This Review is part of a thematic series on **Heterodimerization of Signaling Molecules**, which includes the following articles:

Regulation of G Protein–Coupled Receptor Signaling by Scaffold Proteins

G Protein–Coupled Receptor Heterodimerization

Gerda Breitwieser, Editor

Regulation of G Protein–Coupled Receptor Signaling by Scaffold Proteins

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Abstract—The actions of many hormones and neurotransmitters are mediated through stimulation of G protein–coupled receptors. A primary mechanism by which these receptors exert effects inside the cell is by association with heterotrimeric G proteins, which can activate a wide variety of cellular enzymes and ion channels. G protein–coupled receptors can also interact with a number of cytoplasmic scaffold proteins, which can link the receptors to various signaling intermediates and intracellular effectors. The multicomponent nature of G protein–coupled receptor signaling pathways makes them ideally suited for regulation by scaffold proteins. This review focuses on several specific examples of G protein–coupled receptor-associated scaffolds and the roles they may play in organizing receptor-initiated signaling pathways in the cardiovascular system and other tissues. **(*Circ Res*. 2002;91:672-680.)**

Key Words: GPCR ■ adrenergic ■ heptahelical ■ arrestin ■ phosphorylation

Cardiovascular function is regulated by a wide variety of hormones and neurotransmitters. The vast majority of hormones and neurotransmitters in the cardiovascular system exert their cellular effects through activation of G protein–coupled receptors (GPCRs), which are cell surface receptors also known as heptahelical receptors, 7-transmembrane receptors, and serpentine receptors because of their characteristic transmembrane topology. Agonist stimulation of a GPCR results in a conformational change in the receptor, leading to receptor association with heterotrimeric G proteins within the plasma membrane. This interaction causes conformational changes within the G-protein subunits leading to GDP release and GTP binding to the Gα subunit. The Gα and G$\beta\gamma$ subunits then dissociate from each other and exert regulation over various effectors such as enzymes and ion channels. Due to the multicomponent nature of GPCR signaling pathways, which involve at least a receptor, a G-protein complex, and an effector, these pathways can be made substantially more efficient via localization of the various components in close proximity to each other.

GPCRs associate not only with G proteins, but with a variety of other proteins as well.[1–4] GPCR-associated proteins may play at least four distinct roles in receptor signaling. First, a GPCR-associated protein may directly mediate receptor signaling, as in the case of G proteins. Second, a GPCR-associated protein may regulate receptor signaling through controlling receptor localization and/or trafficking. Third, a GPCR-associated protein may act as an allosteric modulator of receptor conformation, altering receptor pharmacology and/or other aspects of receptor function. Finally, a GPCR-associated protein may act as a scaffold, physically linking the receptor to various effectors. These four roles are by no means mutually exclusive. Each GPCR-associated protein may fill one, two, three, or all four of these roles.

Scaffold proteins may be defined as proteins that associate with two or more partners to enhance the efficiency and/or specificity of cellular signaling pathways. Interest in scaffold proteins has increased dramatically over the past decade, due to an explosion of technological advances in the detection and analysis of protein-protein interactions. These advances have

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List of GPCR/Scaffold Interactions

G Protein–Coupled Receptor	Scaffold	Scaffold-Associated Proteins
Drosophila rhodopsin	InaD[12]	PLC, PKC, TRP, calmodulin[8]
β_1-Adrenergic receptor	PSD-95[20]	NMDAR,[95] Kv1.4 channels,[96] nNOS,[97] neuroligins,[98] Fyn[99]
β_1-Adrenergic receptor	MAGI-2[21]	NMDAR,[100] β-catenin,[101] PTEN,[102] δ-catenin,[103] atrophin[104]
β_2-Adrenergic receptor	NHERF-1/2[23]	NHE3,[29] ezrin,[105] CFTR,[24,106–108] PDGFR,[109] Taz[110]
β_2-Adrenergic receptor	AKAP79/250[37–40]	PKA, PKC, calcineurin[36]
Angiotensin AT_1 receptor	Jak2[51]	STAT1[51]
Metabotropic glutamate receptors 1 and 5	Homer1/2/3[54]	Shank,[30] IP3R,[56] syntaxin 13[65]
Metabotropic glutamate receptors 1, 2, 3, and 5	Tamalin[31]	ARNO[31]
Metabotropic glutamate receptor 7	PICK1[32–34]	PKC,[111] AMPA receptors,[112] monoamine transporters[113]
Many G protein–coupled receptors	β-Arrestin1/2[68]	Src,[75] Jnk3,[81] ASK1,[81] Arf, ARNO,[73] Mdm2,[74] ERKs[77,79,83]
Serotonin 5-HT2C receptor	MUPP1[84,85]	NG2,[114] c-kit,[115] TAPP1[116]
Somatostatin SSTR2 receptor	CortBP1[86]	Cortactin[117]
Prolactin-releasing hormone receptor	PICK1[87]	PKC,[111] AMPA receptors,[112] monoamine transporters[113]
Dopamine D2,3 receptors	α-Filamin[88,89]	Actin,[118] integrins[119]
Calcium-sensing receptor	α-Filamin[90,91]	Actin,[118] integrins[119]
Cannabinoid CB1 receptor	FAN[92]	TNF receptor[120]
Prostaglandin EP3 receptor	Muskelin[93]	Thrombospondin-1[121]
α_2-Adrenergic receptors	14-3-3[94]	PKC,[122] Raf-1,[123] ASK1[124]

Scaffold proteins known to associate with various G protein–coupled receptors are listed along with selected other proteins known to associate with the scaffolds. This list includes all of the receptor/scaffold interactions described in the text as well as a number of other recently described interactions between GPCRs and scaffold proteins. Listing of scaffold-associated proteins does not imply that these proteins have been shown to exist in a cellular complex with the listed receptors in a scaffold-dependent manner. In fact, for many of the cases listed here, the function of the receptor-associated proteins as scaffolds is still hypothetical. Nonetheless, all of the proteins listed here as scaffolds are multidomain proteins that most likely serve scaffolding functions for the G protein–coupled receptors with which they associate.

led to the realization that many proteins have multiple binding partners and may therefore function as scaffold proteins. There are many different classes of scaffold protein, and over the past several years there have been a number of reviews on this subject.[5–9]

This review article will not attempt to exhaustively list all of the proteins that have been reported to associate with various G protein–coupled receptors.[1–4] Instead, the focus will be on describing a few specific cases where GPCR-associated proteins appear to be acting as scaffolds (Table). Furthermore, this review will not focus exclusively on GPCRs involved in regulating cardiovascular function. Several of the best examples of regulation of GPCR signaling by scaffold proteins come from outside the cardiovascular system, and these will be discussed as prototypes of GPCR/scaffold interactions. It is likely that there are many interactions between cardiovascular GPCRs and scaffold proteins that are physiologically important but that have not yet been characterized. Work on the prototypical examples of GPCR/scaffold interactions described in this review have helped to lay the foundation for understanding the structural determinants and functional importance of other GPCR/scaffold interactions that may be discovered in the future.

As illustrated in Figure 1, a GPCR can associate with a cytoplasmic scaffolding protein via one of three intracellular loops or via the receptor's intracellular carboxyl-terminus. The distal portions of many GPCR carboxyl-termini are known to interact with scaffold proteins containing PDZ domains,[9] and these interactions will be discussed in the first section of this review. The carboxyl-termini of many GPCRs are also known to be involved in associations with various non-PDZ scaffold proteins, and examples of these interactions will be discussed in the second section of this review. Finally, the third intracellular loops of most GPCRs contain key determinants for association with β-arrestins, important scaffold proteins that will be discussed in the third section of this review. The two types of GPCR that have been studied

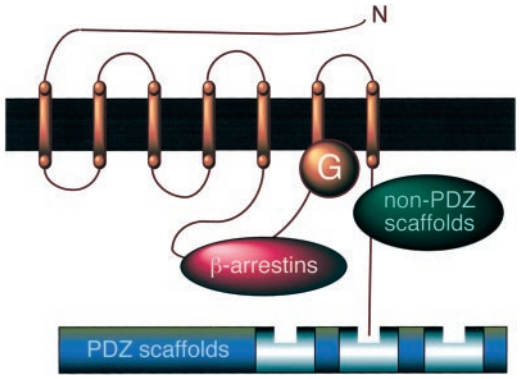

Figure 1. Schematic of GPCR/scaffold interactions. Scaffold proteins that interact with GPCRs may be divided into three broad categories: (1) PDZ scaffolds, which associate with the distal portions of GPCR carboxyl-termini; (2) various non-PDZ scaffolds, which associate with GPCR carboxyl-termini or other GPCR cytoplasmic regions; and (3) β-arrestins, which associate with many GPCRs and typically recognize determinants on GPCR third cytoplasmic loops.

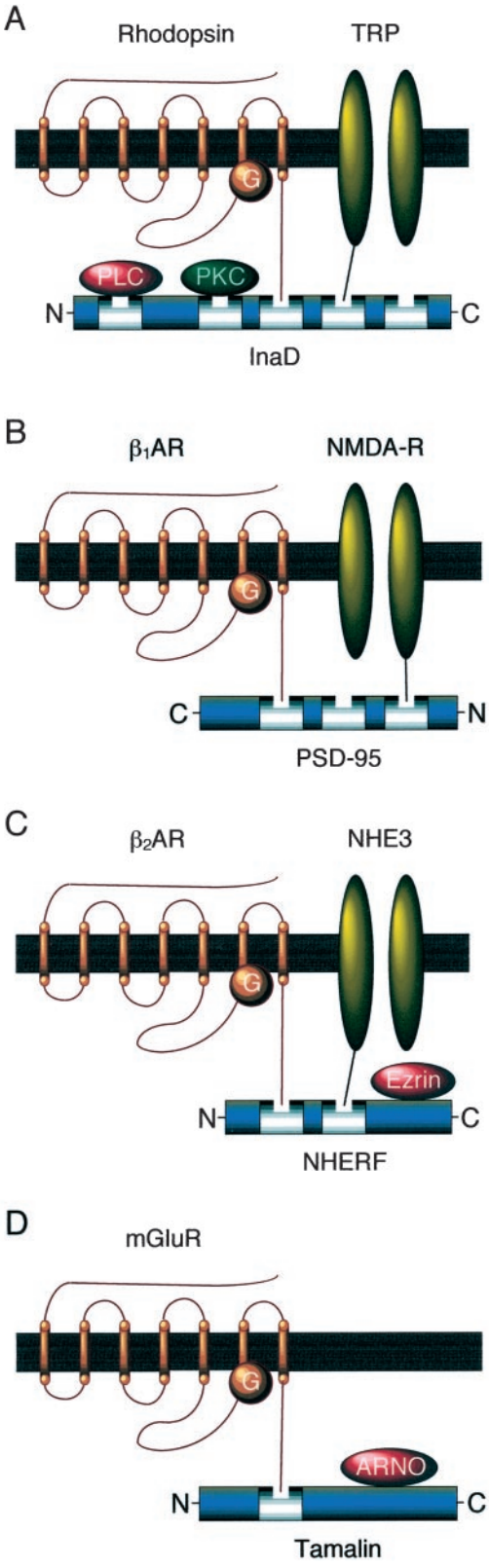

Figure 2. Prototypical GPCR/PDZ interactions. PDZ domain–containing scaffold proteins are shown in blue, with the PDZ domain regions indicated in gray. A, Multi-PDZ protein InaD can link *Drosophila* rhodopsin to key effectors, such as phospholipase C (PLC), protein kinase C (PKC), and the TRP calcium channel. B, Multi-PDZ protein PSD-95 can link the mammalian β_1-adrenergic receptor (β_1AR) to key effectors such as NMDA-type glutamate receptor channels. C, Multi-PDZ protein NHERF can bind to the β_2-adrenergic receptor (β_2AR) as well as to other proteins such as the Na^+-H^+ exchanger 3 (NHE3) and the actin-associated protein ezrin. D, PDZ protein tamalin can link various metabotropic glutamate receptor (mGluR) subtypes to cytoplasmic signaling proteins such as the ADP-ribosylation factor (ARF) nucleotide binding site opener (ARNO).

most intensively as model systems of the regulation of GPCR function are rhodopsin and β-adrenergic receptors,[10] and therefore these two receptor classes will be discussed first.

GPCR Carboxyl-Terminal Interactions With PDZ Scaffold Proteins

Rhodopsin, the light receptor, is a G protein–coupled receptor found in the retina. Accurate vision requires the rapid processing of visual images, and the cellular signaling pathways underlying vision therefore must be extremely fast. Given the unique demands of the visual system, it is perhaps no surprise that rhodopsin offers one of the clearest examples of G protein–coupled receptor signaling made more rapid and more efficient by means of an associated scaffold protein.

Drosophila rhodopsin physically associates with a scaffold protein named InaD.[11,12] InaD is a large cytoplasmic protein with five PSD-95/Discs-large/ZO-1 homology (PDZ) domains, which are specialized domains for mediating interactions with the carboxyl-termini of other proteins.[9] Two of the InaD PDZ domains mediate the interaction with rhodopsin,[12] whereas the other domains mediate interactions with a variety of other proteins involved in visual signaling. Mammalian visual signaling is quite different from that of *Drosophila*, and the role of rhodopsin-associated scaffold proteins in mammalian visual signaling is unclear at present.

Stimulation of *Drosophila* rhodopsin promotes coupling to a G_q protein that activates an isoform of phospholipase C, leading to increased intracellular calcium and the activation of protein kinase C as well as the opening of a calcium-regulated channel known as TRP. Most of the components of this signaling pathway (rhodopsin, phospholipase C, protein kinase C, and the calcium channel TRP) have been found to associate with InaD[8,11–18] (Figure 2A). With all of these molecules bound to the same scaffold and located in close spatial proximity to each other, visual signaling is less reliant on the slow speed of diffusion and can proceed with lightning quickness. As proof of the physiological importance of the scaffolding function of InaD, it has been shown that *Drosophila* mutants lacking InaD exhibit visual signaling that is both reduced in magnitude and dramatically slowed relative to wild-type flies: the amplitudes of quantum bumps in response to singe photons of light by mutants lacking InaD are less than one-fifth of the amplitudes observed in control flies, and the latencies of visual responses are slowed by more than 6-fold in the InaD mutants.[19]

β-Adrenergic receptors (βARs) exist as three distinct subtypes and mediate physiological responses to epinephrine, a key hormone involved in the regulation of cardiovascular function in response to stress. Like *Drosophila* rhodopsin, mammalian βARs can associate with PDZ domain–containing scaffold proteins. The β_1-adrenergic receptor associates with two related PDZ proteins, postsynaptic density protein

95 (PSD-95)[20–22] and membrane-associated guanylate kinase-like protein inverted-2 (MAGI-2).[21] The β_2-adrenergic receptor, in contrast, does not associate with PSD-95 or MAGI-2, but rather associates specifically with two other PDZ proteins, the Na^+-H^+ exchanger regulatory factor proteins NHERF-1 and NHERF-2.[23–25]

There is good evidence that the βAR-associated PDZ proteins can function as scaffolds. The association of the β_1AR with PSD-95[20] has been shown to physically link the β_1AR to effectors such as the *N*-methyl-D-aspartate (NMDA) class of glutamate receptor channels, which are known to be regulated by β_1AR stimulation in neurons[26–28] (Figure 2B). The agonist-promoted association of the β_2AR with the NHERF proteins,[23] in contrast, facilitates regulation of the Na^+-H^+ exchanger type 3 (NHE3), a cell surface transporter that is inhibited by NHERF proteins[29] (Figure 2C). Increases in cellular cAMP typically inhibit NHE3 activity, but β_2AR stimulation, which increases cellular cAMP, paradoxically enhances NHE3 activity.[29] This enhancement of NHE3 activity is blocked if the β_2AR cannot bind NHERF,[23] suggesting that either β_2AR association with NHERF prevents NHERF regulation of NHE3 or that association with NHERF links β_2AR to a cellular signaling pathway important for regulation of Na^+-H^+ exchange.

βAR associations with PDZ proteins are dependent on specialized motifs at the receptors' carboxyl-termini: E-S/T-x-V in the case of the β_1AR[20,21] and D-S/T-x-L in the case of the β_2AR.[23,24] Interestingly, the associations of β_1AR with PSD-95[22] and β_2AR with NHERF-1[25] have both been shown to be disrupted via receptor phosphorylation by G protein–coupled receptor kinase 5 (GRK5). PDZ domain interactions are often critically dependent on a serine or threonine at the -2 position of the target protein's carboxyl-terminus,[9] and it is therefore possible that many G protein–coupled receptor interactions with PDZ proteins will be regulated by GRK5 phosphorylation. Phosphorylation-dependent regulation of receptor association with scaffolds represents a mechanism by which cellular responsiveness to certain hormones, neurotransmitters, and other stimuli can be rapidly and reversibly fine-tuned.

Glutamate is the primary excitatory neurotransmitter in the mammalian central nervous system, and many of glutamate's physiological actions are mediated through a family of G protein–coupled receptors known as metabotropic glutamate receptors (mGluRs). The carboxyl-termini of metabotropic glutamate receptors can associate with a variety of PDZ domain–containing scaffold proteins. For example, mGluR1 and mGluR5 can interact directly with the PDZ domain of Shank proteins,[30] whereas mGluR1, mGluR2, mGluR3, and mGluR5 have been shown to associate with the PDZ protein tamalin[31] (Figure 2D). The motif S-S/T-L at the mGluR carboxyl-terminus is critical for both of these interactions. Tamalin is a cellular binding partner of the ADP-ribosylation factor (ARF) nucleotide binding site opener (ARNO), and has been shown to act as a scaffold to facilitate the physical linkage of mGluRs to ARNO in cells.[31] Finally, mGluR7 associates specifically with a PDZ protein referred to as the protein that interacts with C-kinase (PICK1).[32–35] The mGluR7 carboxyl-terminus ends in the motif L-V-I, which is critical for the interaction with PICK1[32,33] and is quite distinct from the mGluR1/2/3/5 motif that mediates interaction with tamalin. PICK1 has been shown to link mGluR7 to protein kinase C in cells, revealing a scaffolding function for this interaction.[33]

GPCR Carboxyl-Terminal Interactions With Non-PDZ Scaffold Proteins

The family of A-kinase anchoring proteins (AKAPs) was one of the first classes of proteins to be recognized as scaffold proteins.[36] These proteins associate with protein kinase A (PKA) and a variety of other proteins to organize cellular signaling pathways. β-Adrenergic receptors and many other G protein–coupled receptors can couple to G_s to increase cAMP and activate PKA downstream, and thus, the association of AKAPs with G protein–coupled receptors would seem to be an attractive potential mechanism for enhancing the efficiency of G_s-mediated signaling. Two different AKAPs have been found to interact with β-adrenergic receptors. AKAP250, also known as gravin, has been reported to bind to the β_2AR carboxyl-terminus, promoting receptor association with PKA and regulating receptor desensitization (Figure 3A).[37,38] AKAP79 has also been shown to interact with the β_2AR, promoting β_2AR phosphorylation and downstream mitogenic signaling.[39,40] The AKAP-binding motif on the β_2AR carboxyl-terminus has not been defined in detail, and thus it is not clear at present if interaction with AKAPs is unique to the β_2AR or if other GPCRs may also associate with certain AKAPs to organize signaling pathways involving PKA.

Angiotensin II is a potent hemodynamic regulator that exerts most of its physiological actions through a receptor known as AT_1. Stimulation of the AT_1 receptor has been found to activate not only traditional G-protein pathways but also the Janus kinase (Jak)-signal transducers and activators of transcription (STAT) signaling pathway,[41–52] which is typically activated by cytokine or growth factor receptors but not by GPCRs. Jaks are tyrosine kinases and STATs are transcription factors that can shuttle between the cytoplasm and the nucleus to regulate the expression of various genes.[53] The ability of the AT_1 receptor to regulate Jak/STAT signaling has been found to be dependent on a direct interaction between the AT_1R and Jak2.[45,49,51,52] Association of Jak2 with the AT_1R not only promotes Jak2 phosphorylation of STAT1, but also leads to recruitment of STAT1 into a complex with AT_1R,[51,52] revealing a function of Jak2 as a scaffold protein (Figure 3B). The interaction of Jak2 with the AT_1R is dependent on a specialized motif (Y-I-P-P) found in the AT_1R carboxyl-terminus[45] but not present in the carboxyl-termini of most other GPCRs.

The metabotropic glutamate receptor subtypes mGluR1 and mGluR5 have been found to associate via a specialized carboxyl-terminal motif (P-P-x-x-F-R) with the Homer family of proteins.[54–64] Homer 1b, 2, and 3 contain coiled-coil domains that mediate Homer multimerization, whereas Homer 1a is an immediate early gene that does not contain coiled-coil domains and thus can disrupt Homer 1b, 2, and 3 multimeric complexes when its expression is induced.[55,57] Association with Homer proteins has significant conse-

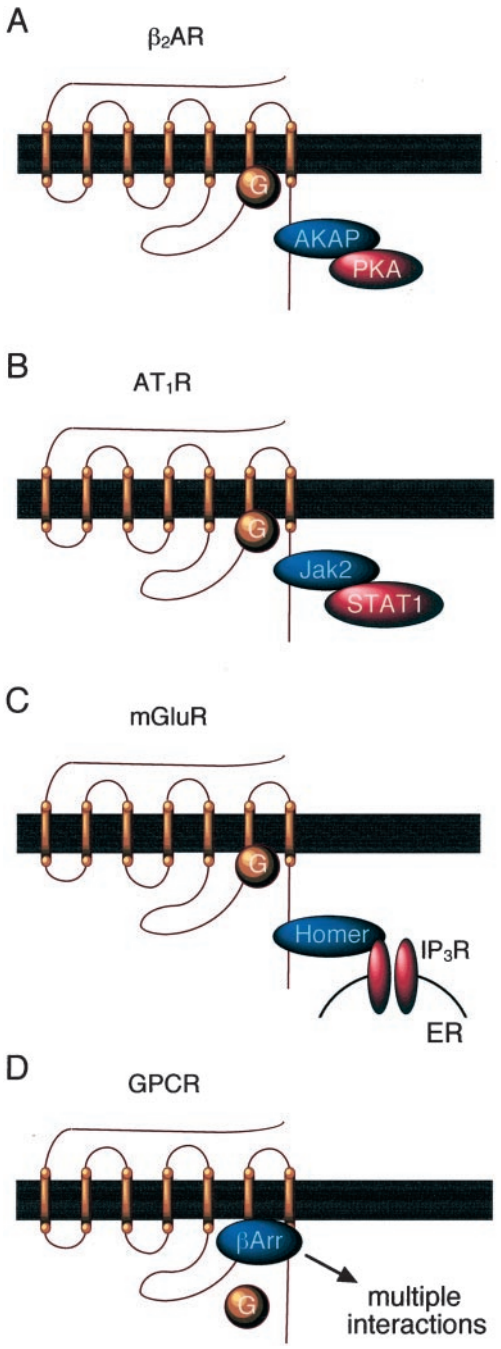

Figure 3. Prototypical GPCR interactions with non-PDZ scaffolds. A, AKAPs such as gravin and AKAP79 can link the β_2-adrenergic receptor (β_2AR) to protein kinase A. B, Jak2 can facilitate the association of angiotensin AT1 receptors with STAT1. C, Scaffold protein Homer can couple various metabotropic glutamate receptor (mGluR) subtypes to the inositol triphosphate receptor (IP3R), which is found in the endoplasmic reticulum (ER). D, β-Arrestins (βArr) associate with many GPCRs, disrupting G-protein coupling and also acting as scaffold proteins to facilitate multiple interactions between GPCRs and cytoplasmic proteins.

quences for mGluR1 and mGluR5 function, and many of these effects are likely due to actions of Homer proteins as scaffolds (Figure 3C). Homer proteins can bind to a variety of other proteins beyond themselves and mGluRs, including intracellular inositol triphosphate (IP3) receptors,[56] syntaxin 13,[65] and the Shank family of scaffold proteins.[30] Because mGluR1 and mGluR5 are both G_q-coupled receptors that lead to increased cellular levels of IP3 when stimulated, the Homer-dependent linkage of these receptors to IP3 receptors may be especially important for regulating their signaling.[56] Homer proteins also exert a strong effect on regulating the level of constitutive G-protein coupling to mGluR1 and mGluR5: association with Homer3 dampens down mGluR1/5 constitutive activity, whereas expression of Homer1a enhances constitutive activity of mGluR1 and mGluR5, probably by disrupting mGluR1/5 association with Homer3.[63] It is not certain at present if these effects on mGluR constitutive activity are dependent on the action of Homer proteins as scaffolds or if they are due instead to direct allosteric effects of Homer proteins on receptor conformation.

GPCR Interactions With β-Arrestins

All of the GPCR/scaffold interactions discussed thus far are dependent on the presence of specialized motifs in the GPCR carboxyl-termini. The requirement of a defined motif for receptor/scaffold interaction is of interest, because it reveals a molecular mechanism by which different subtypes of receptors may be specifically linked to different intracellular effectors via differential association with scaffold proteins. However, the requirement of a defined motif for scaffold interaction with GPCRs also limits the potential generality of each GPCR/scaffold interaction, because only a small number of GPCRs are likely to possess a specialized motif required for interaction with a particular scaffold protein.

The exception to the rule of specificity in GPCR/scaffold interactions is the β-arrestin family of proteins. β-Arrestin1 and β-arrestin2 were first identified as proteins involved in the desensitization of β-adrenergic receptors,[66,67] but it is now known that β-arrestins can associate with the majority of G protein–coupled receptors. Agonist activation of most G protein–coupled receptors results in receptor phosphorylation by GRKs, which promotes receptor association with β-arrestins and receptor uncoupling from G proteins.[68] Key determinants for interaction with β-arrestins are found in the third cytoplasmic loops of many GPCRs, although determinants in other intracellular GPCR regions may also contribute to β-arrestin association.[68] β-arrestins are known to associate with proteins involved in endocytosis such as clathrin,[69] AP-2,[70,71] *N*-ethylmaleimide–sensitive factor (NSF),[72] ARF6,[73] ARNO,[73] and Mdm2,[74] and these interactions facilitate agonist-promoted GPCR internalization into clathrin-coated pits. Thus, β-arrestins act as scaffolds to physically link G protein–coupled receptors to the endocytic machinery inside the cell. Unlike all of the other interactions described above, the interactions of β-arrestins with G protein–coupled receptors are not dependent on a specialized motif unique to one or several receptors, and it is therefore likely that β-arrestins act as scaffold proteins for most G protein–coupled receptors (Figure 3D).

β-Arrestins can associate with a variety of proteins other than G protein–coupled receptors and endocytic proteins. For example, β-arrestin1 can associate with the tyrosine kinase Src.[75,76] β-Arrestin–dependent recruitment of Src plays a key role in mitogenic signaling by the β_2-adrenergic receptor[75] as

well as in mitogenic signaling by other receptors such as neurokinin receptors,[77] interleukin receptors,[78] protease-activated receptors,[79] and endothelin receptors.[80] In light of these findings, β-arrestins are no longer viewed simply as proteins involved in G protein–coupled receptor desensitization, but rather as multipurpose scaffolds that can turn off some receptor-initiated signaling pathways while simultaneously activating other pathways.

Other proteins known to associate with β-arrestins include members of two distinct mitogen activated kinase (MAP) kinase cascades, c-Jun N-terminal kinase 3 (JNK3) and extracellular signal regulated kinases 1 and 2 (ERK1/2). In the case of JNK3, β-arrestin2, but not β-arrestin1, is a high-affinity binding partner of both JNK3 and apoptosis-stimulating kinase 1 (ASK1), a kinase upstream of JNK activation.[81,82] JNK3 activity is potently regulated by β-arrestin2 as well as by the recruitment of β-arrestin2 to activated angiotensin AT_1 receptors.[81] In the case of ERK1/2, both β-arrestins appear to be capable of scaffolding the ERKs in close proximity to various G protein–coupled receptors, facilitating receptor-mediated ERK activation.[77,79,83] Thus, β-arrestin1 and β-arrestin2 can have differential scaffolding activities with very different consequences for G protein–coupled receptor signaling.

Scaffold Versus Nonscaffold Actions of Receptor-Associated Proteins

As mentioned earlier, a protein may be considered a scaffold if it associates with two or more members of a signaling pathway to help increase the efficiency and/or specificity of the pathway. Not all receptor-associated proteins act as scaffolds, and even for those that do, it can often be difficult to determine whether a particular effect of the associated protein on receptor function is due to a scaffolding action or not. For example, the interactions of both β_1AR and β_2AR with their PDZ binding partners have effects on receptor internalization,[20,21,25] and the interactions of mGluR1 and mGluR5 with Homer proteins have significant consequences for receptor trafficking[52,53,55,56,59] and coupling to G proteins.[58] However, it is unclear if these effects are due to (1) a true scaffolding function of the receptor-associated proteins, (2) a direct allosteric action of the receptor-associated proteins on receptor conformation, or (3) a combination of scaffolding effects and direct allosteric effects. It is likely that many receptor-associated proteins act simultaneously as both scaffolds and direct allosteric modulators of receptor function, so these actions can therefore be difficult to tease apart experimentally.

Agonist-Dependence of Receptor/Scaffold Interactions

One of the most interesting questions to ask about scaffold proteins associated with G protein–coupled receptors is whether or not their interactions with receptors are regulated by agonist stimulation. It is well-known that agonist binding causes significant alterations in G protein–coupled receptor conformation, leading to profoundly enhanced association of receptor intracellular domains with G proteins.[10] Of the receptor/scaffold interactions discussed, several have been found to be enhanced by agonist stimulation, including β_2AR/NHERF, β_1AR/MAGI-2, AT_1R/Jak2, and the interaction of many receptors with β-arrestins. In contrast, there is no evidence at present for agonist regulation of some of the other receptor/scaffold interactions discussed, including rhodopsin/InaD, β_2AR/AKAP, β_1AR/PSD-95, mGluR/Homer, and mGluR/PDZ. The extent of agonist regulation of a receptor/scaffold interaction probably plays a significant role in determining the way in which the scaffold protein can regulate receptor signaling, especially in determining the temporal characteristics of the regulation.

Problems in Studying Receptor/Scaffold Interactions

There are several problems inherent in studying the biology of scaffold proteins. One problem is that measurements of the function of such proteins must always be indirect, because scaffold proteins are defined not by any intrinsic activity of their own but rather by their ability to enhance the interactions of other proteins. The activities of G proteins can be accurately assessed by measuring their GTPase activity, and the activities of kinases can be accurately quantified by measuring their phosphorylation of a substrate; unfortunately, however, there is no simple assay for the quantification of scaffolding activity. Rough estimates of scaffold protein efficiency can be derived only for signaling systems as a whole, examined in the absence or presence of a given scaffold protein. The speed of a signaling pathway is probably the easiest parameter to quantify, as in the case of rhodopsin signaling in the absence or presence of InaD.[19]

A second problem inherent in studying scaffold proteins is that the signaling pathways they organize can be very complex. For example, the PDZ domain-containing scaffold protein PSD-95 is known to associate with at least 50 distinct signaling proteins.[9] Studying the association of even two proteins in isolation can be extremely complicated, and studying the association of more than two proteins introduces numerous extra layers of complexity with each protein that is added into the mix. Scaffolds bind to multiple proteins, by definition, and to fully understand the function of a scaffold protein, it is important to understand in detail such issues as whether or not the various binding partners can bind to the scaffold simultaneously in cells. If the partners cannot all bind simultaneously, it is important to understand the sequence of binding events and the time course of each association, as well as how the binding of one protein might influence the binding or dissociation of all the other proteins. Questions such as these can be difficult to address experimentally in a quantitative fashion. Recent advances in real-time imaging of multiprotein cellular signaling complexes are likely to be very helpful in helping to answer such questions about the cellular functions of scaffold proteins.

Concluding Thoughts

Agonist-stimulated G protein–coupled receptors initiate cellular signaling pathways involving multiple components. Some G protein–coupled receptors have been found to associate with scaffold proteins, which also interact with components involved in downstream receptor signaling.

These scaffold proteins can have significant effects on the efficiency of receptor signaling. The differential distributions of various scaffold proteins may help to explain why certain receptors exhibit differential activity in distinct tissues. G protein–coupled receptors are extremely common targets for therapeutic pharmaceuticals in the treatment of cardiovascular diseases and other disorders, and disruption of receptor interactions with scaffold proteins represents an intriguing potential therapeutic approach to the treatment of various disease states.

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