Published in final edited form as:

Prog Mol Biol Transl Sci. 2018 ; 160: 47–61. doi:10.1016/bs.pmbts.2018.07.007.

Arrestins: Introducing Signaling Bias Into Multifunctional Proteins

Vsevolod V. Gurevich¹, Qiuyan Chen², and Eugenia V. Gurevich

Department of Pharmacology, Vanderbilt University, Nashville, TN, United States

Abstract

Arrestins were discovered as proteins that bind active phosphorylated G protein-coupled receptors (GPCRs) and block their interactions with G proteins, i.e., for their role in homologous desensitization of GPCRs. Mammals express only four arrestin subtypes, two of which are largely restricted to the retina. Two nonvisual arrestins are ubiquitous and interact with hundreds of different GPCRs and dozens of other binding partners. Changes of just a few residues on the receptor-binding surface were shown to dramatically affect GPCR preference of inherently promiscuous nonvisual arrestins. Mutations on the cytosol-facing side of arrestins modulate their interactions with individual downstream signaling molecules. Thus, it appears feasible to construct arrestin mutants specifically linking particular GPCRs with signaling pathways of choice or mutants that sever the links between selected GPCRs and unwanted pathways. Signaling-biased “designer arrestins” have the potential to become valuable molecular tools for research and therapy.

1. WILD-TYPE ARRESTINS: TOO MANY FUNCTIONS

The first arrestin, visual (systematic name arrestin-1^a), was discovered for its ability to specifically bind light-activated phosphorylated rhodopsin and suppress G protein activation.^{1,2} The first nonvisual arrestin, originally called β -arrestin, as it preferred β 2-adrenergic receptor (β 2AR) over rhodopsin (systematic name—arrestin-2), was discovered due to similar function: suppression of G protein activation by β 2AR.^{3,4} The second nonvisual arrestin, arrestin-3, was also discovered as a protein that specifically binds active phosphorylated G protein-coupled receptors (GPCRs),^{5–7} whereas cone-specific arrestin-4 was cloned by homology.^{8,9} It was shown in all cases that prior phosphorylation of GPCRs by GPCR kinases that specifically target active receptors¹⁰ was a prerequisite for high-affinity arrestin binding.¹¹ For some time, it appeared that the only function of all arrestins was the binding to active phosphorylated GPCRs, which precludes receptor interactions with G proteins.^{12,13} Thus, arrestins play a critical role in the second step of GPCR homologous desensitization.¹⁴ The findings that nonvisual arrestins bind major components of the internalization machinery of the coated pit clathrin¹⁵ and clathrin adaptor AP2¹⁶ were first

¹Corresponding author: vsevolod.gurevich@vanderbilt.edu.

²Current address: Department of Biological Sciences, Purdue University, West Lafayette, IN 47906, United States.

^aWe use systematic names of arrestin proteins, where the number after the dash indicates the order of cloning: arrestin-1 (historic names S-antigen, 48 kDa protein, visual or rod arrestin), arrestin-2 (β -arrestin or β -arrestin1), arrestin-3 (β -arrestin2 or hTHY-ARRX), and arrestin-4 (cone or X-arrestin).

viewed as an extension of their desensitizing activity because the removal of GPCRs off the plasma membrane reduces cellular response. However, the discoveries that receptor-bound arrestin facilitates the activation of c-Src,¹⁷ as well as of MAP kinases JNK3¹⁸ and ERK1/2,¹⁹ suggested that many more interactions were likely. In fact, each nonvisual arrestin was found to interact with more than a hundred diverse proteins²⁰ and the list of their binding partners keeps growing. Among other things, arrestins were implicated in recruiting signaling proteins to microtubules,²¹ in cytochrome C release during apoptosis,²² focal adhesion dynamics,²³ activation of small GTPases,^{24–27} glucose maintenance,²⁸ and many other cellular functions,^{29,30} including even heterologous desensitization of GPCRs phosphorylated by second messenger-activated kinases.^{31,32}

Structurally, all four vertebrate arrestins are quite similar: elongated two-domain molecules, with the long axis of no more than 90 Å and the short axis of ~30–35 Å^{33–38} (Fig. 1). Even taking into account their conformational flexibility,³⁹ arrestins are not big enough to accommodate more than 4–5 binding partners at the same time.⁴⁰ This is particularly true considering that many signaling proteins interact with receptor-bound arrestins, where one side of the molecule is engaged, and therefore shielded, by the GPCR⁴¹ (Figs. 1 and 2). Thus, there must be distinct interaction sites for each individual partner, and its binding likely excludes the binding of many others.

This notion implies two things: (a) targeted manipulation of individual sites can bias arrestin toward or away from particular partners; and (b) when these specific sites are identified, smaller elements with limited functional capabilities extracted from multifunctional arrestin proteins can be used as molecular tools to promote or suppress certain branches of signaling.

2. RECEPTOR SPECIFICITY: SELECTIVE TARGETING OF CERTAIN GPCRS?

Mammals express hundreds of different GPCRs,⁴² but only four arrestins.⁴³ However, evolution shows that an arrestin protein can be specific for a particular receptor. For example, arrestin-1 demonstrates remarkable preference for rhodopsin over other GPCRs.^{3,44} Experimental swapping of various elements⁴⁴ and individual residues⁴⁵ between arrestin-1 and nonvisual arrestin-2 identified a limited set of side chains that determine receptor preference. Mutagenesis of every residue in arrestin-1^{46,47} coupled with analysis of the crystal structure of the arrestin-1 complex with rhodopsin^{48,49} (Fig. 2) revealed several additional residues that play important roles in GPCR binding and/or directly contact the receptor, and therefore might contribute to receptor specificity. Collectively, these studies identified more than a dozen “suspects.” Placing every one of the 20 possible residues in each position would require an enormous number of combinations that could not be tested experimentally within a reasonable period of time. Luckily, the analysis of arrestin evolution^{29,43} shows that there were very few different residues in each of the critical positions, which substantially decreases the number of necessary mutations and combinations. The mutations of these “receptor discriminator” residues were actually introduced into the most promiscuous of the two nonvisual subtypes, arrestin-3.³⁸ Mutants were tested for their binding to β2AR, M2 muscarinic receptor, D1 and D2 dopamine

receptors, and Y1 and Y2 neuropeptide Y receptors.^{50–52} The results were encouraging. Replacement of just two residues enhanced arrestin-3 preference for some receptors over others ~50–60-fold.⁵¹ Certain mutations selectively affected arrestin interactions with inactive (predocking) and activated GPCRs.^{50,52} The good news is that these data suggest that the construction of nonvisual arrestins with relatively narrow receptor specificity is feasible. The bad news is that testing even a few dozen arrestin mutants with ~400 nonodorant GPCRs expressed in every mammalian species is hardly realistic. Thus, this work should specifically target GPCRs with known disease-causing gain-of-function mutations.^{53,54}

3. MAKING ARRESTINS PHOSPHORYLATION INDEPENDENT

Receptor binding induces a global conformational change in arrestins,⁵⁵ which includes the movement of the two domains relative to each other^{48,49,56–59} (Fig. 3). In all arrestins relatively few intramolecular interactions hold the molecule in its basal conformation. The most important among these are the polar core, an arrangement of five charged residues in the center of the molecule between the two domains,^{34,61} and a hydrophobic three-element interaction that involves β -strand I and α -helix in the N-domain and β -strand XX of the C-terminus.^{34,62} Destabilization of either of these interactions in the visual arrestin-1^{61–65} or in nonvisual arrestins^{66–69} yields “enhanced” mutants that bind the receptor more readily and demonstrate high binding even to unphosphorylated GPCRs.^{64,66–70} Therefore, these enhanced arrestins have the potential to suppress excessive signaling of GPCR mutants and, specifically, compensate for the defects in receptor phosphorylation. Thus, the construction of receptor-specific arrestins should focus on these enhanced mutants and GPCR subtypes where gain-of-function mutations cause human disorders.^{53,54} This work should follow the existing precedent of partial compensation of excessive signaling caused by the defects in rhodopsin phosphorylation by enhanced phosphorylation-independent version of visual arrestin-1, achieved in vivo in rod photoreceptors.^{71,72} After all, the approach that yields measurable compensation in rods with single photon sensitivity and subsecond signaling shutoff is likely to yield near-complete compensation in any other GPCR-driven signaling system, all of which are a significantly less sensitive and much slower.

4. CHANNELING THE SIGNALING TOWARD OR AWAY FROM SELECTED PATHWAYS

Arrestins have been implicated in the activation of numerous signaling proteins, including protein kinases c-Src,¹⁷ JNK3,¹⁸ and ERK1/2¹⁹ that play important roles in cell proliferation and death. Although arrestin-dependent signaling was long considered to be G protein independent,³⁰ recent studies showed that arrestin-mediated signaling in many cases (possibly always, although generalizations are dangerous) depends on the activation of G proteins.^{73,74} In fact, this is biologically understandable, as the previous model of arrestin-dependent signaling did not explain how the upstream-most kinases, such as c-Raf1 in case of ERK1/2, or ASK1 in case of JNK3, were activated. These kinases are not activated by arrestin-dependent scaffolding and must come to the scaffold already active. Therefore, their activation might require G protein-mediated signaling. As c-Src and ERK1/2 usually

transduce prosurvival and proliferation signals, whereas JNK family kinases mediate antiproliferative, sometimes proapoptotic signaling, selective channeling of arrestin-mediated signaling in one direction, but not the other, appears a tempting way of telling the cell to live or die in its own language. Targeted enhancement of pro- and antisurvival signaling has therapeutic potential in degenerative disorders and cancer, respectively.

However, to attempt this we need to understand the molecular mechanisms involved. Originally the activation of both ERK1/2 and JNK3 was reported to be the result of arrestin binding to the receptor.^{18,19} Subsequent experiments confirmed this in the case of ERK1/2—this kinase has fairly low affinity for free arrestins⁷⁵ and gets activated by phosphorylation only upon receptor activation.^{76,77} It turned out that JNK3 can be activated by free arrestin-3,^{75,77–79} and even by its receptor binding-deficient mutant^{75,77} in cells. Experiments with purified arrestin-3 and kinases in vitro left no doubt that neither GPCRs nor any other proteins are necessary.^{80,81} Since receptors were not required, it made sense to identify JNK3-binding elements in arrestin-3 that did not contain the GPCR-binding parts.⁸² Interestingly, one of these JNK3-binding peptides, containing the N-terminal residues 1–25, effectively facilitated JNK3 activation in vitro and in cells⁸³ (Fig. 4). This short peptide (smaller than 3 kDa) is so far the smallest known scaffold of any three-tier MAP kinase activation cascade. The smallest scaffold previously described was MP1 (~13.5 kDa), which appears to act in complex with its partner p14, which is not related by sequence, but very similar by 3D structure, making the whole scaffold ~27 kDa.⁸⁴ The other known MAP kinase scaffolds in yeast (Ste5p and Pbs2p) and mammals (KSR1, JIP1/2/3/4/5, and arrestin-2/3) range in size from 45 to 147 kDa (reviewed in⁸⁵). Interestingly, homologous arrestin-2-derived peptide, that differs from its arrestin-3-derived counterpart only in five positions (Fig. 4), does not facilitate JNK3 activation,⁸³ recapitulating the functional differences between parental full-length arrestin-2 and arrestin-3 proteins. The ability of the arrestin-3-derived mini-scaffold to suppress cell proliferation and/or support other JNK3-dependent biological functions needs to be tested. Mechanistically, its identification is an important breakthrough, as this is only the second example of a monofunctional element extracted from a multifunctional arrestin protein. The first one was the arrestin C-terminus, carrying both clathrin- and AP2-binding sites, that was shown to out-compete the receptor–arrestin complexes from coated pits, thereby specifically inhibiting arrestin-dependent GPCR internalization.⁸⁶ Two other arrestin-3-based tools were also developed: a V343T point mutant with severely impeded ability to activate JNK3⁷⁹ and a receptor binding-deficient multiple mutant that interacted with JNK3 but did not facilitate its activation.⁷⁷ The latter was even shown to act as a dominant-negative silent scaffold, suppressing arrestin-3-dependent JNK3 activation in cells.⁷⁷ As it was found to bind not only JNK3 but also upstream kinases ASK1 and MKK4, the most logical explanation of its dominant-negative action is that it recruits these kinases away from productive scaffolds, thereby suppressing signal transduction in the cascade. However, both of these are unlikely to be clean monofunctional tools, as they contain the whole arrestin-3 molecule, and therefore very likely retain many of its functions unrelated to the JNK signaling.

Other signaling-biased arrestin-based molecular tools were developed in which individual functions were disabled by mutagenesis. Arrestin-2-R307A mutant was shown to be defective in c-Raf1 binding,⁷⁶ whereas arrestin-2-D26A/D29A double mutant did not bind

MEK1.⁸⁷ As both c-Raf1 and MEK1 are upstream kinases in the ERK1/2 activation cascade, these mutants failed to promote ERK1/2 activation. Several arrestin-3 mutants that do not bind phosphodiesterase but appeared to retain other functions, including GPCR binding, were also described.⁸⁸ Collectively, these data prove that individual functions of arrestins can be eliminated, yielding signaling bias. Relatively short peptides that effectively bind clathrin and AP2⁸⁶ or facilitate the activation of JNK3⁸³ show that, in some cases, monofunctional elements of arrestin proteins can be created and used for targeted manipulation of cellular signaling.

5. “DESIGNER ARRESTINS”: A ROADMAP OR A DREAM?

Chimera construction,^{38,44,89} site-directed mutagenesis,^{45–47,50–52,90} peptide interaction studies,⁹¹ EPR,^{21,92} NMR,⁹³ and X-ray crystallography^{48,49,56,94} all suggest that the receptors engage the concave sides of both arrestin domains (Figs. 1 and 2). With few exceptions (such as calmodulin⁹⁵ and microtubules²¹), nonreceptor partners bind to the other side of the molecule or the C-terminus released upon receptor binding, although the binding sites were fully or partially localized, out of many dozens of proteins that bind arrestins,²⁰ only for clathrin,¹⁵ AP2,¹⁶ c-Raf1,⁷⁶ MEK1,⁸⁷ PDE,⁸⁸ and JNK3.^{82,83} In general, any protein that binds the arrestin–receptor complex has to engage the elements that are not shielded by the receptor⁴¹ (Figs. 1 and 2). Experimental evidence shows that targeted mutations in distinct parts of arrestins can modulate receptor preference and the interactions with downstream signaling proteins. The next obvious step would be to combine these two to construct true “designer arrestins” that link the receptor we want to the signaling pathway of our choosing. Potential scientific and therapeutic utility of such mutants is obvious. Although there might be certain limitations of what can be constructed, due to apparent allosteric connections between the receptor-binding sites and elements where downstream signaling proteins dock,^{59,60} only experimental work in this direction can reveal what is possible. Even a limited set of designer arrestins channeling the signaling from particular GPCRs to defined pathways would greatly enrich our toolbox used in research and medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Supported in part by NIH RO1 Grants EY011500, GM077561, and GM109955 (the latter two RO1s were replaced by R35 GM122491) (V.V.G.), NS065868 and DA030103 (E.V.G.).

ABBREVIATIONS

ASK1	apoptosis signal-regulating kinase 1
EPR	electron paramagnetic resonance
ERK	extracellular signal-regulated kinase

GPCR	G protein-coupled receptor
JNK	c-Jun N-terminal kinase
MAPK	mitogen-activated protein kinase
NMR	nuclear magnetic resonance

REFERENCES

1. Kuhn H, Hall SW, Wilden U. Light-induced binding of 48-kDa protein to photoreceptor membranes is highly enhanced by phosphorylation of rhodopsin. *FEBS Lett.* 1984;176:473–478. [PubMed: 6436059]
2. Wilden U, Hall SW, Kühn H. Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments. *Proc Natl Acad Sci USA.* 1986;83:1174–1178. [PubMed: 3006038]
3. Lohse MJ, Andexinger S, Pitcher J, et al. Receptor-specific desensitization with purified proteins. Kinase dependence and receptor specificity of beta-arrestin and arrestin in the beta 2-adrenergic receptor and rhodopsin systems. *J Biol Chem.* 1992;267:8558–8564. [PubMed: 1349018]
4. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. Beta-arrestin: a protein that regulates beta-adrenergic receptor function. *Science.* 1990;248:1547–1550. [PubMed: 2163110]
5. Attramadal H, Arriza JL, Aoki C, et al. Beta-arrestin2, a novel member of the arrestin/ beta-arrestin gene family. *J Biol Chem.* 1992;267:17882–17890. [PubMed: 1517224]
6. Sterne-Marr R, Gurevich VV, Goldsmith P, et al. Polypeptide variants of beta-arrestin and arrestin3. *J Biol Chem.* 1993;268:15640–15648. [PubMed: 8340388]
7. Rapoport B, Kaufman KD, Chazenbalk GD. Cloning of a member of the arrestin family from a human thyroid cDNA library. *Mol Cell Endocrinol.* 1992;84:R39–R43. [PubMed: 1587386]
8. Murakami A, Yajima T, Sakuma H, McLaren MJ, Inana G. X-arrestin: a new retinal arrestin mapping to the X chromosome. *FEBS Lett.* 1993;334:203–209. [PubMed: 8224247]
9. Craft CM, Whitmore DH, Wiechmann AF. Cone arrestin identified by targeting expression of a functional family. *J Biol Chem.* 1994;269:4613–4619. [PubMed: 8308033]
10. Gurevich EV, Tesmer JJ, Mushegian A, Gurevich VV. G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. *Pharmacol Ther.* 2012; 133:40–46. [PubMed: 21903131]
11. Gurevich VV, Gurevich EV. The molecular acrobatics of arrestin activation. *Trends Pharmacol Sci.* 2004;25:105–111. [PubMed: 15102497]
12. Wilden U. Duration and amplitude of the light-induced cGMP hydrolysis in vertebrate photoreceptors are regulated by multiple phosphorylation of rhodopsin and by arrestin binding. *Biochemistry.* 1995;34:1446–1454. [PubMed: 7827093]
13. Krupnick JG, Gurevich VV, Benovic JL. Mechanism of quenching of phototransduction. Binding competition between arrestin and transducin for phosphorhodopsin. *J Biol Chem.* 1997;272:18125–18131. [PubMed: 9218446]
14. Carman CV, Benovic JL. G-protein-coupled receptors: turn-ons and turn-offs. *Curr Opin Neurobiol.* 1998;8:335–344. [PubMed: 9687355]
15. Goodman OB, Jr, Krupnick JG, Santini F, et al. Beta-arrestin acts as a clathrin adaptor in endocytosis of the beta2-adrenergic receptor. *Nature.* 1996;383:447–450. [PubMed: 8837779]
16. Laporte SA, Oakley RH, Zhang J, et al. The 2-adrenergic receptor/arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. *Proc Natl Acad Sci USA.* 1999;96:3712–3717. [PubMed: 10097102]
17. Luttrell LM, Ferguson SS, Daaka Y, et al. Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science.* 1999;283:655–661. [PubMed: 9924018]
18. McDonald PH, Chow CW, Miller WE, et al. Beta-arrestin 2: a receptor-regulated MAPK scaffold for the activation of JNK3. *Science.* 2000;290:1574–1577. [PubMed: 11090355]

19. Luttrell LM, Roudabush FL, Choy EW, et al. Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc Natl Acad Sci USA*. 2001;98:2449–2454. [PubMed: 11226259]
20. Xiao K, McClatchy DB, Shukla AK, et al. Functional specialization of beta-arrestin interactions revealed by proteomic analysis. *Proc Natl Acad Sci USA*. 2007;104: 12011–12016. [PubMed: 17620599]
21. Hanson SM, Cleghorn WM, Francis DJ, et al. Arrestin mobilizes signaling proteins to the cytoskeleton and redirects their activity. *J Mol Biol*. 2007;368:375–387. [PubMed: 17359998]
22. Kook S, Zhan X, Cleghorn WM, Benovic JL, Gurevich VV, Gurevich EV. Caspase-cleaved arrestin-2 and BID cooperatively facilitate cytochrome C release and cell death. *Cell Death Differ*. 2014;21:172–184. [PubMed: 24141717]
23. Cleghorn WM, Branch KM, Kook S, et al. Arrestins regulate cell spreading and motility via focal adhesion dynamics. *Mol Biol Cell*. 2015;26(4):622–635. [PubMed: 25540425]
24. Bhattacharya M, Anborgh PH, Babwah AV, et al. Beta-arrestins regulate a Ral-GDS Ral effector pathway that mediates cytoskeletal reorganization. *Nature Cell Biol*. 2002;4:547–555. [PubMed: 12105416]
25. Hunton DL, Barnes WG, Kim J, et al. Beta-arrestin 2-dependent angiotensin II type 1A receptor-mediated pathway of chemotaxis. *Mol Pharmacol*. 2005;67:1229–1236. [PubMed: 15635042]
26. Ma X, Espana-Serrano L, W-j K, Thayerle Purayil H, Nie Z, Daaka Y. β Arrestin1 regulates the guanine nucleotide exchange factor RasGRF2 expression and the small GTPase Rac-mediated formation of membrane protrusion and cell motility. *J Biol Chem*. 2014;289:13638–13650. [PubMed: 24692549]
27. Cleghorn WM, Bulus N, Kook S, Gurevich VV, Zent R, Gurevich EV. Non-visual arrestins regulate the focal adhesion formation via small GTPases RhoA and Rac1 independently of GPCRs. *Cell Signal*. 2018;42:259–269. [PubMed: 29133163]
28. Zhu L, Rossi M, Cui Y, et al. Hepatic β -arrestin 2 is essential for maintaining euglycemia. *J Clin Invest*. 2017;127:2941–2945. [PubMed: 28650340]
29. Gurevich EV, Gurevich VV. Arrestins are ubiquitous regulators of cellular signaling pathways. *Genome Biol*. 2006;7:236. [PubMed: 17020596]
30. Peterson YK, Luttrell LM. The diverse roles of arrestin scaffolds in G protein-coupled receptor signaling. *Pharmacol Rev*. 2017;69:256–297. [PubMed: 28626043]
31. Tóth AD, Prokop S, Gyombolai P, et al. Heterologous phosphorylation-induced formation of a stability lock permits regulation of inactive receptors by β -arrestins. *J Biol Chem*. 2018;293:876–892. [PubMed: 29146594]
32. Luo J, Busillo JM, Stumm R, Benovic JL. 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. [PubMed: 28331048]
33. Granzin J, Wilden U, Choe HW, Labahn J, Krafft B, Buldt G. X-ray crystal structure of arrestin from bovine rod outer segments. *Nature*. 1998;391:918–921. [PubMed: 9495348]
34. Hirsch JA, Schubert C, Gurevich VV, Sigler PB. The 2.8 Å crystal structure of visual arrestin: a model for arrestin's regulation. *Cell*. 1999;97:257–269. [PubMed: 10219246]
35. Han M, Gurevich VV, Vishnivetskiy SA, Sigler PB, Schubert C. Crystal structure of beta-arrestin at 1.9 Å: possible mechanism of receptor binding and membrane translocation. *Structure*. 2001;9:869–880. [PubMed: 11566136]
36. Milano SK, Pace HC, Kim YM, Brenner C, Benovic JL. Scaffolding functions of arrestin-2 revealed by crystal structure and mutagenesis. *Biochemistry*. 2002;41: 3321–3328. [PubMed: 11876640]
37. Sutton RB, Vishnivetskiy SA, Robert J, et al. Crystal structure of cone arrestin at 2.3 Å: evolution of receptor specificity. *J Mol Biol*. 2005;354:1069–1080. [PubMed: 16289201]
38. Zhan X, Gimenez LE, Gurevich VV, Spiller BW. Crystal structure of arrestin-3 reveals the basis of the difference in receptor binding between two non-visual arrestins. *J Mol Biol*. 2011;406:467–478. [PubMed: 21215759]
39. Gurevich VV, Gurevich EV. Extensive shape shifting underlies functional versatility of arrestins. *Curr Opin Cell Biol*. 2014;27:1–9. [PubMed: 24680424]

40. Gurevich VV, Gurevich EV. The structural basis of arrestin-mediated regulation of G protein-coupled receptors. *Pharm Ther.* 2006;110:465–502.
41. Gurevich VV, Gurevich EV. The new face of active receptor bound arrestin attracts new partners. *Structure.* 2003;11:1037–1042. [PubMed: 12962621]
42. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J.* 1999;18:1723–1729. [PubMed: 10202136]
43. Indrischek H, Prohaska SJ, Gurevich VV, Gurevich EV, Stadler PF. Uncovering missing pieces: duplication and deletion history of arrestins in deuterostomes. *BMC Evol Biol.* 2017;17:163. [PubMed: 28683816]
44. Vishnivetskiy SA, Hosey MM, Benovic JL, Gurevich VV. Mapping the arrestin-receptor interface: structural elements responsible for receptor specificity of arrestin proteins. *J Biol Chem.* 2004;279:1262–1268. [PubMed: 14530255]
45. Vishnivetskiy SA, Gimenez LE, Francis DJ, et al. Few residues within an extensive binding interface drive receptor interaction and determine the specificity of arrestin proteins. *J Biol Chem.* 2011;286:24288–24299. [PubMed: 21471193]
46. Ostermaier MK, Peterhans C, Jaussi R, Deupi X, Standfuss J. Functional map of arrestin-1 at single amino acid resolution. *Proc Natl Acad Sci USA.* 2014;111: 1825–1830. [PubMed: 24449856]
47. Peterhans C, Lally CC, Ostermaier MK, Sommer ME, Standfuss J. Functional map of arrestin binding to phosphorylated opsin, with and without agonist. *Sci Rep.* 2016;6:28686. [PubMed: 27350090]
48. Kang Y, Zhou XE, Gao X, et al. Crystal structure of rhodopsin bound to arrestin determined by femtosecond X-ray laser. *Nature.* 2015;523:561–567. [PubMed: 26200343]
49. Zhou XE, He Y, de Waal PW, et al. Structural identification of phosphorylation codes for arrestin recruitment by G protein-coupled receptors. *Cell.* 2017;170:457–469. [PubMed: 28753425]
50. Gimenez LE, Babilon S, Wanka L, Beck-Sickinger AG, Gurevich VV. Mutations in arrestin-3 differentially affect binding to neuropeptide Y receptor subtypes. *Cell Signal.* 2014;26:1523–1531. [PubMed: 24686081]
51. Gimenez LE, Vishnivetskiy SA, Baameur F, Gurevich VV. Manipulation of very few receptor discriminator residues greatly enhances receptor specificity of non-visual arrestins. *J Biol Chem.* 2012;287:29495–29505. [PubMed: 22787152]
52. Prokop S, Perry NA, Vishnivetskiy SA, et al. Differential manipulation of arrestin-3 binding to basal and agonist-activated G protein-coupled receptors. *Cell Signal.* 2017;36:98–107. [PubMed: 28461104]
53. Schoneberg T, Schulz A, Biebermann H, Hermsdorf T, Rompler H, Sangkuhl K. Mutant G-protein-coupled receptors as a cause of human diseases. *Pharmacol Ther.* 2004;104:173–206. [PubMed: 15556674]
54. Stoy H, Gurevich VV. How genetic errors in GPCRs affect their function: possible therapeutic strategies. *Genes Dis.* 2015;2:108–132. [PubMed: 26229975]
55. Schleicher A, Kuhn H, Hofmann KP. Kinetics, binding constant, and activation energy of the 48-kDa protein-rhodopsin complex by extra-metarhodopsin II. *Biochemistry.* 1989;28:1770–1775. [PubMed: 2719933]
56. Shukla AK, Manglik A, Kruse AC, et al. Structure of active β -arrestin-1 bound to a G-protein-coupled receptor phosphopeptide. *Nature.* 2013;497:137–141. [PubMed: 23604254]
57. Vishnivetskiy SA, Hirsch JA, Velez M-G, Gurevich YV, Gurevich VV. Transition of arrestin in the active receptor-binding state requires an extended interdomain hinge. *J Biol Chem.* 2002;277:43961–43968. [PubMed: 12215448]
58. Modzelewska A, Filipek S, Palczewski K, Park PS. Arrestin interaction with rhodopsin: conceptual models. *Cell Biochem Biophys.* 2006;46:1–15. [PubMed: 16943619]
59. Chen Q, Iverson TM, Gurevich VV. Structural basis of arrestin-dependent signal transduction. *Trends Biochem Sci.* 2018;43:412–423. [PubMed: 29636212]
60. Chen Q, Perry NA, Vishnivetskiy SA, et al. Structural basis of arrestin-3 activation and signaling. *Nat Commun.* 2017;8:1427. [PubMed: 29127291]

61. Vishnivetskiy SA, Paz CL, Schubert C, Hirsch JA, Sigler PB, Gurevich VV. How does arrestin respond to the phosphorylated state of rhodopsin? *J Biol Chem.* 1999;274: 11451–11454. [PubMed: 10206946]
62. Vishnivetskiy SA, Schubert C, Climaco GC, Gurevich YV, Velez M-G, Gurevich VV. An additional phosphate-binding element in arrestin molecule: implications for the mechanism of arrestin activation. *J Biol Chem.* 2000;275:41049–41057. [PubMed: 11024026]
63. Gurevich VV, Benovic JL. Visual arrestin binding to rhodopsin: diverse functional roles of positively charged residues within the phosphorylation-recognition region of arrestin. *J Biol Chem.* 1995;270:6010–6016. [PubMed: 7890732]
64. Gurevich VV, Benovic JL. Mechanism of phosphorylation-recognition by visual arrestin and the transition of arrestin into a high affinity binding state. *Mol Pharmacol.* 1997;51: 161–169. [PubMed: 9016359]
65. Gurevich VV. The selectivity of visual arrestin for light-activated phosphorhodopsin is controlled by multiple nonredundant mechanisms. *J Biol Chem.* 1998;273: 15501–15506. [PubMed: 9624137]
66. Celver J, Vishnivetskiy SA, Chavkin C, Gurevich VV. Conservation of the phosphate-sensitive elements in the arrestin family of proteins. *J Biol Chem.* 2002;277:9043–9048. [PubMed: 11782458]
67. Kovoov A, Celver J, Abdryashitov RI, Chavkin C, Gurevich VV. Targeted construction of phosphorylation-independent β -arrestin mutants with constitutive activity in cells. *J Biol Chem.* 1999;274:6831–6834. [PubMed: 10066734]
68. Pan L, Gurevich EV, Gurevich VV. The nature of the arrestin×receptor complex determines the ultimate fate of the internalized receptor. *J Biol Chem.* 2003;278: 11623–11632. [PubMed: 12525498]
69. Gurevich VV, Pals-Rylaarsdam R, Benovic JL, Hosey MM, Onorato JJ. Agonist-receptor-arrestin, an alternative ternary complex with high agonist affinity. *J Biol Chem.* 1997;272:28849–28852. [PubMed: 9360951]
70. Gray-Keller MP, Detwiler PB, Benovic JL, Gurevich VV. Arrestin with a single amino acid substitution quenches light-activated rhodopsin in a phosphorylation-independent fashion. *Biochemistry.* 1997;36:7058–7063. [PubMed: 9188704]
71. Song X, Vishnivetskiy SA, Gross OP, et al. Enhanced arrestin facilitates recovery and protects rod photoreceptors deficient in rhodopsin phosphorylation. *Curr Biol.* 2009;19:700–705. [PubMed: 19361994]
72. Samaranyake S, Song X, Vishnivetskiy SA, Chen J, Gurevich EV, Gurevich VV. Enhanced mutant compensates for defects in rhodopsin phosphorylation in the presence of endogenous arrestin-1. *Front Mol Neurosci.* 2018;11:203. [PubMed: 29973866]
73. Grundmann M, Merten N, Malfacini D, et al. Lack of beta-arrestin signaling in the absence of active G proteins. *Nat Commun.* 2018;9:341. [PubMed: 29362459]
74. Alvarez-Curto E, Inoue A, Jenkins L, et al. Targeted elimination of G proteins and arrestins defines their specific contributions to both intensity and duration of G protein-coupled receptor signaling. *J Biol Chem.* 2016;291:27147–27159. [PubMed: 27852822]
75. Song X, Coffa S, Fu H, Gurevich VV. How does arrestin assemble MAPKs into a signaling complex? *J Biol Chem.* 2009;284:685–695. [PubMed: 19001375]
76. Coffa S, Breitman M, Spiller BW, Gurevich VV. A single mutation in arrestin-2 prevents ERK1/2 activation by reducing c-Raf1 binding. *Biochemistry.* 2011;50:6951–6958. [PubMed: 21732673]
77. Breitman M, Kook S, Gimenez LE, et al. Silent scaffolds: inhibition of c-Jun N-terminal kinase 3 activity in the cell by a dominant-negative arrestin-3 mutant. *J Biol Chem.* 2012;287:19653–19664. [PubMed: 22523077]
78. Miller WE, McDonald PH, Cai SF, Field ME, Davis RJ, Lefkowitz RJ. Identification of a motif in the carboxyl terminus of beta-arrestin2 responsible for activation of JNK3. *J Biol Chem.* 2001;276:27770–27777. [PubMed: 11356842]
79. Seo J, Tsakem EL, Breitman M, Gurevich VV. Identification of arrestin-3-specific residues necessary for JNK3 activation. *J Biol Chem.* 2011;286:27894–27901. [PubMed: 21715332]

80. Zhan X, Kaoud TS, Dalby KN, Gurevich VV. Non-visual arrestins function as simple scaffolds assembling MKK4–JNK3 α 2 signaling complex. *Biochemistry*. 2011;50: 10520–10529. [PubMed: 22047447]
81. Zhan X, Kaoud TS, Kook S, Dalby KN, Gurevich VV. JNK3 binding to arrestin-3 differentially affects the recruitment of upstream MAP kinase kinases. *J Biol Chem*. 2013;288:28535–28547. [PubMed: 23960075]
82. Zhan X, Perez A, Gimenez LE, Vishnivetskiy SA, Gurevich VV. Arrestin-3 binds the MAP kinase JNK3 α 2 via multiple sites on both domains. *Cell Signal*. 2014;26:766–776. [PubMed: 24412749]
83. Zhan X, Stoy H, Kaoud TS, et al. Peptide mini-scaffold facilitates JNK3 activation in cells. *Sci Rep*. 2016;6:21025. [PubMed: 26868142]
84. Lunin VV, Munger C, Wagner J, Ye Z, Cygler M, Sacher M. The structure of the MAPK scaffold, MP1, bound to its partner, p14. A complex with a critical role in endosomal map kinase signaling. *J Biol Chem*. 2004;279:23422–23430. [PubMed: 15016825]
85. Brown MD, Sacks DB. Protein scaffolds in MAP kinase signalling. *Cell Signal*. 2009;21:462–469. [PubMed: 19091303]
86. Orsini MJ, Benovic JL. Characterization of dominant negative arrestins that inhibit beta2-adrenergic receptor internalization by distinct mechanisms. *J Biol Chem*. 1998;273:34616–34622. [PubMed: 9852134]
87. Meng D, Lynch MJ, Huston E, et al. MEK1 binds directly to betaarrestin1, influencing both its phosphorylation by ERK and the timing of its isoprenaline-stimulated internalization. *J Biol Chem*. 2009;284:11425–11435. [PubMed: 19153083]
88. Baillie GS, Adams DR, Bhari N, et al. Mapping binding sites for the PDE4D5 cAMP-specific phosphodiesterase to the N- and C-domains of beta-arrestin using spot-immobilized peptide arrays. *Biochem J*. 2007;404:71–80. [PubMed: 17288540]
89. Gurevich VV, Dion SB, Onorato JJ, et al. Arrestin interaction with G protein-coupled receptors. Direct binding studies of wild type and mutant arrestins with rhodopsin, b2-adrenergic, and m2 muscarinic cholinergic receptors. *J Biol Chem*. 1995;270: 720–731. [PubMed: 7822302]
90. Hanson SM, Gurevich VV. The differential engagement of arrestin surface charges by the various functional forms of the receptor. *J Biol Chem*. 2006;281:3458–3462. [PubMed: 16339758]
91. Pulvermuller A, Schroder K, Fischer T, Hofmann KP. Interactions of metarhodopsin II. Arrestin peptides compete with arrestin and transducin. *J Biol Chem*. 2000;275: 37679–37685. [PubMed: 10969086]
92. Hanson SM, Francis DJ, Vishnivetskiy SA, et al. Differential interaction of spin-labeled arrestin with inactive and active phosphorhodopsin. *Proc Natl Acad Sci USA*. 2006;103: 4900–4905. [PubMed: 16547131]
93. Zhuang T, Chen Q, Cho M-K, et al. Involvement of distinct arrestin-1 elements in binding to different functional forms of rhodopsin. *Proc Natl Acad Sci USA*. 2013;110:942–947. [PubMed: 23277586]
94. Szczepek M, Beyriere F, Hofmann KP, et al. Crystal structure of a common GPCR-binding interface for G protein and arrestin. *Nat Commun*. 2014;5:4801. [PubMed: 25205354]
95. Wu N, Hanson SM, Francis DJ, et al. Arrestin binding to calmodulin: a direct interaction between two ubiquitous signaling proteins. *J Mol Biol*. 2006;364:955–963. [PubMed: 17054984]

Fig. 1.

The structure of arrestin. Two views are shown: (A) side view; (B) view down the concave sides of both domains (based on the crystal structure of bovine arrestin-2 solved with the resolution of $1.9 \text{ \AA}$; PDB ID: 1G4M³⁵). The molecule is colored by secondary structure, as follows: *light blue*, β -strand; *green*, β -turn; *red*, α -helix. The long axis of the arrestin molecule is smaller than $90 \text{ \AA}$, whereas its short axis is $\sim 30\text{--}35 \text{ \AA}$. N- and C-domains, their concave sides, as well as the central crest on the receptor-binding side of the molecule are indicated. The N-terminus (N-t) and C-terminus (C-t) of the part resolved in crystal are also indicated. Note that several N-terminal residues, the C-terminus extending beyond β -strand XX, as well as the linker between the C-domain and β -strand XX are not resolved in any arrestin structures.

Fig. 2.

Residues responsible for the receptor specificity of arrestins. The residues in arrestin-2 (PDB ID: 1G4M) in positions identified as important for the receptor preference of different arrestin proteins are shown as CPK models colored as follows: *light blue*, those identified by reduced mobility of the spin label in receptor-bound arrestins using EPR (Val70, Leu71, Leu73, Val167, Leu191, Ser234, Thr246, Tyr249); *green*, those identified by site-directed mutagenesis and receptor binding in vitro and in live cells (Leu48, Glu50, Arg51, Asp240, Cys251, Pro252, Asp259, Thr261); *dark blue*, those identified by both of these methods (Leu68, Tyr238); *magenta*, those identified by direct contact with the receptor in the crystal structure of the arrestin-1 complex with rhodopsin (Lys138, Asn245, Ala247, Gln248). Note that all receptor-binding residues identified by different methods are localized on the concave sides of the two arrestin domains. Many of these are localized in the central crest of the receptor-binding side of arrestins.

Fig. 3.

Activation-induced conformational changes in arrestins. The most notable conformational changes upon arrestin activation are shown here using arrestin-3 as an example. These include the release of the arrestin C-tail (*magenta*; *magenta arrow* shows the direction of the movement) anchored to the body of the molecule via the three-element interaction in the basal state (PDB ID: 3P2D³⁸), the movement to the receptor of the finger loop and helix formation in it (*red*; *red arrow* shows the direction of the movement), as well as the rotation of the two domains relative to each other (N-domain, *gray*; C-domain, *teal*). Rearrangements of several loops are also shown: 139-loop (middle loop in nonvisual arrestins; dark blue; dark blue arrow indicates the direction of movement); interdomain hinge (*yellow with yellow arrowhead* in a circle), one of the connectors between the N-domain and the α -helix (*green with green arrowhead* in a circle), register-shifted β -strand XI (*dark brown*), and the lariat loop (*orange with orange arrow*; note that in the active structure (PDB ID: 5TV1⁶⁰) it is not resolved). In Movie 1 in the online version at <https://doi.org/10.1016/bs.pmbts.2018.07.007> arrestin-3 transitions from basal to active conformation are shown. The elements are color coded, as indicated earlier, except that the C-tail is not shown and register-shifted β -strand XI is shown in *magenta*.

Fig. 4.

Mini-scaffold facilitating JNK3 activation. The structure of the arrestin-3-derived T1A peptide, encompassing residues 1–25 (only residues 5–25 are resolved in the crystal structure, PDB ID: 3P2D), are shown. This peptide facilitated JNK3 activation in vitro and in cells, apparently scaffolding ASK1-MKK4/7-JNK3 cascade.⁸³ The residues that differ from closely related arrestin-2, which does not promote JNK3 activation (Ser14, Pro15, Cys17), are shown as ball-and-stick models, with the atoms colored, as follows: *gray*, carbon; *red*, oxygen; *blue*, nitrogen; *yellow*, sulfur. The conformation shown is of the T1A peptide in the context of fully folded basal arrestin-3 (PDB ID: 3P2D³⁸), which is not necessarily the conformation of this peptide when it acts as a scaffold for the ASK1-MKK4/7-JNK3 cascade.