RESEARCH HIGHLIGHT

GPCRs and β-arrestins — an on-off relationship

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Traditionally, G protein-coupled receptor (GPCR)-mediated G protein signaling has been thought to be terminated by receptor phosphorylation followed by recruitment of β-arrestins, which uncouples G protein from the receptor. However, new detailed insights published in *Cell* by Grimes et al. suggest that the initial agonist-stimulated β-arrestin accumulation at the cell membrane and GPCR–β-arrestin association are mechanistically distinct and much more dynamic than previously appreciated.

G protein-coupled receptors (GPCRs) are ubiquitously expressed cell surface biosensors that regulate many physiological processes and are considered important drug targets.[1] Agonist stimulation of GPCRs results in activation of heterotrimeric G proteins, which initiates an intracellular signaling cascade that leads to a cellular response.[2] In order to terminate G protein signaling, cells have devised a specialized desensitization mechanism that includes receptor phosphorylation by GPCR kinases and subsequent recruitment of β-arrestins (βarrs) to the phosphorylated receptors. Traditionally, the GPCR–βarr interaction is considered to block the G protein-binding site at the receptor core, promote receptor endocytosis, and initiate βarr-mediated signaling.[3] Furthermore, based on their affinity for βarrs, GPCRs are divided into two groups: Class A GPCRs, which contain sparse intracellular phosphorylation sites, and Class B GPCRs with intracellular phosphorylation site clusters.[4–6] Upon agonist stimulation, Class A GPCRs recruit βarrs transiently resulting in brief receptor internalization followed by recycling back to the cell surface, whereas Class B GPCRs are internalized into endosomes for prolonged periods of time.[4–6]

In a new study published in *Cell*, Grimes et al. used single-molecule imaging, biophysical, and molecular dynamics (MD) simulation approaches to provide a detailed and fascinating view into the initial accumulation of βarr2 at the plasma membrane upon GPCR activation[7] (Fig. 1). One of the most striking observations from this work was that βarr2 at resting states is not strictly confined to the cytosol as traditionally assumed. Rather, there appears to be a continuous and stochastic translocation of βarr2 molecules to the plasma membrane where they insert themselves into the lipid bilayer via their C-edge domain. Once embedded in the membrane, βarr2 diffuses laterally before detaching again shortly thereafter. The investigators noted that membrane-embedded βarr2 occasionally collides with free receptors to form a transient association. Unexpectedly, the rate at which βarr2 translocates from the cytosol to the plasma membrane did not change upon receptor stimulation, nor did the transient nature of the GPCR–βarr2 association. The only change detected in response to receptor stimulation was an increase in the rate of association between βarr2 and GPCRs. This rate increase was more substantial for Class B GPCRs as compared to Class A GPCRs. However, the transient GPCR–βarr2 association was equally short-lived for both classes of receptors.

Although the GPCR–βarr2 complex stability is not enhanced upon agonist challenge, the total time that βarr2 is embedded in the plasma membrane increases by almost 10-fold. The authors hypothesized that this longer plasma membrane residence time is a result of βarr2 "activation," which is accompanied with conformational changes that stabilize the protein within the membrane lipid environment. The net result is an accumulation of βarr2 at the cell membrane, instead of a direct recruitment to active and phosphorylated receptors. Interestingly, this accumulation was reduced for βarr2 mutants with disrupted C-edge domain, suggesting that the initial receptor-independent translocation from the cytosol to the cell membrane is necessary for its activation and stabilization at the cell surface. In addition, MD simulations proposed that a region of βarr2, called the finger loop (FL), places itself in the lipid bilayer where it potentially prolongs the association of active βarr2 and the plasma membrane. Although the authors demonstrated that a βarr2 mutant with the FL deleted (βarr2-ΔFL) was not accumulated in the plasma membrane upon activation of the Class A GPCR β_2-adrenergic receptor, it has been previously shown that stimulation of Class B GPCRs such as the vasopressin type 2 receptor leads to similarly robust recruitment of βarr1-ΔFL to that of the wild-type βarr1.[8] Thus, it is not clear whether the potential insertion of the FL in the lipid bilayer contributes to the prolonged association of active βarrs with the plasma membrane.

The authors also observed that βarr2, upon dissociation from the receptor, diffuses laterally to clathrin-coated pits (CCPs). Using a βarr2 mutant with its clathrin/AP2-binding site deleted, they demonstrated that the CCP confinement of βarr2 is a result of an interaction with clathrin/AP2. Surprisingly, agonist-stimulated GPCRs were also visualized to diffuse to CCPs by themselves after βarr2 dissociation. Although some receptors co-localized with βarr2 at CCPs, the investigators observed receptors at these structures without any visible βarr2. The mechanism behind this receptor transport to CCPs is not obvious from the study. However, it depends on βarrs as no receptors accumulate at CCPs in βarr1/2 knockout CHO cells. As the single-molecule experiments were done in wild-type CHO cells, it cannot be ruled out that endogenous unlabeled βarrs either transport or attract the receptors to CCPs. In fact, this scenario fits well with the study showing that the Class B GPCRs were more effectively recruited to CCPs than Class A GPCRs.

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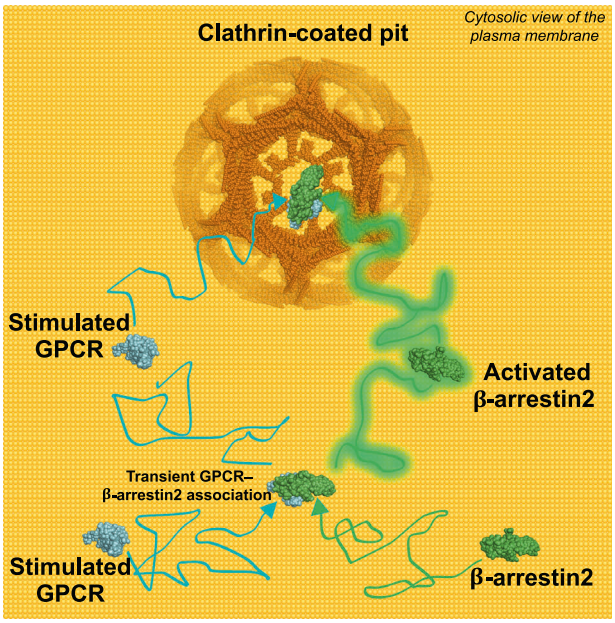

Fig. 1 A cytosolic view of how a membrane-embedded βarr2 diffuses freely and encounters an agonist-stimulated GPCR to form a transient complex. This short-lived association activates βarr2, which diffuses toward a CCP independently of the GPCR.

The newly discovered, highly dynamic association between GPCRs and βarrs suggests that our traditional model of initial GPCR activation needs to be reconsidered as this association was thought to be much more stable. However, once GPCRs and βarrs reach CCPs, they are likely to become trapped due to the high local presence of binding partners such as clathrin, AP2, and βarrs (for receptors). Beyond this step, the GPCR–βarr association was not further interrogated in this study due to technical limitations. Thus, it is not known whether the highly dynamic nature of the initial GPCR–βarr interaction persists upon receptor internalization. On one hand, it is not clear why the GPCR–βarr association should not be as dynamic at internalized compartments such as endosomes as at the plasma membrane. On the other hand, once internalized, it is well documented that Class A and Class B GPCRs behave very differently with respect to their association with βarrs. Class B GPCRs internalize into endosomes in complex with βarrs and can stay associated there for prolonged periods of time.[4–6] In contrast, Class A GPCRs lose their association with βarrs shortly after having been internalized.[4–6] Despite not interacting with βarrs in endosomes, some Class A GPCRs maintain their activity in this compartment and can stimulate G proteins.[9] Based on this, it is not straightforward to reconcile how dynamic βarr association with internalized Class A and Class B GPCRs can lead to these two distinct scenarios, and thus, the nature of the GPCR–βarr association in internalized compartments will have to be investigated in further detail.

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ADDITIONAL INFORMATION

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