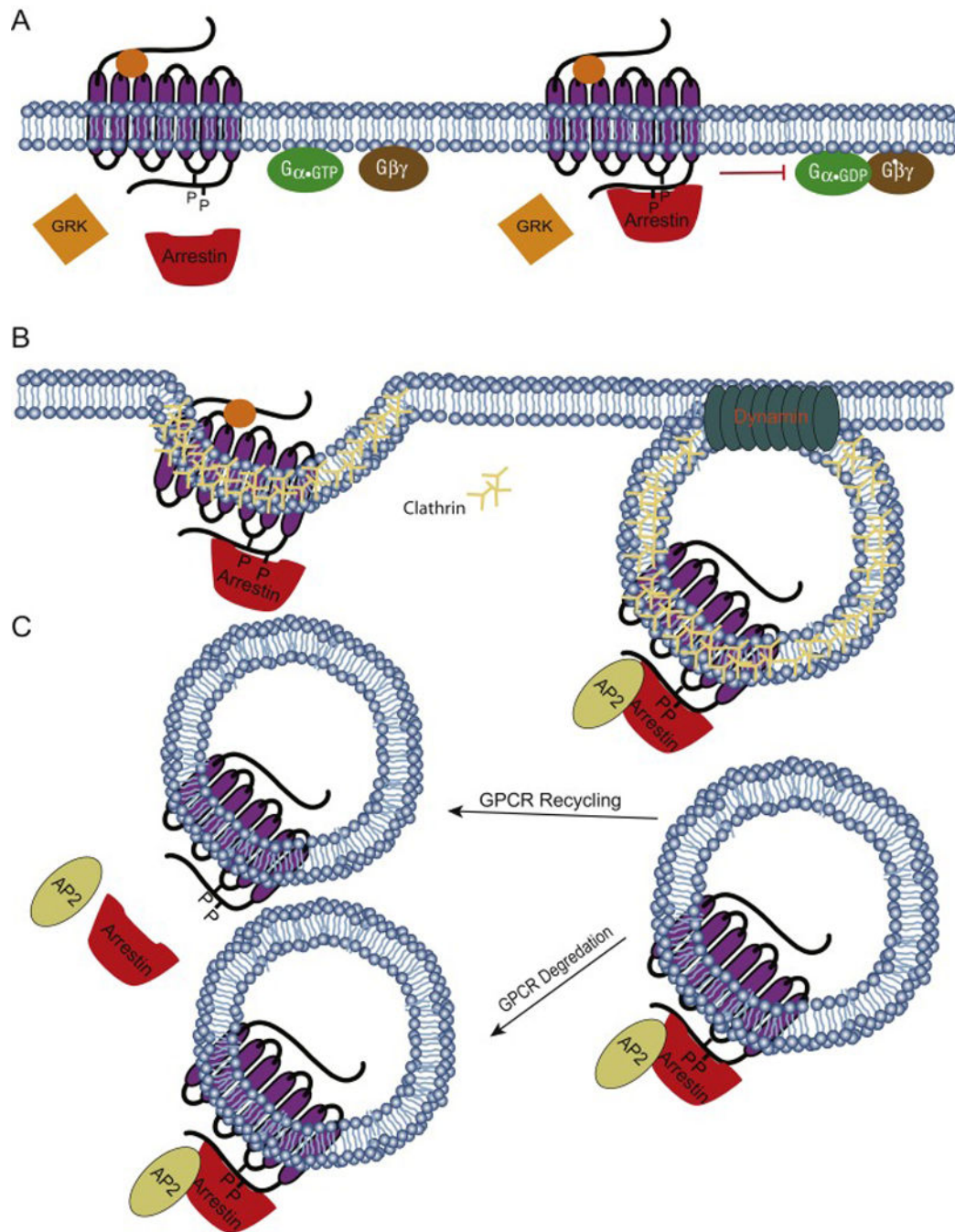


Figure 2. 7TMR/GPCR signal regulation

A) Receptor desensitization. GRKs phosphorylate the C-terminal of activated GPCRs. Arrestin binds to the phosphorylated receptor that is then sterically blocked from interacting with cognate G protein. B) Receptor sequestration. Arrestin serve as an adaptor and recruit endocytic machinery proteins to mediate GPCR internalization into vesicles. GPCR internalization sequesters the activated receptors from further stimulation. C) Arrestin may dissociate or remain bound to the receptor in vesicle. Early dissociation of arrestin promotes receptor recycling. Failure of the arrestin-GPCR complex to dissociate marks that receptor

for further processing that results in either receptor degradation or delayed receptor recycling.

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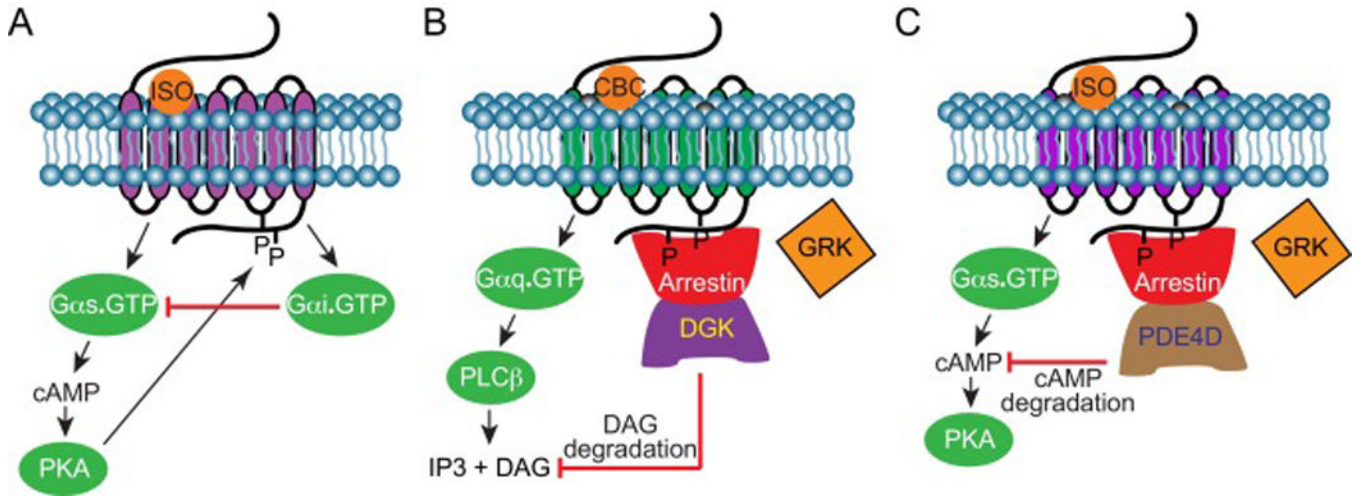


Figure 3. GPCR feedback regulation

A) G protein switching. Upon initial stimulation, β_2 AR activates the G_s protein to increase adenylyl cyclase signaling and PKA activity to propagate the cellular response. PKA initiates G protein switching by phosphorylating site-specific residues on β_2 AR. PKA phosphorylation of β_2 AR alters the receptor coupling from G_s to G_i . Activated G_i yields a decrease in cAMP production and PKA activity. B) Arrestins partake in G_q -coupled M1 muscarinic receptor feedback regulation. Arrestin scaffolds with diacylglycerol kinases (DGKs) and recruits them to the plasma membrane. These events activate DGKs to degrade DAG, promoting arrestin-mediated G_q negative feedback regulation. C) Upon β_2 AR activation, arrestin scaffolds with PDE4D and promotes its translocation to the plasma membrane. PDE4D translocation and activation negatively regulates the β_2 AR-cAMP cascade by degrading the substrate necessary for PKA activation

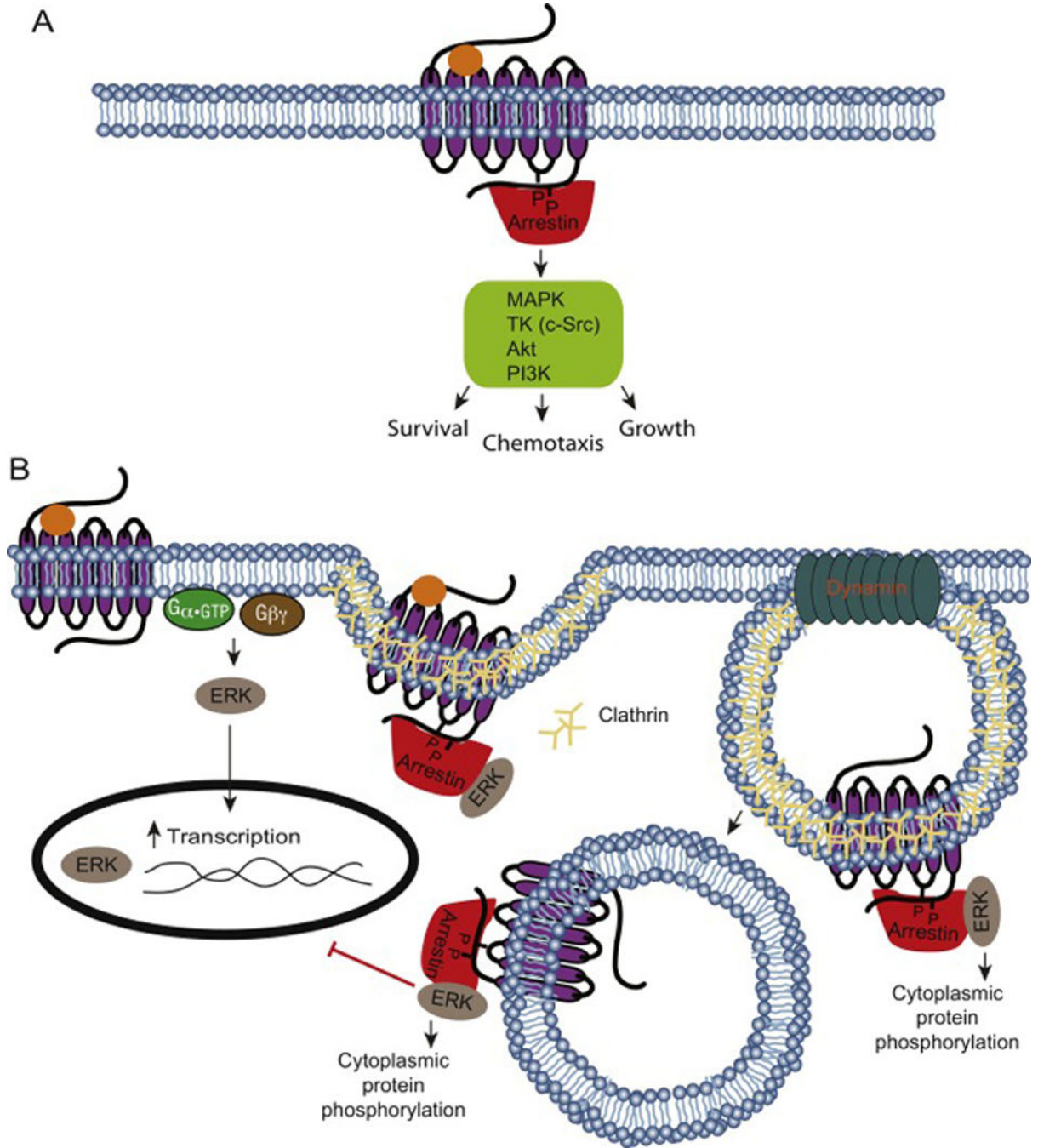


Figure 4. Arrestin mediated signaling

A) Arrestins extend the GPCR signaling pathway beyond G proteins by scaffolding with kinases. GPCR ligand activation induces receptor conformational changes that can result in arrestin signaling activation. Arrestin mediated signaling is independent of G protein coupling and can regulate diverse cellular processes. B) Arrestins regulate protein spatiotemporal distribution. GPCR stimulation promotes ERK activation via G protein mediated mechanisms. This pathway promotes nuclear translocation of ERK and subsequent activation of downstream transcription factors. Arrestin mediates an alternative pathway

wherein ERK complexes with GPCR-bound arrestin. Vesicle-bound ERK is sequestered in the cytoplasm and activates a separate set of effectors.

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