

MINIREVIEW

β -Arrestin-Dependent Actin Reorganization: Bringing the Right Players Together at the Leading Edge

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ABSTRACT

First identified as mediators of G-protein-coupled receptor desensitization and internalization and later as signaling platforms, β -arrestins play a requisite role in chemotaxis and reorganization of the actin cytoskeleton, downstream of multiple receptors. However, the precise molecular mechanisms underlying their involvement have remained elusive. Initial interest in β -arrestins as facilitators of cell migration and actin reorganization stemmed from the known interplay between receptor endocytosis and actin filament formation, because disruption of the actin cytoskeleton inhibits these β -arrestin-dependent events. With growing interest in the mechanisms by which cells can sense a gradient of agonist during cell migration, investigators began to hypothesize that β -arrestins may contribute to directed migration by controlling chemotactic receptor turnover

at the plasma membrane. Finally, increasing evidence emerged that β -arrestins are more than just clathrin adaptor proteins involved in turning off receptor signals; they are actually capable of generating their own signals by scaffolding signaling molecules and controlling the activity of multiple cellular enzymes. This new role of β -arrestins as signaling scaffolds has led to the hypothesis that they can facilitate cell migration by sequestering actin assembly activities and upstream regulators of actin assembly at the leading edge. This *Minireview* discusses recent advances in our understanding of how β -arrestin scaffolds contribute to cell migration, focusing on recently identified β -arrestin interacting proteins and phosphorylation targets that have known roles in actin reorganization.

Introduction

β -Arrestins play a requisite role in chemotaxis and reorganization of the actin cytoskeleton, downstream of multiple receptors (for review, see DeFea, 2007; DeWire et al., 2007; Cotton and Claing, 2009). The ability of β -arrestins to orchestrate actin cytoskeletal rearrangements seems to depend in large part upon their role as signaling scaffolds, as evidenced by the identification of specific actin assembly scaffolds and

the observation that β -arrestin biased ligands can induce chemotaxis in the absence of G-protein engagement (Hunton et al., 2005b; Xiao et al., 2010; Zoudilova et al., 2010). However, the precise mechanisms by which they regulate actin reorganization and chemotaxis have continued to elude investigators. Over the past few years, a number of new β -arrestin scaffolds involving proteins with well established roles in the regulation of actin polymerization have been identified. In addition, new roles for “old” β -arrestin scaffolds, such as members of the mitogen-activated protein kinase (MAPK) family have emerged. Of particular interest as targets of β -arrestin signaling are proteins that create free actin

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ABBREVIATIONS: MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor; LIMK, Lin11/Is1-1/Mec-3 domain kinase 1; WASp, Wiskott-Aldrich-family protein; ERK, extracellular signal-regulated kinase; siRNA, small interfering RNA; AT1AR, angiotensin II type 1A receptor; PI3K, phosphatidylinositol 3-kinase; fMLP, *N*-formyl-methionine-leucine-phenylalanine; GDS, guanine nucleotide dissociation stimulator; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase; Jnk, c-Jun NH₂-terminal kinase; MLCK, myosin light chain kinase; PAR₂, proteinase-activated receptor 2; CXCR, C-X-C chemokine receptor; MKK, mitogen-activated protein kinase kinase; PAFR, platelet activating factor receptor; Arp, actin-related protein; GEF, guanine nucleotide exchange factor; PAK4, p21 protein (Cdc42/Rac)-activated kinase 4; PIP5K, phosphatidylinositol 4-phosphate 5-kinase; PIP₂, phosphatidylinositol bisphosphate; ARNO, Arf nucleotide binding site opener; ELMO, engulfment and cell motility protein; WS, WHIM syndrome; WHIM, warts, hypogammaglobulinemia, infections, and myelokathexis.

barbed ends for polymerization, facilitate formation or reorganization of focal contacts, or facilitate the nucleation of new actin filaments.

The question of how β -arrestins regulate chemotaxis through scaffolding is a difficult one to address, because there are multiple factors to consider. First, most studies are conducted using a specific GPCR to activate β -arrestin-dependent signaling; however, there is a considerable amount of variation among GPCRs in terms of which scaffolds they promote (DeWire et al., 2007; DeFea, 2008, 2011; Luttrell and Gesty-Palmer, 2010). Second, even within β -arrestin/MAPK scaffolds, there is considerable variation between receptors in composition of the scaffolds, their subcellular localization, their mechanism of MAPK activation, and the downstream consequences of their formation. For example, not all GPCRs that promote β -arrestin-dependent MAPK activation also promote its cytoplasmic sequestration or β -arrestin-dependent chemotaxis (DeFea et al., 2000a,b). Thus, β -arrestin binding partners and β -arrestin-dependent phosphorylation events identified for one receptor may not be involved in signaling by another. Third, β -arrestins have been shown to both promote and inhibit some of the same downstream signaling moieties in a receptor-specific fashion. This means that two receptors can promote association of β -arrestins with the same proteins with opposite signaling outcomes.

Multiple theories remain as to how β -arrestins contribute to actin reorganization and chemotaxis. Although some studies strongly support the hypothesis that β -arrestins scaffold signaling molecules at the leading edge to enhance actin assembly, others suggest a more reciprocal role of the actin cytoskeleton in receptor internalization. The former hypothesis is supported by the identification of signaling scaffolds that enhance actin polymerization and membrane protrusion and by the fact that β -arrestin biased agonists can promote chemotaxis. A list highlighting β -arrestin binding partners and phosphorylation targets with known roles in chemotaxis is provided in Table 1. The latter hypothesis is supported by evidence that many of the targets of β -arrestin-dependent scaffolding are cytoskeletal proteins involved in clathrin-mediated endocytosis. Because tight spatiotemporal control over both actin assembly and receptor internalization is important for chemotaxis, both theories still seem to be valid.

Role of β -Arrestins in Actin Assembly

Actin assembly within a cell is the primary driving force behind directed cell movement and can be both directly and indirectly regulated by various proteins. De novo actin assembly is a spontaneous but slow process and the rate limiting step is thought to be the formation of a stable nucleus of three actin monomers. When the barbed end of a filament is free from capping proteins, addition of monomers onto pre-assembled filaments is very rapid. The major means by which a cell speeds up actin assembly are 1) activation of proteins that break existing filaments into smaller fragments creating a free barbed end at each break and 2) activation of nucleators (i.e., proteins that overcome the rate-limiting step in actin assembly by facilitating association of actin monomers into filament seeds). Cofilin is one of the primary actin filament severing proteins, and its activation is often an early event in cell migration. Cofilin rapidly disassembles existing filaments, providing seeds for elongation and, in

coordination with activation of nucleating proteins (discussed in the next paragraph), can lead to the formation of a leading edge that drives the direction of cell migration. Spatial control over cofilin activity is essential to allow for treadmilling of filaments at the leading edge while protecting filaments at just behind it. Formation of β -arrestin scaffolds containing β -arrestin-2, cofilin, and its upstream phosphatases, slingshot and chronophin, have been observed downstream of PAR₂ and AT1AR (Zoudilova et al., 2007, 2010; Xiao et al., 2010). Association with β -arrestin facilitates dephosphorylation of cofilin by its upstream phosphatase and localizes cofilin activity to the leading edge of the cell to promote barbed end formation and membrane protrusion (Xiao et al., 2010; Zoudilova et al., 2010) (Fig. 1). The scaffold containing cofilin, chronophin, and β -arrestins-1 and -2 has been demonstrated in primary white blood cells. In the absence of β -arrestin-2, association of cofilin and chronophin, cofilin dephosphorylation, and cell migration were abolished, pointing to the physiological significance of this scaffold (Zoudilova et al., 2010). Furthermore, β -arrestins can directly bind LIMK and antagonize its ability to phosphorylate and inhibit cofilin, further contributing to localized actin filament severing activity (Zoudilova et al., 2007).

To date, the cofilin scaffolds are the only β -arrestin scaffolds for which a role in actin assembly has been confirmed experimentally. However, there are studies that suggest β -arrestins could affect actin nucleation as well. The primary nucleators in mammalian cells are the Arp2/3 complex, formins, and p150spiral (Campellone and Welch, 2010; Firat-Karalar and Welch, 2011). Arp2/3 is crucial for the formation of branched actin filaments, such as those observed in the leading edge of migrating cells, and requires a second family of proteins, Wiskott-Aldrich-family proteins (WASPs), for activation. WASPs bind to Arp2/3 and induce a conformational change resulting in apposition of Arp2 and -3 and its subsequent activation. Although no direct connection between β -arrestins and Arp2/3-mediated nucleation has been demonstrated, Arp2/3 complex components and WASP family proteins have been identified in a proteomics screen as potential β -arrestin-interacting proteins (Xiao et al., 2007) and phosphorylation targets of MAPK (Christensen et al., 2010) (discussed in the next section). An interesting hypothesis is that β -arrestin binding to Arp2/3 induces a conformational change similar to that observed with WASPs (Firat-Karalar and Welch, 2011). Another possibility is that β -arrestins can scaffold Arp2/3 with WASP family members, resulting in actin nucleation, either synergizing with or independent of, input from other more well characterized pathways (Fig. 2). Furthermore, phosphorylation of Arp2 on threonine residues has been reported and is essential for its ability to nucleate actin and form branched filaments and activation of WASP family proteins is enhanced by serine/threonine phosphorylation of WASP-family proteins by ERK1/2 (Nakanishi et al., 2007; Mendoza et al., 2011). Thus, Arps and WASP family proteins are putative targets for β -arrestin-dependent MAPK regulation (discussed below) (Fig. 2). Additional actin nucleating proteins are formins and p150spiral, which are primarily responsible for de novo assembly of unbranched actin filaments (Firat-Karalar and Welch, 2011). To date it remains unknown whether β -arrestins regulate these proteins, although a formin-like protein was identified as a putative β -arrestin-dependent phosphorylation target (Christensen et al., 2010).

Actin cytoskeletal proteins regulated by β -arrestins

β arr, β -arrestin; co-IP, coimmunoprecipitation; N.R., not reported.

***β*-Arrestins and the Regulation of Monomeric GTPases.**

mation; Cdc42 induces filopodia formation; and Rac-1 is important for membrane ruffling and lamellipodia formation. Demonstrating a role for β -arrestin-1 in the regulation of RhoA, siRNA knockdown of β -arrestin-1 but not β -arrestin-2 significantly reduces RhoA activation and stress-fiber formation by ATIAR (Barnes et al., 2005). It is noteworthy that unlike many other β -arrestin-dependent signaling events, RhoA activity required cooperation between G_{α_q} -dependent and β -arrestin-dependent pathways. There are a number of potential mechanisms by which β -arrestins might affect RhoGTPase activity, including potentiation of guanine nucleotide exchange factor (GEF) activity or inhibition of GTPase

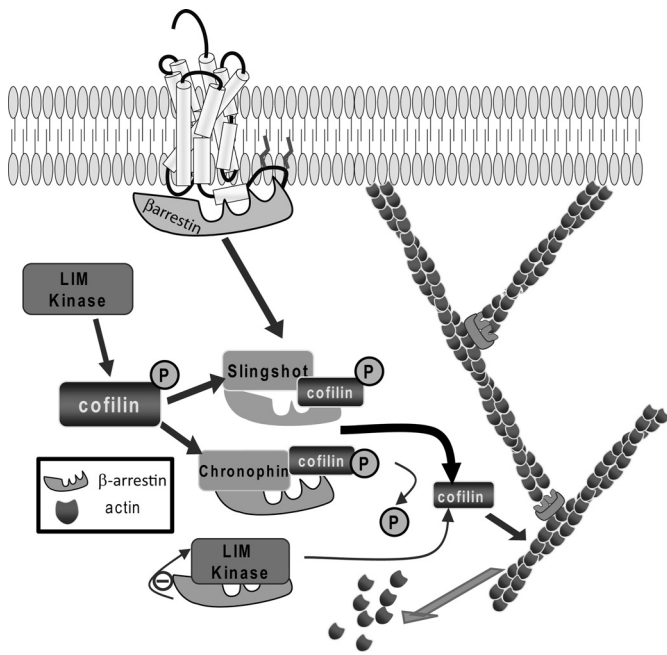


Fig. 1. Model for β -arrestin-dependent regulation of actin filament severing at the leading edge. β -Arrestin scaffolding of cofilin with its upstream phosphatases (Chronophin and Slingshot) leads to enhanced cofilin activity. In addition, inhibition of LIMK by β -arrestins contributes to the increased cofilin activity. Cofilin specifically severs older filaments creating new filament seeds and, in combination with Arp2/3, allows for continual formation of branched filaments at the leading edge.

activating proteins. Direct binding to and inhibition of a novel Rho GTPase activating protein (ARHGAP21) by β -arrestin-1 has recently been demonstrated. This interaction is stimulated by AT1AR activation and disruption of this complex inhibits AT1AR-mediated stress fiber formation and RhoA activation (Anthony et al., 2011). PIP₃, which is generated by PI3K (discussed below) can also activate a number of GEFs for RhoA Cdc42 and Rac, and β -arrestins have been shown to regulate PI3K activity. Whether PI3K is also involved in AT1AR-mediated RhoA activation has not been addressed. β -Arrestin-1-dependent p38MAPK activation has been reported to elicit F-actin rearrangement via a Rac-1-dependent mechanism, downstream of β_2 -adrenergic receptors (Gong et al., 2008), and so regulation of RhoA GTPases may also lie downstream of the MAPK scaffolds (discussed under *Tyrosine and Lipid Kinase Scaffolds and β -Arrestin-Dependent Cell Migration*). β -Arrestin-dependent regulation of RhoA GTPases has also been implicated in inhibition of

cell migration. The type III transforming growth factor- β receptor inhibits migration and alters actin cytoskeleton via forming a β -arrestin-2 scaffolding complex with Cdc42 in both cancer and epithelial cells (Mythreye and Blobel, 2009a,b).

Another GTPase, RalA, induces membrane blebbing in response to the fMLP receptor in neutrophils and lysophosphatidic acid in cancer cells (Bhattacharya et al., 2002, 2007; Li et al., 2009). Ral-GDS is a guanine exchange factor that activates RalA and can exist in an inactive complex with β -arrestin-1 in the cytosol of resting cells. Activation of the fMLP or lysophosphatidic acid receptor recruits the β -arrestin-1/Ral complex to the membrane and upon receptor/ β -arrestin-1 binding, Ral-GDS is released and activates RalA. fMLP receptor-induced membrane ruffling is blocked by a mutant Ral-GDS that cannot bind β -arrestin-1, suggesting that the ability of β -arrestin-1 to traffic it to the membrane is crucial for its activity. Subsequent studies have demonstrated that expression of RalA and β -arrestins-1 and -2 are increased in metastatic cancers, and expression of a Ral-GDS mutant that is deficient in β -arrestin binding inhibits RalA activation in tumor cells, leading to decreased cell migration. RalA itself is important for targeting filamin, another actin binding protein regulated by β -arrestins, to the membrane (Ohta et al., 1999). Filamin plays a role in adhesion turnover and kinase sequestration at the leading edge. Downstream of AT1AR, filamin associates with β -arrestin-2 and MAPK, and this complex is thought to play a role in membrane ruffling (Scott et al., 2006). Regulation of filamin in the context of β -arrestin-dependent MAPK scaffolding is discussed below.

β -Arrestin-Scaffolded MAPKs and Cytoskeletal Reorganization

Among the first identified β -arrestin scaffolds were those containing MAPK modules (ERK1/2/MEK1/2/Raf and Jnk/Ask1/MKK4) that regulate subsequent MAPK activation. A number of receptors that require β -arrestins for chemotaxis and actin cytoskeleton reorganization also promote β -arrestin-dependent association with MAPKs; however, the precise mechanism by which this pathway contributes to chemotaxis remains unclear (DeFea et al., 2000b; DeFea, 2007). A requirement for MAPKs in cell migration and cytoskeletal reorganization has been well established, and β -arrestins can sequester ERK1/2 at the leading edge of cells during cell migration in multiple cell types (Ge et al., 2003, 2004). Therefore, it has been hypothesized that sequestration of ERK1/2 and other MAPKs may facilitate the phosphorylation of cy-

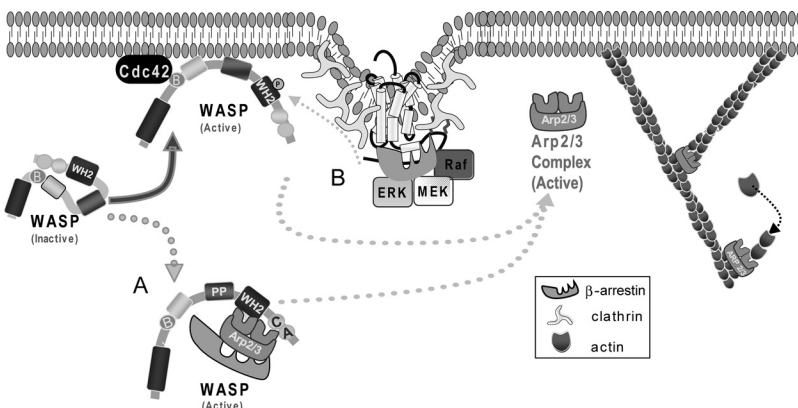


Fig. 2. Models for putative involvement of β -arrestin actin nucleation at the leading edge. A, proteomics screens identified p16 of the Arp2/3 complex as a β -arrestin-interacting protein. In this model, β -arrestins may either directly activate Arp2/3 or enhance its association with WASPs to facilitate actin nucleation. B, β -arrestin-sequestered MAPK may phosphorylate WASPs at the membrane to activate them, facilitate their association with Arp2/3, and increase actin nucleation.

tosolic proteins involved in chemotaxis. Unfortunately, studies identifying specific MAPK targets and proof of a requirement for their phosphorylation in chemotaxis have been slow to emerge. In fact, identifying such targets is far more complicated than it may appear. Many receptors trigger both β -arrestin-dependent and -independent activation of MAPKs; in some cases, only the β -arrestin-dependent pathway contributes to chemotaxis, whereas in others, there is synergy from both pathways. One would need to identify proteins whose receptor-dependent phosphorylation is inhibited by both knockdown/knockout of β -arrestins and inhibition of ERK1/2 activation. It would then be necessary to confirm that chemotaxis downstream of that same receptor was inhibited by β -arrestin knockdown, ERK1/2 inhibition, and mutation of putative β -arrestin-dependent ERK1/2 phosphorylation sites on the identified protein. To date, no such substrates have been rigorously identified, although some MAPK targets involved in cytoskeletal reorganization have been identified either as β -arrestin binding partners or proteins whose activity is regulated by β -arrestins.

One approach to predicting possible β -arrestin-dependent MAPK targets involved in chemotaxis has been to examine proteins that are known to affect actin dynamics. For example, phosphorylation of myosin light chain kinase (MLCK) and filamin by MAPK has been reported, as has an effect of phosphorylation on their activity and subsequent cytoskeletal reorganization (He et al., 2003; Nakagawa et al., 2010; Kim and McCulloch, 2011). As discussed above, filamin has been identified in a complex with β -arrestins and ERK1/2. It is noteworthy, however, that the proposed role of filamin in β -arrestin- and ERK1/2-dependent cytoskeletal changes is upstream, rather than as a MAPK substrate (Scott et al., 2006). The role of filamin in cell migration is a compound one, and the manner in which it is modulated by β -arrestins is still not completely clear. Like β -arrestins, filamin can sequester kinases at the leading edge. Thus, it is possible that it is synergistically involved in the previously described sequestration of ERK1/2 at the leading edge by β -arrestins. In addition, it plays an important role in turnover of adhesion proteins such as integrins during cell migration (Kim and McCulloch, 2011) and in internalization of dopamine receptor (Kim et al., 2005; Cho et al., 2007). Thus, β -arrestin-dependent regulation of filamin may contribute to gradient sensing by controlling receptor number at the membrane. MLCK phosphorylation by MAPK has been implicated as an important regulator of stress fiber contraction during cell migration (Klemke et al., 1997). MLCK phosphorylation downstream of PAR₂ was reported and seemed to correlate with changes in tight junction-associated actin reorganization and epithelial permeability. PAR₂-stimulated actin reorganization at tight junctions was inhibited by siRNA knockdown of β -arrestins (Cenac et al., 2004; Eutamene et al., 2005; Jacob et al., 2005). Thus, although it stands to reason that both filamin and MLCK are β -arrestin-dependent ERK1/2 substrates, this has not yet been definitively proven.

Other MAPKs have also been shown to be involved in β -arrestin-dependent cell migration. Suppression of β -arrestin-2 expression and inhibition of p38MAPK attenuated cell migration downstream of both AT1AR and CXCR4 (Sun et al., 2002; Hunton et al., 2005a), although both receptors promote β -arrestin-dependent ERK1/2 activation. Downstream of PAFR, β -arrestin1 recruits apoptosis signal-regu-

lating kinase-1, MKK3, and p38MAPK into a scaffold, and inhibition of p38MAPK or β -arrestin-1 inhibits PAFR-stimulated actin bundle formation. The Arp2/3 complex also localizes to the membrane in the same region upon PAFR activation, raising the question of whether it is a substrate for the sequestered p38MAPK (Velculescu et al., 1995). Another putative β -arrestin-dependent p38MAPK substrate is the 27-kDa heat shock protein, which binds to the barbed end of actin filaments and regulates chemotaxis through a phosphorylation-dependent process (Pichon et al., 2004; Jog et al., 2007). It has also been shown that β_2 -adrenergic receptors can promote G-protein-independent, β -arrestin-1/p38 MAPK-dependent 27-kDa heat shock protein phosphorylation and F-actin reorganization (Rojanathammanee et al., 2009).

Putative Targets of β -Arrestin-Dependent MAPK Phosphorylation

Three global proteomics screens have identified novel β -arrestin binding partners and β -arrestin-dependent phosphoproteins downstream of angiotensin II type 1A receptor activation that may be involved in cell migration (Xiao et al., 2007, 2010; Christensen et al., 2010). Among the β -arrestin phosphoprotein targets identified in these proteomics screens, a few are potentially phosphorylated by cytoplasmic/membrane-sequestered ERK1/2, as evidenced by the presence of a proline-directed kinase motif. Many of these proteins are members of the MAPK modules themselves or are involved in endocytosis, suggesting a reciprocal role for MAPKs and β -arrestins, in terms of each regulating the other's activity; however, many are known regulators of actin assembly. Although these putative targets have not been confirmed, we provided an analysis of likely targets and their possible role in actin assembly. One study, using siRNA knockdown of β -arrestin-2, identified a number of proteins associated with the actin cytoskeleton as putative β -arrestin-dependent MAPK substrates, including paxillin, RhoGEF7, PAK4, and α -adducin. Rap-GEF2 and RhoGEF7 are GEFs that activate the small GTPases Rap1 and RhoA, respectively. Both proteins are involved in cytoskeletal reorganization chemotaxis; β -arrestin-dependent regulation of RhoA downstream of AT1AR was discussed above (Barnes et al., 2005; Bhattacharya et al., 2007). Furthermore, there is evidence that phosphorylation of other RhoGEFs plays a role in AT1AR-stimulated RhoA activation (Ying et al., 2009). PAK4 is a downstream target of the RhoA pathway, and it can phosphorylate LIMK, which in turn inhibits the actin filament-severing protein cofilin (discussed above). It would be interesting to determine whether β -arrestins can inhibit PAK4 to further contribute to inhibition of LIMK activity or increased cofilin activity. Rap1 and PAK4 have also been implicated as upstream regulators of ERK1/2; thus, these proteins may also represent targets for negative feedback on MAPK pathways. Adducin is an actin-barbed end-capping protein, and studies suggest that phosphorylation by other kinases, such as protein kinase C and Rho-activated kinase, diminish its affinity for actin, increasing the pool of free barbed ends and facilitating actin polymerization. Thus, β -arrestin/ERK1/2-dependent phosphorylation may have a similar effect. The other study, using a biased agonist of AT1AR that activates only the β -arrestin-dependent signaling pathway, confirmed the β -arrestin-dependent phosphor-

ylation of α -adducin and identified a number of RhoGEFs as well as identifying other potential substrates (Christensen et al., 2010). Among the proteins identified was a WASp family member (WASP-2) as a potential β -arrestin-specific MAPK target. This result is particularly interesting, given that other groups have also reported MAPK-dependent phosphorylation of several WASps. In particular, phosphorylation of WAVE2 promotes its functional interaction with Arp2/3 and actin and is indispensable for membrane protrusion in response to epidermal growth factor stimulation (Nakanishi et al., 2007; Mendoza et al., 2011). Thus, Ser/Thr phosphorylation of WASps by β -arrestin-sequestered ERK1/2 may represent another novel mechanism by which β -arrestins regulate actin nucleation and subsequent formation of branched filaments. Another possible β -arrestin target is the actin binding protein known as eplin (Christensen et al., 2010; Xiao et al., 2010), identified both as a β -arrestin binding protein and a β -arrestin specific ERK1/2 phosphorylation target. Eplin binds actin filaments and has been reported to disassemble stress fibers in response to ERK1/2 phosphorylation (Han et al., 2007). Thus, β -arrestins may contribute to cytoskeletal reorganization through coordinated ERK/eplin-mediated disassembly and RhoA-mediated formation of stress fibers.

With a bit of luck, future studies will confirm whether these proteins do in fact represent long-sought-after β -arrestin-dependent cytoplasmic ERK1/2 substrates. It is important to bear in mind that the same pattern of β -arrestin-dependent phosphorylation may not be observed downstream of other receptors that promote β -arrestin-dependent ERK1/2 activation and chemotaxis. Because ATL1AR does not require ERK1/2 activation for chemotaxis, it is important to do similar studies with receptors known to promote ERK1/2-dependent chemotaxis.

Tyrosine and Lipid Kinase Scaffolds and β -Arrestin-Dependent Cell Migration

A number of other kinases with known roles in chemotaxis are targets of β -arrestin scaffolding. Over a decade ago, src, the nonreceptor tyrosine kinase, was identified as a β -arrestin binding partner downstream of the several GPCRs, linking them to ERK1/2 activation. In some cases, β -arrestin/src complexes are specifically linked to nuclear translocation rather than to cytoplasmic sequestration of ERK1/2, and do not promote cell migration (DeFea et al., 2000a). However, src-like tyrosine kinases and other nonreceptor tyrosine kinases play integral roles in the regulation of the actin cytoskeleton and cell migration. Two studies have demonstrated that prostaglandin E2 receptors promote cancer cell migration via β -arrestin-dependent recruitment of src into a signaling complex that then transactivates the epidermal growth factor Receptor (Kim et al., 2010). Similar β -arrestin-dependent transactivation pathways have been linked to proliferation as well. Another study demonstrated that macrophage inflammatory protein 1 β stimulation of the chemokine receptor CCR5 promotes cell migration through the coordinated activation and membrane recruitment of tyrosine kinases (pyk2 and Lyn) as well as PI3K and ERK1/2 (Cheung et al., 2009). Membrane recruitment and association of pyk2, Lyn, PI3K, and ERK1/2 is impaired by siRNA knockdown of both β -arrestins. Likewise, β -arrestin knockdown abolishes macrophage inflammatory protein 1 β -induced chemotaxis. It is noteworthy that formation of this complex is independent

of $G\alpha_i$, suggesting that this represents an alternate signaling pathway for CCR5-mediated chemotaxis, the more traditional pathway being through $\beta\gamma$ subunits released from $G\alpha_i$. These results suggest that β -arrestins serve to bring multiple kinase cascades together at the membrane; however, the precise order of phosphorylation in this particular complex is not yet known. Previous studies have shown that src-like kinases and focal adhesion kinases can phosphorylate actin-binding proteins directly, including vinculin, talin, paxillin, and tensin, to modulate cytoskeletal rearrangement and cell migration (Huvener and Danen, 2009). β -Arrestins possibly facilitate phosphorylation of key actin-binding proteins by scaffolded tyrosine kinases.

Phosphatidylinositol kinases PI3K and PIP5K can stimulate actin reorganization and cell migration through multiple downstream mediators including Rac, Cdc42, RhoA, WASps, Akt, and other proteins. PIP5K phosphorylates phosphatidylinositol 4-phosphate to create phosphatidylinositol bisphosphate (PIP₂). PI3K phosphorylates PIP₂ to generate PIP₃; thus, spatially restricted PI3K and PIP5K activities can contribute to the pool of PIP₂ and PIP₃ available for activation of downstream effectors. β -Arrestins can spatially regulate the activity of PI3K and its downstream effectors through direct inhibition or indirect stimulation and can directly bind and facilitate PIP5K activity (Beaulieu et al., 2005; Wang and DeFea, 2006; Wang et al., 2007; Nelson et al., 2008; Kendall et al., 2011). In vitro, β -arrestins directly bind to and inhibit PI3K activity (Wang and DeFea, 2006; Wang et al., 2007). β -Arrestins can also facilitate PI3K activity, although whether this involves direct interaction is unclear (Povsic et al., 2003). The concept that β -arrestins can both promote and inhibit PI3K activity depending on the receptor pathway activated is not as paradoxical as it seems. PI3K activity, like many other activities associated with cell migration, must be tightly controlled. Generation of PIP₃ is necessary for the activation of numerous proteins involved in chemotaxis, such as GEFs for RhoA GTPases and Akt. PIP₂, which is created by PIP5K and converted to PIP₃ by PI3K, is also needed for correct activation and localization of nucleation-promoting factors (Cantley, 2002; Firat-Karalar and Welch, 2011). β -Arrestin-dependent inhibition of PI3K and simultaneous stimulation of PIP5K may be important for maintaining a pool of PIP₂ available for WASp activation (Fig. 3), whereas β -arrestin-dependent activation of PI3K may contribute to RhoA activation and stress-fiber formation. Both activities are essential for cell migration but must be temporally and spatially controlled.

Reciprocal β -Arrestin/Internalization/Actin Assembly Pathways

There are two major theories as to how β -arrestins regulate cell migration and chemotaxis: one involving their classic role in receptor desensitization and chemotaxis and one involving their ability to scaffold and localize signaling proteins. It is noteworthy that many studies suggest these two activities also regulate each other. Although it has long been proposed that β -arrestin signaling complexes are linked to endocytosis to facilitate their trafficking and localization within the cell, investigators have also proposed that β -arrestin-dependent actin reorganization is required for receptor internalization. A prime example of this phenomenon is seen with PI3K. PI3K activity is required for early endocy-

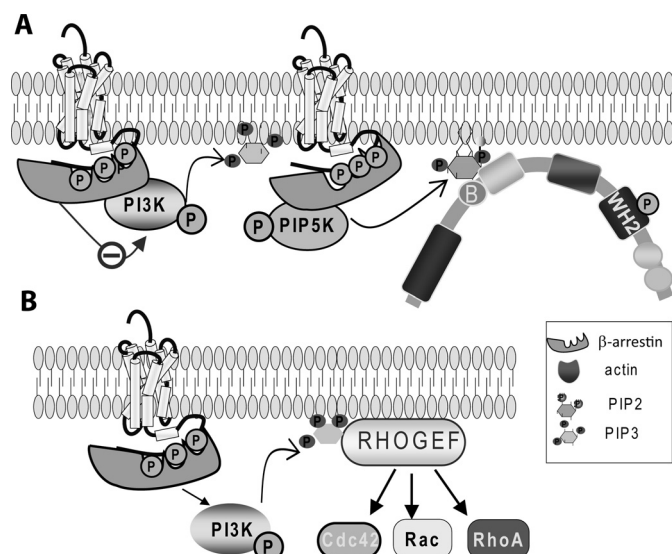


Fig. 3. Model for regulation of PI3K by β -arrestins at the leading edge. **A**, β -arrestin-dependent inhibition of PI3K and stimulation of PIP5K may result in localized pockets of increased PIP₂ concentration, leading to activation of WASPs. **B**, β -arrestin-dependent activation of PI3K may lead to activation of Rho-GEFs and subsequent Cdc42, RhoA, and Rac-1 activity.

otic vesicle formation, as well as the early steps in phagocytosis. PIP5K is essential for recruiting PIP₂ to clathrin-coated pits during initial formation of an early endosome (Naga Prasad et al., 2002; Nienaber et al., 2003). One theory is that β -arrestins facilitate early endosome formation through their interaction with clathrin as well as through their ability to regulate PI3K and PIP5K activities, which in turn facilitates membrane budding. Similarly, cofilin activity has been proposed to play a role in the rapid formation of actin filaments to facilitate retrograde trafficking of early endosomes (Okreglak and Drubin, 2007). The calcium-sensing receptor was also shown to trigger plasma membrane ruffling via a pathway that involves β -arrestin-1, Arf nucleotide binding site opener (ARNO), ADP-ribosylating factor 6 (ARF6), and engulfment and cell motility protein (ELMO) (Bouschet et al., 2007). Previous studies have also shown that β -arrestin/ARNO interactions are important for desensitization of β_2 -adrenergic receptor and luteinizing hormone/choriogonadotropin receptor (Mukherjee et al., 2000; Claing et al., 2001). Thus the β -arrestin scaffolding complex containing ARNO/ARF6/ELMO could connect extracellular signals to the cytoskeleton or facilitate receptor desensitization (Bouschet et al., 2007). With this reciprocal nature of β -arrestins in receptor endocytosis and desensitization in mind, it is likely that β -arrestins are capable both of promoting actin assembly to form leading edge and in desensitizing receptors to facilitate gradient sensing and directional cytoskeletal reorganization. Depending on the receptor, one or the other of these two functions may dominate, or both may be acting simultaneously.

Arrestin Scaffolds in Health and Disease

Inappropriate regulation of cytoskeleton can have pathological consequences, leading to tumor cell metastasis, uncontrolled inflammation, and developmental defects. A prime example is the proposed role of β -arrestin-mediated chemotaxis in cancer progression and metastasis (Sun et al.,

2002; Ge et al., 2004; Li et al., 2009). Two studies have shown that β -arrestin-dependent signaling contributes to cancer cell migration: CXCR4-mediated p38 activation in HeLa and human embryonic kidney 293 cells (Sun et al., 2002) and PAR₂-activated ERK1/2 activation in MDA-MB-231 breast cancer cells (Ge et al., 2004). Silencing of siRNA or MAPK (either p38MAPK or ERK1/2) reduced constitutive migration of tumor cells and β -arrestin levels and basal ERK1/2 activation were higher in metastatic than non-metastatic cell lines. More recent studies have suggested that lysophosphatidic acid receptor stimulates cell migration and cytoskeletal reorganization in MDA-MB-231, and this is reduced with expression of a mutant of RalGDS (RalGDS⁶¹⁶⁻⁷⁶⁸) that is deficient in β -arrestin binding (Li et al., 2009). Another study showed that prostaglandin E₂ induces lung cancer cell migration via EP4/ β -arrestin-1/c-Src signaling complex (Kim et al., 2010), suggesting that β -arrestin can result in differential modulation of signaling by bringing target proteins into scaffolding complexes in response to extracellular signals.

Evidence that β -arrestins promote leukocyte chemotaxis and that allergic asthma is reduced in β -arrestin-2(-/-) mice suggests that they are also important mediators of inflammation (Walker et al., 2003; Dickey et al., 2010). A requirement for β -arrestins in chemotaxis was first demonstrated in 2002 with the observation that chemokine receptor CXCR4-mediated lymphocyte chemotaxis was defective in β -arrestin-2 and G protein-coupled receptor kinase 6 knockout mice (Fong et al., 2002) or after small interfering RNA knockdown of β -arrestin-2. Studies have also demonstrated that β -arrestin-2 augments activation of MAPKs and cofilin in leukocytes (Fong et al., 2002; Sun et al., 2002; Zoudilova et al., 2007, 2010). A recent study (Lagane et al., 2008) also proposed a role for a β -arrestin scaffold in CXCR4-mediated chemotaxis in WHIM (warts, hypogammaglobulinemia, infections, and myelokathexis) syndrome (WS). WHIM syndrome is an immunodeficiency syndrome linked to heterozygous mutations of the CXCR4, resulting in a truncated receptor that lacks the last 15 residues of the C-tail. In the aforementioned study, it was shown that in leukocytes from patients with WS, the mutant receptor was recruited more slowly and to a lesser extent than wild-type CXCR4, suggesting that defective β -arrestin signaling may contribute to WS (Lagane et al., 2008).

Conclusions

Although we still do not fully understand the mechanism by which β -arrestins regulate the actin cytoskeleton, it is clear that this previously unappreciated activity of β -arrestins has widespread implications. We have focused here on the role of β -arrestin scaffolds in regulating actin assembly, severing and bundling events, rather than their putative role in receptor endocytosis. β -Arrestins clearly play an important role in the overall generation of free actin barbed ends at the leading edge, and scaffolding of cofilin with its upstream activators is important for this to occur. Additional studies are needed to address the possible role of β -arrestins in actin nucleation, despite the wealth of putative targets involved in this process. Likewise, there is considerable evidence that β -arrestins regulate actin-binding proteins involved in other aspects of cell morphology such as stress-fiber formation and filament bundling. There is also support for the model wherein β -arrestins regulate RhoA GTPases RalA and filamin to promote membrane protrusions necessary for cell

migration. How these activities are coordinated with cofilin activity has not yet been investigated. However, given the reciprocal relationship between signaling pathways such as cofilin and PI3K and endocytosis, it is important not to discount the traditional β -arrestin-associated events when assessing their contribution to cell migration. Many groups have demonstrated that the ability of a cell to sense direction is dependent upon its sensitivity to chemokine concentration gradients. Gradient sensing is regulated in part by desensitization of receptors, which in turn controls the number of receptors at the cell surface. As more information regarding the role of β -arrestins in the regulation of the cytoskeleton emerges, our understanding of how they regulate pathological processes such as inflammation and metastasis will increase. Given the potential proinflammatory and prometastatic effects of β -arrestins, the possibility that biased targeting of specific β -arrestin signaling pathways becomes more enticing. However, because of the importance of maintaining their function as the primary regulators of GPCR signal duration, a complete understanding of all of the ramifications of tampering with β -arrestin-dependent cytoskeletal reorganization is needed.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Min and DeFea.

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