GPCRs In Intracellular Compartments: Implications For Drug Discovery

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The architecture of eukaryotic cells is defined by extensive membrane-delimited compartments, which entails separate metabolic processes that would otherwise interfere with each other, leading to functional differences between cells. G protein-coupled receptors (GPCRs) are the largest class of cell surface receptors, and their signal transduction is traditionally viewed as a chain of events initiated from the plasma membrane. Furthermore, their intracellular trafficking, internalization, and recycling were considered only to regulate receptor desensitization and cell surface expression. On the contrary, accumulating data strongly suggest that GPCRs also signal from intracellular compartments. GPCRs localize in the membranes of endosomes, nucleus, Golgi and endoplasmic reticulum apparatuses, mitochondria, and cell division compartments. Importantly, from these sites they have shown to orchestrate multiple signals that regulate different cell pathways.

G protein-coupled receptors nuclear membrane mitochondria

1. Introduction

The best current estimate claims that cells appeared on Earth ~3.0–3.3 billion years ago [1]. The process of cellularization is still highly debated, with specialists suggesting that cells were originated by the evolution of the cytoplasm inside a primordial lipid vesicle, while others argue the possibility that the cytoplasm was instead developed outside the surface of early vesicles, and thus, cells were simply the result of an invagination process of this proto-cytosol into those vesicles that had an early functional cytoskeleton [2]. The acquisition of the cell membrane was the first crucial step in biological evolution that defined the boundaries of a cell and allowed controlled communications between intracellular and extracellular environments to produce those relative stable equilibriums crucial for life to evolve. In fact, besides its function as structural support and defense, the cell membrane regulates exchanges of molecules from and to the external environment, responding to environmental physical and chemical changes, and thus plays a crucial role in the interactions with other cells and/or the extracellular environments; moreover, the cell membranes are involved in energy formation and other chemical reactions. Notably, even though some prokaryotes also contain intracellular structures that can be seen as primitive organelles, prokaryotes and archaea have maintained the mono-compartmental structure of their ancestor cells [3].

2. G protein-Coupled Receptors (GPCRs) and Their Role in Modulating Different Types of Stimuli

GPCRs are the largest family of membrane receptors, with nearly 800 genes coding for these proteins. They are involved in many physiological processes such as sensing light, taste and smell, neurotransmission, metabolism, endocrine and exocrine secretion, cell growth, and migration. In a simplistic way, GPCRs are composed of seven transmembrane domains, three extracellular and three intracellular loops, an extracellular N-terminus, and an intracellular C-terminus [4]. The extracellular loop and the extracellular portion of the seven transmembrane domains are deputed to agonist recognition, while the rest of the transmembrane core and the intracellular loops convey the receptor activation to intracellular transducers.

The evolutionary success of GPCRs is thought to depend upon the interplay between the distinctive properties of their extracellular domains to be readily adaptable for new sensory functions, as shown by the ability to be activated by such different types of stimuli as photons, odorants, neurotransmitters, and hormones, and the conservation of a transmembrane core and intracellular signal transduction mechanisms [5][6]. In fact, most of the vertebrate physiology is based on the signal transduction of GPCRs. G proteins are the first and more characterized partners of GPCRs. They are trimeric proteins composed of α, β, and γ subunits and are activated by the nucleotide GTP. The α and β/γ subunits dissociate upon GPCR activation and are regarded as two independent functional units that regulate different downstream effectors [7]. To date, eighteen α, five β, and twelve γ G protein subunits have been discovered. Based on their similarity and functional activities, the α subunits are further subdivided into four different families: (i) Gαs, that stimulates adenylyl cyclase; (ii) Gαi that inhibits the activity of adenylyl cyclase; (iii) Gαq, whose activation stimulates the phospholipase enzyme; and (iv) Gα12 that activates the small GTPase Rho. Instead, Gβ/γ subunits form a tightly bound dimeric complex that plays a critical role in regulating the catalytic activity of the G protein α subunits, and in modulating the activity of several enzymes and ion channels [8]. In contrast to G protein α subunits, the βγ do not have a catalytic activity on their own but modulate signaling through protein–protein interactions. The first effector found to be activated by Gβγ was the inwardly rectifying K^+ channel (GIRK) in atrial myocytes after muscarinic M2 receptor activation [9][10]. At the beginning, the idea that βγ dimers alone could be the primary mediators of signal activation was controversial, but today, the list of Gβγ interacting proteins comprises a substantial number of targets, such as enzymes and channels [8]. It is worth mentioning that G proteins can also be activated in a GPCR-independent way [11]. Receptor-independent activators of G protein signaling (AGS) play surprising roles in signal processing and have opened new areas of research related to the role of G proteins in signal transduction [12].

The functional complexity of GPCRs could not be attributed to only the exclusive activation of G proteins. Evidence has gradually emerged that GPCRs can signal through many other proteins such as β-arrestins and small G proteins, among others [13]. Notably, by means of a membrane yeast two-hybrid system, it has been recently shown that GPCRs can form interactomes connecting more than 686 proteins that regulate diverse cellular functions [14].

3. GPCRs Are Present in Different Cellular Compartments

GPCRs were originally thought to exclusively localize to the plasma membrane and to mediate cellular signaling of stimuli coming from outside the cell. Even though early evidence suggested a subcellular localization and function of some GPCRs, the interest in these “unusual” locations was scarce as it was assumed that activation by ligands

was restricted to the plasma membrane. Progress in this area finally demonstrated that GPCR-mediated signaling occurs not only from the plasma membrane but also from intracellular compartments such as endosomes, Golgi membranes [15], mitochondria [16], cell division compartments (centrosomes, spindle midzone, and midbodies) [17], and nuclear membrane [18].

The functional importance of GPCR subcellular localization was originally shown for the rhodopsin receptor [19][20] that primarily activates the G proteins from the intracellular disk membranes of the rod cell outer segment. In particular, the disk membranes originated from basal evaginations of the plasma membrane of the rod cell outer segment that were retained intracellularly. The subcellular segregation is crucial for the optimal response of rhodopsin to light, as the disk membrane contains six times less cholesterol than the plasma membrane. Rhodopsin is also the major protein of the plasma membrane of rod cells, but the high membrane cholesterol content inhibits rhodopsin participation in the visual transduction cascade at this site [19].

Further evidence suggesting that GPCR signaling also occurred within an internal membrane compartment emerged in studies of β2 adrenergic receptor-mediated activation of the mitogen-activated protein kinases (MAPKs) Erk1/2, which was inhibited by dominant-negative versions of dynamin and β-arrestin-1 that by preventing receptor internalization showed that β2 adrenergic compartmentalized signaling was responsible for ERK activation [21]. Furthermore, overexpression of a mutant β-arrestin-1 that binds c-SRC (a non-receptor tyrosine kinase that regulates the RAS-MAPK/Erk signaling) without promoting β2 adrenergic receptor internalization also shows a reduced phosphorylation of ERK mediated by β2 adrenergic receptor activation, supporting the concept that this receptor signaling occurred within an internal compartment of the cell [22]. Several GPCRs have been known to co-localize on endocytic vesicles together with β-arrestins and Erk1/2, strongly suggesting that endosomes are specific compartments for GPCR–β-arrestin-mediated signaling [15]. Recent evidence suggest that signaling from endosomes may participate in the pathogenesis of cardiac diseases. After prolonged isoproterenol stimulation, β_1 adrenergic receptors decrease on the cell surface by endocytosis, but they are still active and as shown by Morisco et. al., they mediate cardiac hypertrophy [23]. In fact, the inhibition of the β_1 adrenergic receptor internalization process by concanavalin A blocks isoproterenol-induced cardiac hypertrophy, strongly suggesting that the cardiac hypertrophy was caused by the ability of these receptors to signal from endosome compartments.

The Golgi apparatus is another compartment where ligand-dependent GPCR activation has been detected. For instance, the β1 adrenergic receptor is known to activate the Gαs protein canonically in the plasma membrane, but also in the Golgi apparatus where it is sorted to form a pre-existing receptor pool [24]. Physiologically, catecholamines are charged and need transporters to cross the plasma membrane such as organic cation transporter 3 (OCT3) to reach for example the Golgi β1 adrenergic receptor [24]. This internal membrane pool of receptors contributes significantly to the overall production of cellular cAMP elicited by β_1 adrenergic agonists.

Among other receptors activated in the Golgi apparatus, there is the TSH receptor. In this case, the receptor is internalized together with its agonist and when it reaches the Golgi network it activates a local pool of Gαs protein. This critical receptor localization near the nucleus seems required for efficient CREB phosphorylation and gene transcription [25].

Another internal compartment where GPCRs are localized and function is the mitochondrion. Early evidence of this localization came from the cannabinoid CB1 receptor that was shown to be sorted in the outer mitochondrial membrane of skeletal and myocardial cells. The activation of mitochondrial CB1 receptor by its lipophilic agonists in these tissues was associated with mitochondrial regulation of the oxidative activity through relevant enzymes implicated in pyruvate metabolism [16]. Thereafter, melatonin receptors (MT1Rs) were recognized to localize in the outer mitochondrial membrane of neurons to regulate cell respiration. Strikingly, it was shown that the lipophilic melatonin ligand was produced in the mitochondrion to auto-regulate its MT1 receptors and in turn stimulate the Gαi proteins localized in the intermembrane space to inhibit stress-mediated cytochrome c release [26]. These remarkable findings challenge the classical perception of GPCRs' biological function by showing that an intracellular organelle can both synthesize a ligand and directly respond to it through an auto-receptor mechanism. To name this amazing new mechanism, the term "automitocrine" was proposed, in analogy to "autocrine" when a similar phenomenon occurs among cells [26].

Notably, GPCRs are also present in cell division compartments where they regulate cytokinesis [17]. For instance, the odorant OR2A4 receptor localizes to the spindle poles during mitosis and to the cleavage furrow and midbody ring during cytokinesis in HeLa cells [17]. The crucial role played by these receptors in cell division was also established in OR2A4 knockdown experiments where the lack of the receptor caused cytokinesis failure.

Furthermore, over thirty GPCRs have been described to localize at the nuclear membrane and convincing evidence show they play important physiological roles such as gene regulation [18][27][28][29]. In isolated nuclei from rat hearts, isoproterenol through the activation of β3 adrenergic receptors and Ang II through the activation of AT1 and AT2 receptors increase nuclear gene transcription. Importantly, these effects were blocked by the pertussis toxin (PTX), suggesting that the activation of Gi proteins was essential for β3 adrenergic and AT1 and AT2 receptor regulation of gene expression [30][31].

Unexpectedly, GPCRs have also been found in extracellular vesicles produced by eukaryotic cells, such as exosomes [32]. These tiny structures of 50–100 nm in diameter are formed in multivesicular intracellular bodies that are late endosomal compartments situated in the endocytic route between early endosomes and lysosomes. Internal vesicles of multivesicular bodies are generated by inward budding of the membrane and are released in the extracellular milieu following fusion of the multivesicular body with the plasma membranes. Exosomes can carry various types of proteins, lipids, and nucleic acids (mRNA and non-coding RNA) and have been recognized as important tools for cell-to-cell communication [33][34][35]. Specifically, Kwon et al. [32] found that GPRC5B, an orphan GPCR, is present in exosomes released by hepatocyte growth factor (HGF)-treated Madin–Darby canine kidney (MDCK) cell cysts. Exosomal GPRC5B is taken up by nearby MDCK cells and together with HGF promotes Erk phosphorylation and tubulogenesis. Furthermore, the same researchers found that GPRC5B is elevated in urinary exosomes from patients with acute kidney injury, suggesting that the transport of this receptor through exosomes could recapitulate a repairing mechanism. After this initial finding, other GPCRs have been found to be secreted from cells via exosomes in various physiological and pathological contexts [36].

4. GPCR Sorting to the Plasma Membrane and to Intracellular Compartments

Distribution of proteins to different cellular compartments requires protein-sorting codes that are recognized and segregated by cytoplasmic adaptor complexes that regulate protein trafficking. Many proteins are sorted by short signal peptides attached to the N-terminus (or occasionally at the C-terminus or along the internal sequence) of the protein [37]. Integral membrane proteins including GPCRs are first synthesized in the perinuclear endoplasmic reticulum and then transported along the secretory pathway through the Golgi apparatus and the trans-Golgi network to be delivered to the plasma membrane. Once the nascent protein is inserted into the membrane, the signal peptide is normally cleaved off from the mature protein [38]. Most GPCRs lack a cleavable signal peptide and the molecular mechanisms that lead to their targeting to the plasma membrane, or their sorting to intracellular compartments, are poorly understood. Only a small group of GPCRs contains cleavable signal peptides and their removal results in the retention of the receptor in the endoplasmic reticulum [39]. The variety of the cellular destinations of GPCRs raises the question of how GPCRs are delivered to these targets.

Most of the work on GPCR trafficking has focused primarily on their plasma membrane localization and internalization. Several chaperone proteins bind to nascent GPCRs in the endoplasmic reticulum and carry them to the Golgi complex and finally to the plasma membrane [40]. Receptor activity-modifying proteins (RAMPs) are a family of three single pass membrane proteins that were initially discovered as regulators of the calcitonin receptor-like receptor (CLR) function and plasma membrane expression [41]. It is recognized that RAMPs also interact with several other GPCRs to switch ligand selectivity, and to modulate signal transduction and receptor trafficking [42] [43]. Among others, the chaperone effects, first noted for CLR, have been shown for the calcium sensing receptor [44], the secretin receptor [45], the GPR30 receptor [46], and the type 1 corticotrophin releasing factor receptor (CRF1) [47].

Other proteins that have been shown to deliver GPCRs to the membrane are the receptor expression enhancing proteins (REEPs) and the receptor transporting proteins (RTPs), identified for their ability to enhance odorant and taste receptors' cell surface expression [48]. Furthermore, the integral protein calnexin regulates the membrane expression of dopamine D1 and D3 receptors [49], CD4 enhances the plasma membrane expression of the chemokine CCR5 receptor [50], the transmembrane protein 147 (tmem147) reduces the M3 receptors at the membrane levels [51], and Rab43 regulates the expression of adrenergic α2B and muscarinic M3 receptors [52].

This brief overview indicates the heterogeneity in the molecular chaperones involved in GPCR trafficking and the lack of a common thread associated with this phenomenon. Furthermore, GPCR trafficking to the plasma membrane varies depending on the expression of the molecular chaperone and the context in which the two proteins (GPCR and chaperone) are expressed. In addition, the same GPCR can use different sorting mechanisms depending on the cell context in which it is expressed. For example, in primary neurons and in neuronal SH-SY5Y cells, Rab43 selectively regulates the total surface expression of the endogenous adrenergic α2 receptors, but not of the muscarinic M3 receptors. In contrast, the surface transport of both receptors requires Rab43 in non-neuronal NRK49F cells, suggesting that the sorting function of Rab43 is neuronal cell specific [52].

The nuclear membrane is another important target of GPCR sorting. In this compartment, GPCRs regulate nuclear events such as DNA synthesis and gene expression [31][53][54][55], transcription initiation [56], and histone modification [57]. The nuclear membrane is an extension of the endoplasmic reticulum and is formed by three connected membrane domains: the outer nuclear membrane that is a continuation of the ribosome-studded rough endoplasmic reticulum that also contains specific protein complexes; the pore membrane, where large macromolecular assemblies called nuclear pore complexes control the passage of molecules to and from the nucleus; and the inner nuclear membrane that faces the nucleoplasm and hosts a number of specific proteins that directly regulate the genome [58]. Like the plasma membrane, GPCR translocation in the inner nuclear membrane appears to be controlled by several processes including lateral diffusion through the membrane of the nuclear pore and those regulated by proteins of the canonical soluble protein transport machinery [58][59][60].

Some GPCRs localize to the nuclear membrane by using canonical nuclear localization signal (NLS) peptides, short basic sequences that confer specificity for one or more karyopherin nuclear transport proteins [61]. Karyopherin proteins were initially described as carriers of soluble proteins, but they are also recognized to play a major role in the transport of transmembrane proteins. The GPCRs that have been recognized to date to use this mechanism to localize to the nuclear membrane are the adenosine A1 and A2B, the angiotensin AT1, apelin, the bradykinin B2, CXCR2 and CXCR4, the coagulation factor II receptor-like 1 (F2rl1, previously known as Par2), and the oxytocin receptors [62][63][64][65][66][67]. Moreover, some GPCRs contain multiple NLS import sequences in different receptor parts. For example, F2rl1 has two NLS domains, in the first and third intracellular loops; mutations in either loop prevent nuclear translocation, suggesting that both are essential for karyopherin β1 binding [67]. Remarkably, this receptor has an additional C-terminal domain, that does not contain an NLS, but has a prominent role in nuclear transport. It probably interacts with proteins different from karyopherins that still concur with F2rl1 nuclear translocation. Other GPCRs instead translocate to the nucleus though a phosphorylation-mediated mechanism as shown for the glutamate mGlu5 receptor [68].

Interestingly, the Ras superfamily proteins of small GTPases, such as Rab and Arf GTPases, have also emerged as crucial regulators of GPCR localizations [69]. In particular, they control vesicular trafficking, vesicular budding from donor membranes, interactions with cellular motors, and vesicle docking. They are networked to one another through a variety of mechanisms to coordinate the individual events of one stage of transport and to link together the different stages of an entire transport pathway [70]. Among others, Rab11a plays a pivotal role in agonist-independent nuclear translocation of the platelet-activating factor receptor [71]. Interestingly, emerging evidence suggests that several family members of the Ras and Rho small GTPases have putative NLSs. The most prudent assumption is that these proteins complex to GPCRs on one side and to canonical nuclear transport proteins on the other to translocate GPCRs to the nucleus [72].

In general, GPCRs that localize on endosomes, endoplasmic reticulum, and Golgi and outer nuclear membranes have their N-terminus embedded in the lumen of these structures with the carboxyl terminal facing the cytoplasm. On the contrary, activated GPCRs in the inner nuclear membrane signal into the nucleoplasm and directly influence nuclear functions [58]. In mitochondria, GPCRs have been localized in the outer [26][73][74][75] and inner membranes

[76] and they seem to be oriented with their signaling part toward the intermembrane space and the matrix, respectively.

GPCR trafficking to internal compartments can be independent from endocytosis, as it is for the platelet-activating factor receptor (F2RL) that can directly reach the nuclear localization through the trans-Golgi network [71] or receptor internalization in endosomes. While the former is normally agonist independent, the latter is an agonist-dependent process [69].

5. β-Arrestin-Mediated GPCR Compartmentalization

The binding of agonists to GPCRs is characterized by key conformational changes necessary for G protein-dependent signaling transduction and for the exposure of phosphorylation sites to kinases such as GRKs. GPCR phosphorylation is a crucial step for G protein-dependent signal desensitization, leading to the uncoupling of G proteins from the receptor and the recruitment of the β-arrestin proteins. β-Arrestins are crucial for receptor removal from the plasma membrane and thus for the regulation of GPCR endocytic trafficking.

The “barcode” model of β-arrestin/GPCR interaction suggests that the degree and patterns of GPCR phosphorylation match different β-arrestin structural changes, and this matching favors different intracellular signaling [77]. The pattern of phosphorylation directly affects the interaction with the β-arrestin family as described for the β2 adrenergic receptor phosphorylated by GRKs [78][79]. Moreover, besides the canonical GRKs, GPCR phosphorylation could also be mediated by more versatile kinases such as casein kinase 2 (CK2), a kinase that plays per se a crucial role in the cell cycle. In particular, CK2 regulates the M3 muscarinic receptor activity by direct phosphorylation and in β-cells it affects the M3 receptor’s ability to favor insulin secretion [80][81][82]. Protein kinase C (PKC) is another GPCR-phosphorylating enzyme that regulates β-arrestin recruitment as shown for the chemokine receptor CXCR4, at Ser-346 and 347 after agonist stimulation [83]. Therefore, GPCR phosphorylation and the ability to recruit β-arrestin to signal are highly intertwined, especially for their ability to evoke specific intracellular signaling such as Erk phosphorylation, desensitization, and antiapoptosis effects [78].

In cancer and healthy cells, these GPCR–β-arrestin-dependent multiprotein complexes interact with signaling proteins involved in gene transcription, protein ubiquitination, and cytoskeletal remodeling, forming signalosomes. These large supramolecular complexes promote cancer progression and metastasis production by activation of mitogen-activated protein kinase/extracellular signal-regulated kinase, Wnt/β-catenin, nuclear factor κB, and phosphoinositide 3 kinase/Akt [84]. Several in vitro systems have recently been developed to investigate radio- and chemotherapy-resistant cancer cells. In particular, cancer cell lines were exposed to drugs or radiation with the aim of selecting treatment-resistant clones and thus analyze the processes of cancer therapy resistance [85][86]. Notably, based on the major roles played by GPCR–β-arrestin signalosomes in regulating cell growth and survival, the mentioned *in-vitro* approaches could lead to the identification of the GPCR–β-arrestin complex-based mechanisms that promote cancer chemo- or radiotherapy resistance. Intriguingly, it has been observed that the overexpression of GPR35 receptor strongly correlates to drug resistance in epithelial lung cancer cells [87].

While in cancer the GPCR–β-arrestin signalosomes play a crucial role in promoting disease progression, in neurodegeneration they are beneficial by slowing down the development of misfolded proteins involved in neurodegenerative disorders. This is the case of the M1 muscarinic receptor and its ability to efficiently signal through β-arrestin [88][89]. Mutant mice with phosphorylation-deficient M1 receptors have more rapid and pronounced misfolded prion-mediated neurodegeneration progression than controls. This strongly suggests that the M1–β-arrestin complex signal has important neuroprotective effects. Therefore, the next generation of GPCR ligands designed to directly modulate GPCR–β-arrestin-dependent intracellular signaling could pave the way towards, for example, the development of novel neuroprotective and anticancer strategies.

References

1. Brasier, M.; McLoughlin, N.; Green, O.; Wacey, D. A Fresh Look at the Fossil Evidence for Early Archaean Cellular Life. Phil. Trans. R. Soc. B 2006, 361, 887–902.
2. Griffiths, G. Cell Evolution and the Problem of Membrane Topology. Nat. Rev. Mol. Cell Biol. 2007, 8, 1018–1024.
3. Kerfeld, C.A.; Melnicki, M.R. Assembly, Function and Evolution of Cyanobacterial Carboxysomes. Curr. Opin. Plant Biol. 2016, 31, 66–75.
4. Rosenbaum, D.M.; Rasmussen, S.G.F.; Kobilka, B.K. The Structure and Function of G-Protein-Coupled Receptors. Nature 2009, 459, 356–363.
5. Strotmann, R.; Schröck, K.; Böselt, I.; Stäubert, C.; Russ, A.; Schöneberg, T. Evolution of GPCR: Change and Continuity. Mol. Cell Endocrinol. 2011, 331, 170–178.
6. Scarselli, M.; Annibale, P.; Gerace, C.; Radenovic, A. Enlightening G-Protein-Coupled Receptors on the Plasma Membrane Using Super-Resolution Photoactivated Localization Microscopy. Biochem. Soc. Trans. 2013, 41, 191–196.
7. Syrovatkina, V.; Alegre, K.O.; Dey, R.; Huang, X.-Y. Regulation, Signaling, and Physiological Functions of G-Proteins. J. Mol. Biol. 2016, 428, 3850–3868.
8. Smrcka, A.V. G Protein By Subunits: Central Mediators of G Protein-Coupled Receptor Signaling. Cell. Mol. Life Sci. 2008, 65, 2191–2214.
9. Logothetis, D.E.; Kurachi, Y.; Galper, J.; Neer, E.J.; Clapham, D.E. The By Subunits of GTP-Binding Proteins Activate the Muscarinic K+ Channel in Heart. Nature 1987, 325, 321–326.
10. Wickman, K.D.; Iñiguez-Lluhi, J.A.; Davenport, P.A.; Taussig, R.; Krapivinsky, G.B.; Linder, M.E.; Gilman, A.G.; Clapham, D.E. Recombinant G-Protein By-Subunits Activate the Muscarinic-Gated Atrial Potassium Channel. Nature 1994, 368, 255–257.

11. Takesono, A.; Cismowski, M.J.; Ribas, C.; Bernard, M.; Chung, P.; Hazard, S.; Duzic, E.; Lanier, S.M. Receptor-Independent Activators of Heterotrimeric G-Protein Signaling Pathways. J. Biol. Chem. 1999, 274, 33202–33205.
12. Blumer, J.B.; Smrcka, A.V.; Lanier, S.M. Mechanistic Pathways and Biological Roles for Receptor-Independent Activators of G-Protein Signaling. Pharmacol. Ther. 2007, 113, 488–506.
13. Heuss, C.; Gerber, U. G-Protein-Independent Signaling by G-Protein-Coupled Receptors. Trends Neurosci. 2000, 23, 469–475.
14. Sokolina, K.; Kittanakom, S.; Snider, J.; Kotlyar, M.; Maurice, P.; Gandía, J.; Benleulmi-Chaachoua, A.; Tadagaki, K.; Oishi, A.; Wong, V.; et al. Systematic Protein–Protein Interaction Mapping for Clinically Relevant Human GPCR s. Mol. Syst. Biol. 2017, 13, 918.
15. Eichel, K.; von Zastrow, M. Subcellular Organization of GPCR Signaling. Trends Pharmacol. Sci. 2018, 39, 200–208.
16. Mendizabal-Zubiaga, J.; Melser, S.; Bénard, G.; Ramos, A.; Reguero, L.; Arrabal, S.; Elezgarai, I.; Gerrikagoitia, I.; Suarez, J.; Rodríguez De Fonseca, F.; et al. Cannabinoid CB1 Receptors Are Localized in Striated Muscle Mitochondria and Regulate Mitochondrial Respiration. Front. Physiol. 2016, 7, 476.
17. Zhang, X.; Bedigian, A.V.; Wang, W.; Eggert, U.S. G Protein-Coupled Receptors Participate in Cytokinesis. Cytoskeleton 2012, 69, 810–818.
18. O'Malley, K.L.; Jong, Y.-J.I.; Gonchar, Y.; Burkhalter, A.; Romano, C. Activation of Metabotropic Glutamate Receptor MGlu5 on Nuclear Membranes Mediates Intranuclear Ca2+ Changes in Heterologous Cell Types and Neurons. J. Biol. Chem. 2003, 278, 28210–28219.
19. Albert, A.; Boeszebattaglia, K. The Role of Cholesterol in Rod Outer Segment Membranes. Prog. Lipid Res. 2005, 44, 99–124.
20. Palczewski, K. G Protein–Coupled Receptor Rhodopsin. Annu. Rev. Biochem. 2006, 75, 743–767.
21. Daaka, Y.; Luttrell, L.M.; Ahn, S.; Della Rocca, G.J.; Ferguson, S.S.G.; Caron, M.G.; Lefkowitz, R.J. Essential Role for G Protein-Coupled Receptor Endocytosis in the Activation of Mitogen-Activated Protein Kinase. J. Biol. Chem. 1998, 273, 685–688.
22. Luttrell, L.M.; Ferguson, S.S.G.; Daaka, Y.; Miller, W.E.; Maudsley, S.; Della Rocca, G.J.; Lin, F.-T.; Kawakatsu, H.; Owada, K.; Luttrell, D.K.; et al. β-Arrestin-Dependent Formation of β 2 Adrenergic Receptor-Src Protein Kinase Complexes. Science 1999, 283, 655–661.
23. Morisco, C.; Marrone, C.; Galeotti, J.; Shao, D.; Vatner, D.E.; Vatner, S.F.; Sadoshima, J. Endocytosis Machinery Is Required for B1-Adrenergic Receptor-Induced Hypertrophy in Neonatal Rat Cardiac Myocytes. Cardiovasc. Res. 2008, 78, 36–44.

24. Irannejad, R.; Pessino, V.; Mika, D.; Huang, B.; Wedegaertner, P.B.; Conti, M.; von Zastrow, M. Functional Selectivity of GPCR-Directed Drug Action through Location Bias. Nat. Chem. Biol. 2017, 13, 799–806.

25. Godbole, A.; Lyga, S.; Lohse, M.J.; Calebiro, D. Internalized TSH Receptors En Route to the TGN Induce Local Gs-Protein Signaling and Gene Transcription. Nat. Commun. 2017, 8, 443.

26. Suofu, Y.; Li, W.; Jean-Alphonse, F.G.; Jia, J.; Khattar, N.K.; Li, J.; Baranov, S.V.; Leronni, D.; Mihalik, A.C.; He, Y.; et al. Dual Role of Mitochondria in Producing Melatonin and Driving GPCR Signaling to Block Cytochrome c Release. Proc. Natl. Acad. Sci. USA 2017, 114, E7997–E8006.

27. Branco, A.F.; Allen, B.G. G Protein–Coupled Receptor Signaling in Cardiac Nuclear Membranes. J. Cardiovasc. Pharmacol. 2015, 65, 101–109.

28. Campden, R.; Audet, N.; Hébert, T.E. Nuclear G Protein Signaling: New Tricks for Old Dogs. J. Cardiovasc. Pharmacol. 2015, 65, 110–122.

29. Vaniotis, G.; Allen, B.G.; Hébert, T.E. Nuclear GPCRs in Cardiomyocytes: An Insider's View of β-Adrenergic Receptor Signaling. Am. J. Physiol.-Heart Circ. Physiol. 2011, 301, H1754–H1764.

30. Tadevosyan, A.; Maguy, A.; Villeneuve, L.R.; Babin, J.; Bonnefoy, A.; Allen, B.G.; Nattel, S. Nuclear-Delimited Angiotensin Receptor-Mediated Signaling Regulates Cardiomyocyte Gene Expression. J. Biol. Chem. 2010, 285, 22338–22349.

31. Vaniotis, G.; Del Duca, D.; Trieu, P.; Rohlicek, C.V.; Hébert, T.E.; Allen, B.G. Nuclear β-Adrenergic Receptors Modulate Gene Expression in Adult Rat Heart. Cell. Signal. 2011, 23, 89–98.

32. Kwon, S.-H.; Liu, K.D.; Mostov, K.E. Intercellular Transfer of GPRC5B via Exosomes Drives HGF-Mediated Outward Growth. Curr. Biol. 2014, 24, 199–204.

33. Raposo, G.; Stoorvogel, W. Extracellular Vesicles: Exosomes, Microvesicles, and Friends. J. Cell Biol. 2013, 200, 373–383.

34. EL Andaloussi, S.; Mäger, I.; Breakefield, X.O.; Wood, M.J.A. Extracellular Vesicles: Biology and Emerging Therapeutic Opportunities. Nat. Rev. Drug Discov. 2013, 12, 347–357.

35. Longoni, B.; Fasciani, I.; Kolachalam, S.; Pietrantoni, I.; Marampon, F.; Petragnano, F.; Aloisi, G.; Coppolino, M.F.; Rossi, M.; Scarselli, M.; et al. Neurotoxic and Neuroprotective Role of Exosomes in Parkinson's Disease. CPD 2020, 25, 4510–4522.

36. Bebelman, M.P.; Crudden, C.; Pegtel, D.M.; Smit, M.J. The Convergence of Extracellular Vesicle and GPCR Biology. Trends Pharmacol. Sci. 2020, 41, 627–640.

37. Joseph, K.; Spicer, E.K.; Tholanikunnel, B.G. Regulatory Mechanism of G Protein-Coupled Receptor Trafficking to the Plasma Membrane. In Methods in Enzymology; Elsevier: Amsterdam, The Netherlands, 2013; Volume 521, pp. 131–150. ISBN 9780123918628.

38. Blobel, G. Protein Targeting (Nobel Lecture). ChemBioChem 2000, 1, 86–102.
39. Köchl, R.; Alken, M.; Rutz, C.; Krause, G.; Oksche, A.; Rosenthal, W.; Schülein, R. The Signal Peptide of the G Protein-Coupled Human Endothelin B Receptor Is Necessary for Translocation of the N-Terminal Tail across the Endoplasmic Reticulum Membrane. J. Biol. Chem. 2002, 277, 16131–16138.
40. Shirvani, H.; Gätà, G.; Marullo, S. Regulated GPCR Trafficking to the Plasma Membrane: General Issues and the CCR5 Chemokine Receptor Example. In GPCR Signalling Complexes–Synthesis, Assembly, Trafficking and Specificity; Dupré, D.J., Hébert, T.E., Jockers, R., Eds.; Springer: Dordrecht, The Netherlands, 2012; Volume 63, pp. 97–111. ISBN 9789400747647.
41. McLatchie, L.M.; Fraser, N.J.; Main, M.J.; Wise, A.; Brown, J.; Thompson, N.; Solari, R.; Lee, M.G.; Foord, S.M. RAMPs Regulate the Transport and Ligand Specificity of the Calcitonin-Receptor-like Receptor. Nature 1998, 393, 333–339.
42. Hay, D.L.; Walker, C.S.; Gingell, J.J.; Ladds, G.; Reynolds, C.A.; Poyner, D.R. Receptor Activity-Modifying Proteins; Multifunctional G Protein-Coupled Receptor Accessory Proteins. Biochem. Soc. Trans. 2016, 44, 568–573.
43. Hay, D.L.; Pioszak, A.A. Receptor Activity-Modifying Proteins (RAMPs): New Insights and Roles. Annu. Rev. Pharmacol. Toxicol. 2016, 56, 469–487.
44. Bouschet, T.; Martin, S.; Henley, J.M. Receptor-Activity-Modifying Proteins Are Required for Forward Trafficking of the Calcium-Sensing Receptor to the Plasma Membrane. J. Cell Sci. 2005, 118, 4709–4720.
45. Harikumar, K.G.; Simms, J.; Christopoulos, G.; Sexton, P.M.; Miller, L.J. Molecular Basis of Association of Receptor Activity-Modifying Protein 3 with the Family B G Protein-Coupled Secretin Receptor. Biochemistry 2009, 48, 11773–11785.
46. Lenhart, P.M.; Broselid, S.; Barrick, C.J.; Leeb-Lundberg, L.M.F.; Caron, K.M. G-Protein-Coupled Receptor 30 Interacts with Receptor Activity-Modifying Protein 3 and Confers Sex-Dependent Cardioprotection. J. Mol. Endocrinol. 2013, 51, 191–202.
47. Wootten, D.; Lindmark, H.; Kadmiel, M.; Willcockson, H.; Caron, K.; Barwell, J.; Drmota, T.; Poyner, D. Receptor Activity Modifying Proteins (RAMPs) Interact with the VPAC 2 Receptor and CRF 1 Receptors and Modulate Their Function: RAMP Interactions with VPAC 2 and CRF 1 Receptors. Br. J. Pharmacol. 2013, 168, 822–834.
48. Björk, S.; Hurt, C.M.; Ho, V.K.; Angelotti, T. REEPs Are Membrane Shaping Adapter Proteins That Modulate Specific G Protein-Coupled Receptor Trafficking by Affecting ER Cargo Capacity. PLoS ONE 2013, 8, e76366.
49. Free, R.B.; Hazelwood, L.A.; Cabrera, D.M.; Spalding, H.N.; Namkung, Y.; Rankin, M.L.; Sibley, D.R. D1 and D2 Dopamine Receptor Expression Is Regulated by Direct Interaction with the

Chaperone Protein Calnexin. J. Biol. Chem. 2007, 282, 21285–21300.

50. Achour, L.; Scott, M.G.H.; Shirvani, H.; Thuret, A.; Bismuth, G.; Labbé-Jullié, C.; Marullo, S. CD4-CCR5 Interaction in Intracellular Compartments Contributes to Receptor Expression at the Cell Surface. Blood 2009, 113, 1938–1947.
51. Rosemond, E.; Rossi, M.; McMillin, S.M.; Scarselli, M.; Donaldson, J.G.; Wess, J. Regulation of M 3 Muscarinic Receptor Expression and Function by Transmembrane Protein 147. Mol. Pharmacol. 2011, 79, 251–261.
52. Wei, Z.; Xu, X.; Fang, Y.; Khater, M.; Naughton, S.X.; Hu, G.; Terry, A.V.; Wu, G. Rab43 GTPase Directs Postsynaptic Trafficking and Neuron-Specific Sorting of G Protein–Coupled Receptors. J. Biol. Chem. 2021, 296, 100517.
53. Watson, P.H.; Fraher, L.J.; Natale, B.V.; Kisiel, M.; Hendy, G.N.; Hodsman, A.B. Nuclear Localization of the Type 1 Parathyroid Hormone/Parathyroid Hormone-Related Peptide Receptor in MC3T3-E1 Cells: Association with Serum-Induced Cell Proliferation. Bone 2000, 26, 221–225.
54. Savard, M.; Barbaz, D.; Bélanger, S.; Müller-Esterl, W.; Bkaily, G.; D'orléans-Juste, P.; Coté, J.; Bovenzi, V.; Gobeil, F. Expression of Endogenous Nuclear Bradykinin B2 Receptors Mediating Signaling in Immediate Early Gene Activation. J. Cell. Physiol. 2008, 216, 234–244.
55. Jong, Y.-J.I.; Kumar, V.; O'Malley, K.L. Intracellular Metabotropic Glutamate Receptor 5 (MGluR5) Activates Signaling Cascades Distinct from Cell Surface Counterparts. J. Biol. Chem. 2009, 284, 35827–35838.
56. Boivin, B.; Lavoie, C.; Vaniotis, G.; Baragli, A.; Villeneuve, L.; Ethier, N.; Trieu, P.; Allen, B.; Hebert, T. Functional β-Adrenergic Receptor Signalling on Nuclear Membranes in Adult Rat and Mouse Ventricular Cardiomyocytes. Cardiovasc. Res. 2006, 71, 69–78.
57. Saito, T.; Giaid, A. Cyclooxygenase-2 and Nuclear Factor-KappaB in Myocardium of End Stage Human Heart Failure. Congest. Heart Fail. 1999, 5, 222–227.
58. Lusk, C.P.; Blobel, G.; King, M.C. Highway to the Inner Nuclear Membrane: Rules for the Road. Nat. Rev. Mol. Cell Biol. 2007, 8, 414–420.
59. Jong, Y.-J.I.; Harmon, S.K.; O'Malley, K.L. GPCR Signalling from within the Cell: Intracellular GPCR Signaling. Br. J. Pharmacol. 2018, 175, 4026–4035.
60. Zuleger, N.; Kerr, A.R.W.; Schirmer, E.C. Many Mechanisms, One Entrance: Membrane Protein Translocation into the Nucleus. Cell. Mol. Life Sci. 2012, 69, 2205–2216.
61. Wing, C.E.; Fung, H.Y.J.; Chook, Y.M. Karyopherin-Mediated Nucleocytoplasmic Transport. Nat. Rev. Mol. Cell Biol. 2022, 23, 307–328.
62. Morinelli, T.A.; Raymond, J.R.; Baldys, A.; Yang, Q.; Lee, M.; Luttrell, L.; Ullian, M.E. Identification of a Putative Nuclear Localization Sequence within ANG II AT 1A Receptor Associated with

Nuclear Activation. Am. J. Physiol. -Cell Physiol. 2007, 292, C1398–C1408.

63. Lee, D.K.; Lança, A.J.; Cheng, R.; Nguyen, T.; Ji, X.D.; Gobeil, F.; Chemtob, S.; George, S.R.; O'Dowd, B.F. Agonist-Independent Nuclear Localization of the Apelin, Angiotensin AT1, and Bradykinin B2 Receptors. J. Biol. Chem. 2004, 279, 7901–7908.
64. Don-Salu-Hewage, A.S.; Chan, S.Y.; McAndrews, K.M.; Chetram, M.A.; Dawson, M.R.; Bethea, D.A.; Hinton, C.V. Cysteine (C)-X-C Receptor 4 Undergoes Transportin 1-Dependent Nuclear Localization and Remains Functional at the Nucleus of Metastatic Prostate Cancer Cells. PLoS ONE 2013, 8, e57194.
65. Di Benedetto, A.; Sun, L.; Zambonin, C.G.; Tamma, R.; Nico, B.; Calvano, C.D.; Colaianni, G.; Ji, Y.; Mori, G.; Grano, M.; et al. Osteoblast Regulation via Ligand-Activated Nuclear Trafficking of the Oxytocin Receptor. Proc. Natl. Acad. Sci. USA 2014, 111, 16502–16507.
66. Favre, N.; Camps, M.; Arod, C.; Chabert, C.; Rommel, C.; Pasquali, C. Chemokine Receptor CCR2 Undergoes Transportin1-Dependent Nuclear Translocation. Proteomics 2008, 8, 4560–4576.
67. Joyal, J.-S.; Nim, S.; Zhu, T.; Sitaras, N.; Rivera, J.C.; Shao, Z.; Sapieha, P.; Hamel, D.; Sanchez, M.; Zaniolo, K.; et al. Subcellular Localization of Coagulation Factor II Receptor-like 1 in Neurons Governs Angiogenesis. Nat. Med. 2014, 20, 1165–1173.
68. Sergin, I.; Jong, Y.-J.I.; Harmon, S.K.; Kumar, V.; O'Malley, K.L. Sequences within the C Terminus of the Metabotropic Glutamate Receptor 5 (MGluR5) Are Responsible for Inner Nuclear Membrane Localization. J. Biol. Chem. 2017, 292, 3637–3655.
69. Bhosle, V.K.; Rivera, J.C.; Chemtob, S. New Insights into Mechanisms of Nuclear Translocation of G-Protein Coupled Receptors. Small GTPases 2017, 10, 254–263.
70. Mizuno-Yamasaki, E.; Rivera-Molina, F.; Novick, P. GTPase Networks in Membrane Traffic. Annu. Rev. Biochem. 2012, 81, 637–659.
71. Bhosle, V.K.; Rivera, J.C.; Zhou, T.; Omri, S.; Sanchez, M.; Hamel, D.; Zhu, T.; Rouget, R.; Rabea, A.A.; Hou, X.; et al. Nuclear Localization of Platelet-Activating Factor Receptor Controls Retinal Neovascularization. Cell Discov. 2016, 2, 16017.
72. Williams, C.L. The Polybasic Region of Ras and Rho Family Small GTPases: A Regulator of Protein Interactions and Membrane Association and a Site of Nuclear Localization Signal Sequences. Cell Signal. 2003, 15, 1071–1080.
73. Wang, Q.; Zhang, H.; Xu, H.; Guo, D.; Shi, H.; Li, Y.; Zhang, W.; Gu, Y. 5-HTR3 and 5-HTR4 Located on the Mitochondrial Membrane and Functionally Regulated Mitochondrial Functions. Sci. Rep. 2016, 6, 37336.

74. Belous, A.; Wakata, A.; Knox, C.D.; Nicoud, I.B.; Pierce, J.; Anderson, C.D.; Pinson, C.W.; Chari, R.S. Mitochondrial P2Y-Like Receptors Link Cytosolic Adenosine Nucleotides to Mitochondrial Calcium Uptake. J. Cell. Biochem. 2004, 92, 1062–1073.
75. Gbahou, F.; Cecon, E.; Viault, G.; Gerbier, R.; Jean-Alphonse, F.; Karamitri, A.; Guillaumet, G.; Delagrange, P.; Friedlander, R.M.; Vilardaga, J.-P.; et al. Design and Validation of the First Cell-Impermeant Melatonin Receptor Agonist: Cell-Impermeant Melatonin Receptor Agonist. Br. J. Pharmacol. 2017, 174, 2409–2421.
76. Abadir, P.M.; Walston, J.D.; Carey, R.M. Subcellular Characteristics of Functional Intracellular Renin–Angiotensin Systems. Peptides 2012, 38, 437–445.
77. Chen, H.; Zhang, S.; Zhang, X.; Liu, H. QR Code Model: A New Possibility for GPCR Phosphorylation Recognition. Cell Commun. Signal. 2022, 20, 23.
78. Tobin, A.B.; Butcher, A.J.; Kong, K.C. Location, Location, Location…site-Specific GPCR Phosphorylation Offers a Mechanism for Cell-Type-Specific Signalling. Trends Pharmacol. Sci. 2008, 29, 413–420.
79. Nobles, K.N.; Xiao, K.; Ahn, S.; Shukla, A.K.; Lam, C.M.; Rajagopal, S.; Strachan, R.T.; Huang, T.-Y.; Bressler, E.A.; Hara, M.R.; et al. Distinct Phosphorylation Sites on the β 2 -Adrenergic Receptor Establish a Barcode That Encodes Differential Functions of β-Arrestin. Sci. Signal. 2011, 4, ra51.
80. Rabalski, A.J.; Gyenis, L.; Litchfield, D.W. Molecular Pathways: Emergence of Protein Kinase CK2 (CSNK2) as a Potential Target to Inhibit Survival and DNA Damage Response and Repair Pathways in Cancer Cells. Clin. Cancer Res. 2016, 22, 2840–2847.
81. Rossi, M.; Ruiz de Azua, I.; Barella, L.F.; Sakamoto, W.; Zhu, L.; Cui, Y.; Lu, H.; Rebholz, H.; Matschinsky, F.M.; Doliba, N.M.; et al. CK2 Acts as a Potent Negative Regulator of Receptor-Mediated Insulin Release in Vitro and in Vivo. Proc. Natl. Acad. Sci. USA 2015, 112, E6818–E6824.
82. Torrecilla, I.; Spragg, E.J.; Poulin, B.; McWilliams, P.J.; Mistry, S.C.; Blaukat, A.; Tobin, A.B. Phosphorylation and Regulation of a G Protein–Coupled Receptor by Protein Kinase CK2. J. Cell. Biol. 2007, 177, 127–137.
83. Luo, J.; Busillo, J.M.; Stumm, R.; Benovic, J.L. G Protein-Coupled Receptor Kinase 3 and Protein Kinase C Phosphorylate the Distal C-Terminal Tail of the Chemokine Receptor CXCR4 and Mediate Recruitment of β-Arrestin. Mol. Pharmacol. 2017, 91, 554–566.
84. Bagnato, A.; Rosanò, L. New Routes in GPCR/β-Arrestin-Driven Signaling in Cancer Progression and Metastasis. Front. Pharmacol. 2019, 10, 114.
85. Marampon, F.; Di Nisio, V.; Pietrantoni, I.; Petragnano, F.; Fasciani, I.; Scicchitano, B.M.; Ciccarelli, C.; Gravina, G.L.; Festuccia, C.; Del Fattore, A.; et al. Pro-Differentiating and

Radiosensitizing Effects of Inhibiting HDACs by PXD-101 (Belinostat) in in Vitro and in Vivo Models of Human Rhabdomyosarcoma Cell Lines. Cancer Lett. 2019, 461, 90–101.

86. Petragnano, F.; Pietrantoni, I.; Di Nisio, V.; Fasciani, I.; Del Fattore, A.; Capalbo, C.; Cheleschi, S.; Tini, P.; Orelli, S.; Codenotti, S.; et al. Modulating the Dose-Rate Differently Affects the Responsiveness of Human Epithelial Prostate- and Mesenchymal Rhabdomyosarcoma-Cancer Cell Line to Radiation. Int. J. Radiat. Biol. 2020, 96, 823–835.
87. Wang, W.; Han, T.; Tong, W.; Zhao, J.; Qiu, X. Overexpression of GPR35 Confers Drug Resistance in NSCLC Cells by β-Arrestin/Akt Signaling. Onco. Targets Ther. 2018, 11, 6249–6257.
88. Scarpa, M.; Molloy, C.; Jenkins, L.; Strellis, B.; Budgett, R.F.; Hesse, S.; Dwomoh, L.; Marsango, S.; Tejeda, G.S.; Rossi, M.; et al. Biased M1 Muscarinic Receptor Mutant Mice Show Accelerated Progression of Prion Neurodegenerative Disease. Proc. Natl. Acad. Sci. USA 2021, 118, e2107389118.
89. Bradley, S.J.; Molloy, C.; Valuskova, P.; Dwomoh, L.; Scarpa, M.; Rossi, M.; Finlayson, L.; Svensson, K.A.; Chernet, E.; Barth, V.N.; et al. Biased M1-Muscarinic-Receptor-Mutant Mice Inform the Design of next-Generation Drugs. Nat. Chem. Biol. 2020, 16, 240–249.