HHS Public Access

Author manuscript

Prog Mol Biol Transl Sci. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

Prog Mol Biol Transl Sci. 2019 ; 161: 29–45. doi:10.1016/bs.pmbts.2018.09.004.

Arrestin mutations: Some cause diseases, others promise cure

Vsevolod V. Gurevich[1] and **Eugenia V. Gurevich**

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

Abstract

Arrestins play a key role in homologous desensitization of G protein-coupled receptors (GPCRs) and regulate several other vital signaling pathways in cells. Considering the critical roles of these proteins in cellular signaling, surprisingly few disease-causing mutations in human arrestins were described. Most of these are loss-of-function mutations of visual arrestin-1 that cause excessive rhodopsin signaling and hence night blindness. Only one dominant arrestin-1 mutation was discovered so far. It reduces the thermal stability of the protein, which likely results in photoreceptor death via unfolded protein response. In case of the two nonvisual arrestins, only polymorphisms were described, some of which appear to be associated with neurological disorders and altered response to certain treatments. Structure-function studies revealed several ways of enhancing arrestins' ability to quench GPCR signaling. These enhanced arrestins have potential as tools for gene therapy of disorders associated with excessive signaling of mutant GPCRs.

1. Arrestins in mammals: Few subtypes, many functions

The first arrestin described was the visual subtype (systematic name arrestin-1[a]). In fact, it was discovered not for its biological role as we know it today, but as an antigen causing uveitis,[1] hence it was called S-antigen. *Arrestin-1* gene is called SAG (abbreviation for S-antigen) to this day. A year later Kuhn described a "48-kDa" protein in the retina that, along with the visual G protein transducin and rhodopsin kinase, binds rhodopsin in a light-dependent manner.[2] A few years later Kuhn found that rhodopsin phosphorylation (also discovered by his group many years earlier[3]) greatly facilitates the binding of the 48-kDa protein to rhodopsin.[4] The fact that S-antigen and 48-kDa protein are one and the same was established only in mid-1980s.[5,6] Subsequent studies by Dr. Kuhn's group showed that the binding of the 48-kDa protein to phosphorylated rhodopsin quenches light-dependent activation of photoreceptor phosphodiesterase,[7] which was the first evidence for the role of this protein in "arresting" rhodopsin signaling, i.e., in receptor desensitization. Subsequent discoveries of the kinase that selectively phosphorylates agonist-activated β2-adrenergic receptor (β2AR)[8] and the first nonvisual arrestin[9] demonstrated that two-step desensitization, phosphorylation of activated GPCR by a specific receptor kinase followed by arrestin binding to the active phosphorylated receptor, is a common feature of the signaling systems driven by GPCRs (reviewed in ref. 10). Mammals express between ~500

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

[a]We 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).

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

(dolphins) and ~3400 (elephants) different GPCRs (http://sevens.cbrc.jp/), but only 4 arrestin subtypes.[11,12] Two of these four are specialized visual: arrestin-1, expressed at very high levels in both rod and cone photoreceptors,[13–16] and cone-specific arrestin-4, constituting ~2% of total arrestin complement in cones.[13] The other two nonvisual subtypes, arrestin-2 and −3, are ubiquitously expressed and apparently interact with hundreds of different GPCRs.[9,17–19] In most cell types arrestin-2 greatly outnumbers arrestin-3,[20–21] which explains why arrestin-2[9] was cloned years before arrestin-3.[22–24]

Arrestin-1 was shown to suppress G protein activation by phosphorylated light-activated rhodopsin via direct competition.[25,26] Nonvisual arrestins were found to perform exactly the same function, the only difference being that they prefer other GPCRs over rhodopsin.[22,27] Soon after their discovery, both nonvisual arrestins were shown to bind clathrin[28] and clathrin adaptor AP-2,[29] two key players in receptor internalization via coated pits. Many other nonreceptor binding partners of the two nonvisual arrestins were subsequently discovered. Interestingly, some apparently bind both arrestin-2 and −3, whereas others selectively interact with only one of the nonvisual subtypes.[30] Arrestins were implicated in the activation ofc-Src[31] and all three major MAP kinase classes, JNK,[32] ERK,[33] and p38,[34] shown to bind cAMP phosphodiesterase,[35] calmodulin, [36] microtubules,[37,38] ubiquitin ligases Mdm2,[39] AIP4,[40] parkin,[41] and many other signaling proteins (reviewed in refs. 11,42,43). Thus, it appears that arrestins are at the crossroads of numerous vital signaling pathways in the cell.

2. Naturally occurring mutations in visual arrestins

Critical role of arrestin proteins in the visual system was confirmed by abnormally prolonged photoresponses in mice lacking arrestin-1,[44] rhodopsin kinase[45] or expressing rhodopsin without phosphorylation sites necessary for its binding[46] or with insufficient number of these sites.[47] Indeed, in humans frameshift mutation in *SAG* was found to underlie Oguchi disease, a form of stationary night blindness.[48,49] This mutation is a deletion of an adenine in codon 309, which causes a frameshift and premature termination 10 residues later.[48,50] Thus, the resulting protein lacks about half of the C-domain[51] and is unlikely to fold or become functional. Interestingly, the same mutation in the *SAG* gene caused autosomal recessive retinitis pigmentosa (a form of retinal degeneration) in some cases.[50] Although it is not known why the phenotype is so different, certain mechanisms are conceivable. All eukaryotic cells have special mechanisms of “garbage disposal,” at mRNA and protein levels. One of these is nonsense-mediated mRNA decay, i.e., the elimination of mutant or incorrectly spliced mRNA containing premature stop codons.[52–54] The other is the degradation of misfolded and unfolded proteins via ubiquitin-proteasome system (reviewed in ref. 55). Insufficient activity of either can exacerbate the problem caused by this frame-shift in the *SAG* gene. In particular, incomplete elimination of faulty mRNA would make the cell produce too much unfolded mutant arrestin-1, overwhelming ubiquitin-proteasome system and causing cell death via unfolded protein response (see below). This is particularly likely considering that arrestin-1 is the second most abundant protein in rods after rhodopsin,[14–16] so that even if a small fraction of defective mRNA escapes the nucleus and is translated in the cytoplasm, the output of unfolded protein would be large.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Loss-of-function mutations are recessive, i.e., the production of normal arrestin-1 encoded by the second undamaged allele is usually sufficient for health of rod photoreceptors. This is consistent with the results obtained in mice, where hemizygous knockout animals and even the animals expressing arrestin-1 at ~5% of wild-type (WT) level had perfectly healthy photoreceptors and normal rate of photoresponse shutoff after moderately bright flashes.[16,56]

Recently a dominant mutation in, *SAG*, C147F, was discovered[57] (Fig. 1A). Its dominant nature suggested that the mutant itself, rather than the absence of functional arrestin-1, causes the damage that WT arrestin-1 protein generated by the second allele cannot prevent. Although cysteine in this position is conserved in all arrestin subtypes[11,12] and in highly homologous bovine and mouse arrestin-1 and it was shown to be important for arrestin function,[58] in and of itself this did not explain dominant nature of the C147F mutation. Careful inspection of the structure of arrestin-1 in its basal conformation,[51] as well as in complex with rhodopsin[59,60] suggested that the replacements of a cysteine with a relatively small side chain by a bulky phenylalanine in this position can affect protein folding (Fig. 1B).[57] This idea was tested experimentally, and the results turned out to be more complex. It was shown that C147F arrestin-1 folds, but demonstrates much lower thermal stability than the WT protein, denaturing within hours even at physiological temperature of 37°C.[61] Similar loss of thermal stability was demonstrated in case of C147I and C147L, but not with C147A or C147V mutants with smaller side chains, clearly indicating that the bulk of the side chain underlies the problem.[61] The expression of this mutant in photoreceptor-derived 661W cells,[62] in contrast to WT human arrestin-1, was shown to induce unfolded protein response.[61] Thus, considering extremely high expression of arrestin-1 in rods,[14–16] it appears that denaturing C147F mutant overwhelms proteasome system and induces photoreceptor death via unfolded protein response (Fig. 2). It is quite likely that a mutation in the stop codon of *SAG*, which added an extra of 25 amino acids, causes late-onset hereditary retinal degeneration in dogs[64] via a similar mechanism.

Interestingly, no disease-causing mutations or polymorphisms were so far reported in cone-specific *arrestin-4* (*ARR4*), even though it appears to be necessary for proper function of cone photoreceptors.[13]

3. Nonvisual arrestins: Unexpectedly few associations with unclear functional significance

Numerous residues are highly conserved in arrestin evolution,[11,12] which suggests their functional importance. Yet not a single disease-related change in the amino acid sequence has so far been described in human nonvisual arrestins. Several single nucleotide polymorphisms in the arrestin-2 and −3 genes (*ARRB1* and *ARRB2),* which include synonymous mutations in the coding sequence and single nucleotide polymorphisms (SNPs) in the noncoding elements of their mRNAs and in the introns, have been described. The associations of these polymorphisms with various conditions have been extensively tested.

Polymorphism in the promoter region of the *ARRB1* gene was found to be associated with the treatment outcome with antidepressant mirtazapine in Korean patients with major depressive disorder.[65] One haplotype, which includes SNPs in the promoter region and the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

first intron, was found to be associated with the remission status following several weeks of mirtazapine treatment. Two SNPs in *ARRB2*, one in an intron and one in 3′-untranslated region (3′-UTR), were reported to be associated with the effects of antidepressant monotherapy in a group of mostly Caucasian patients with major depression.[66] Interestingly, the same SNP in the 3′-UTR of *ARRB2* mRNA, rs4790694, was found to be associated with two measures of nicotine dependence in European Americans.[67] There was also one major haplotype in both *ARRB2* and *ARRB1* that showed positive association with the indices of nicotine dependence. Interestingly, no such associations were seen in African Americans.[67] A study of association between polymorphism in the *ARRB2* gene and alcohol dependence found no such association in Caucasians.[68] Four SNPs spanning the entire gene were examined (rs4790694 was not included). These human data are somewhat at odds with the finding in animals that demonstrated the existence of an *Arrb2* haplotype consisting of six SNPs in different parts of the gene and one insertion (in the promoter region) that was specifically associated with ethanol preference in rats.[69] The variant haplotype in the ethanol-preferring rat line confers higher arrestin-3 expression both at mRNA and protein levels in several brain regions, but no change in the arrestin-3 protein sequence.

An extensive role of arrestins in signaling as well as in regulation of the GPCR responsiveness drove the investigation of genetic association of arrestin polymorphisms and the effects of addictive drugs. However, investigation of seven *ARRB2* SNPs, including rs4790694, failed to reveal significant association of any individual SNP or haplotype with cocaine or opioid dependence in European Americans.[70] However, polymorphism in *ARRB2* was found to impact the outcome of methadone substitution therapy in Caucasian patients addicted to opioids. The study examined four SNPs in the *ARRB2* gene, from the promoter to 3′-UTR, three of which, in intron 1, exon 11 (synonymous Ser280Ser), and 3′-UTR, were found to be significantly associated with response to methadone therapy, although no association was seen with opioid addiction.[71] These three SNPs formed a haplotype block, and patients homozygous for variant alleles in the block carried an almost threefold higher risk of being nonresponders to the methadone therapy.

A study of four *ARRB2* SNPs failed to detect an association between any of them and schizophrenia in Japanese patients.[72] However, three out of five SNPs (synonymous rs1045280 in exon 11 and rs2036657 and rs4790694 in 3′-UTR) were significantly associated with methamphetamine use disorders such as methamphetamine-induced psychosis. Additionally, a significant association was found between rs1045280 Ser280Ser SNP and tardive dyskinesia, a motor complication induced by long-term treatment with typical antipsychotics.[73]

Overall, these association studies revealed a lot less than one would expect. Considering vital role of nonvisual arrestins in many cellular functions, one would expect to find numerous disease-associated mutations and polymorphisms. However, very few have been described so far. The major problem with interpretation of the existing findings is, of course, the lack of obvious functional significance of these genetic variants. None of the SNPs change the proteins sequence of arrestins. All known SNPs in the exons are synonymous. Many SNPs found associated with human diseases are located in introns or in 3′-UTR, with

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

unknown functional impact. Out of all *ARRB2* SNPs examined so far, only one SNP, rs34230287 (–159C/T), located in the gene promoter, has been shown to affect the promoter activity and *ARRB2* expression: the C variant confers a significantly higher promoter activity and is associated with higher level of *ARRB2* mRNA in Caucasians.[74] However, this SNP was either not examined or, when it was, showed no association with the outcome measures.[71]

Although in most cases this was not tested, the most likely effect of SNPs in coding and noncoding regions is a change in expression. The most extreme change in protein expression is produced by the knockout of its gene. If we look at mouse in vivo studies, the phenotypes detected in single nonvisual arrestin knockouts are fairly mild: mice lacking either *Arrb1* or *Arrb2* are overall normal, albeit demonstrate altered functional responses such as increased sensitivity to adrenergic stimulation of the heart[75] or enhanced locomotor responsiveness to amphetamine[76] in *Arrb1* knockout mice or enhanced morphine analgesia[77] and reduced locomotor response to amphetamine[76,78] and morphine[79] in *Arrb2* knockout mice. Interestingly, simultaneous knockout of both nonvisual subtypes is embryonic lethal because of lung and heart development problems.[80,81] The elimination of the only nonvisual arrestin, *kurtz,* in *Drosophila* is also embryonic lethal,[82] even though in flies kurtz inhibits proproliferative MAP kinase ERK,[83] in contrast to mammalian cells, where nonvisual arrestins facilitate ERK activation via c-Src[31] and by scaffolding of c-Raf1-MEK1-ERK1/2 cascade.[33,84]

One reason for these observations in knockout mice and humans with SNPs is that the two nonvisual subtypes in mammals can partially compensate for one another. Mild phenotypes *of Arrb1* and *Arrb2* knockout mice appear to support this argument. However, in certain aspects these two subtypes are quite different and engage distinct sets of signaling proteins.[30] A recent study suggests that arrestin-2 and arrestin-3 play distinct roles in amphetamine-induced hyperlocomotion and that their effects are dose dependent.[76] While the mechanistic basis for the distinct roles of the two nonvisual arrestins was not determined in that study, there are several well-known functional differences: arrestin-3 has higher affinity than arrestin-2 for clathrin[28] and several GPCRs,[18,80] arrestin-3 is less selective than arrestin-2 for the active phosphorylated form of cognate GPCRs,[85] and arrestin-3, but not arrestin-2, facilitates JNK3 activation in cells.[32,86,87] The latter difference was preserved even in short arrestin-derived N-terminal peptides.[88] One of these differences, or some functional difference that has not been elucidated yet, might underlie distinct roles of arrestin-2 and arrestin-3 in amphetamine-induced hyperlocomotion.[76] However, mild phenotypes of single subtype knockout mice suggest that either nonvisual arrestin can perform most of the biologically important functions of the other subtype.

4. Enhanced arrestins: Compensation of excessive GPCR activity

Numerous mutations in various GPCRs were reported to cause different kinds of pathological conditions in humans (reviewed in refs. 89,90). Some of these mutations are loss of function, so conceptually the strategy for gene therapy is clear: the delivery of a normal coding sequence of a functional GPCR to the affected cells should solve the problem. However, other identified disease-causing mutations are gain of function, where

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

the mutant gene encodes an overactive receptor. Unlike recessive loss-of-function, gain-of-function mutations are dominant, as perfectly normal second allele cannot suppress excessive signaling by the mutant. One possible therapeutic strategy is the expression of an arrestin with enhanced ability to dampen the signaling, so that, on balance, the signal might become near normal. This compensational approach was so far tested only in rod photoreceptors, where the important GPCR is rhodopsin, which is quenched exclusively by arrestin-l.[86] Extensive structure—function studies of arrestin-1 (reviewed in refs. 85,91) yielded several enhanced versions that bind both phosphorylated and unphosphorylated light-activated rhodopsin tighter than parental WT protein (Fig. 3). As usual, translation of the in vitro findings to the in vivo situation yielded both good and bad news. On the positive side, transgenic expression of enhanced arrestin-1 in mouse rods defective in rhodopsin phosphorylation improves the morphology of photoreceptors, their functional performance, and results in signal shutoff that is much faster than with WT arrestin-1.[86] Importantly, facilitation of the shutoff is also documented in mice with defects of rhodopsin phosphorylation expressing normal complement or WT arrestin-1, similar to human patients with rhodopsin mutations.[92] However, neither parameter in "compensated" rods came even close to that of normal rods where rhodopsin was phosphorylated and quenched by WT arrestin-1.[86] As far as the visual system is concerned, with its unrivaled single photon sensitivity and subsecond shutoff of the response, these results suggested that while in principle the strategy is working, more powerful enhanced mutants are necessary for better compensation.[93] However, something that works only partially in rods might be sufficient to fully compensate for excessive signaling in any other GPCR-driven system, where the sensitivity is much lower and the shutoff takes minutes, rather than 200–300 ms.

The mechanism of GPCR binding is well conserved in the arrestin family,[91] so both nonvisual arrestins can be enhanced by the mutations homologous to those that preactivate arrestin-1.[94–96] Yet in case of nonvisual GPCRs there is another catch: both nonvisual arrestins are quite promiscuous and bind pretty much every GPCR tested.[17–19,97] As virtually every cell in the body expresses numerous GPCR subtypes, only one of which is an overactive mutant in patients, introduction of a promiscuous enhanced nonvisual arrestin would likely suppress the signaling not only of the "bad guy" but of all the other perfectly normal receptors coexpressed in the same cell. This is likely to cause unwanted side effects. Thus, to make the same compensational strategy usable, mutant nonvisual arrestins with narrow receptor specificity are needed. While the receptor–arrestin interface is extensive, involving many residues on the concave sides of both arrestin domains,[59,98–100] only select few appear to play a role in receptor preference[100] (Fig. 4). The results of the targeted manipulation of these putative receptor-discriminator residues suggest that the construction of nonvisual arrestins with narrow receptor specificity is feasible: certain double mutants demonstrate 50–60-fold preference for some GPCRs over others,[19] and a number of mutations differentially affect arrestin interactions with distinct functional forms of several GPCRs.[101–103] Generally speaking, preactivating mutations often reduce receptor specificity of arrestins.[94,96] However, this is so in case of active phosphorylated receptors, whereas the same mutations enhance binding only to cognate unphosphorylated GPCRs.[94,96] Moreover, enhanced mutants actually compete with GRKs, suppressing the phosphorylation of GPCRs they target.[95] Thus, while receptor specificity of the mutants combining preactivating

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

mutations with those that make them specific for particular GPCRs needs to be tested experimentally, this compensational approach appears to be a feasible method to rein in excessive signaling by many disease-causing gain-of-function receptor mutants.

5. Conclusions

Several mutations in *SAG* were reported to cause visual disorders in humans and dogs. Loss-of-function mutations are usually recessive, so that only compound heterozygotes are affected, whereas mutations causing misfolding and/or protein instability are dominant and cause severe disorders, such as retinal degeneration. Unexpectedly, no mutations in either nonvisual arrestin were associated with any disease, while polymorphisms in noncoding regions and synonymous base substitutions in exons were found to be associated with the response to treatment of several neurological disorders. The phenotypes of single nonvisual arrestin knockout in mice are mild, whereas simultaneous knockout of both, as well as the knockout of the only nonvisual arrestin in *Drosophila,* causes embryonic lethality. Thus, the most logical explanation of mild effects of single knockouts in mammals is that arrestin-2 and arrestin-3 can compensate for the missing subtype, taking over each other's duties. Preactivated arrestin mutants with enhanced ability to quench GPCR signaling appear to be a viable tool for the gene therapy of disorders caused by gain-of-function mutations in GPCRs. The use of this strategy to suppress excessive signaling by nonvisual GPCRs requires engineering of receptor-specific nonvisual arrestin mutants.

Acknowledgments

This work was supported in part by NIH RO1 grants EY011500, GM077561, and GM109955 (the latter two RO1s were replaced by R35 GM122491) (V.V.G.), and NS065868 and DA030103 (E.V.G.).

Abbreviations

ERK	extracellular signal-regulated kinase
GPCR	G protein-coupled receptor
GRP78/BIP	glucose-regulated protein of 78 kDa/immunoglobulin binding protein
JNK	c-Jun N-terminal kinase
MAP kinase	mitogen-activated protein kinase
WT	wild type
β2AR	β2-adrenergic receptor

References

1. Wacker WB, Donoso LA, Kalsow CM, Yankeelov JA, Jr, Organisciak DT. Experimental allergic uveitis. Isolation, characterization, and localization of a soluble uveitopathogenic antigen from bovine retina. J Immunol 1977;119:1949–1958. [PubMed: 334977]
2. Kuhn H Light-regulated binding of rhodopsin kinase and other proteins to cattle photoreceptor membranes. Biochemistry 1978;17:4389–4395. [PubMed: 718845]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

3. Kuhn H, Dreyer WJ. Light dependent phosphorylation of rhodopsin by ATP. FEBS Lett 1972;20:1–6. [PubMed: 11946367]
4. 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]
5. Pfister C, Chabre M, Plouet J, et al. Retinal S antigen identified as the 48K protein regulating light-dependent phosphodiesterase in rods. Science 1985;228:891–893. [PubMed: 2988124]
6. Pfister C, Dorey C, Vadot E, et al. Identification of the so-called 48 K protein that interacts with illuminated rhodopsin in retinal rods, and the retinal S antigen, inductor of experimental autoimmune uveoretinitis. C R Acad Sci III 1984;299:261–265. [PubMed: 6439387]
7. 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]
8. Benovic JL, DeBlasi A, Stone WC, Caron MG, Lefkowitz RJ. Beta-adrenergic receptor kinase: primary structure delineates a multigene family. Science 1989; 246:235–240. [PubMed: 2552582]
9. 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]
10. Carman CV, Benovic JL. G-protein-coupled receptors: turn-ons and turn-offs. Curr Opin Neurobiol 1998;8:335–344. [PubMed: 9687355]
11. Gurevich EV, Gurevich VV. Arrestins are ubiquitous regulators of cellular signaling pathways. Genome Biol 2006;7:236. [PubMed: 17020596]
12. 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]
13. Nikonov SS, Brown BM, Davis JA, et al. Mouse cones require an arrestin for normal inactivation of phototransduction. Neuron 2008;59:462–474. [PubMed: 18701071]
14. Strissel KJ, Sokolov M, Trieu LH, Arshavsky VY. Arrestin translocation is induced at a critical threshold of visual signaling and is superstoichiometric to bleached rhodopsin. J Neurosci 2006;26:1146–1153. [PubMed: 16436601]
15. Hanson SM, Gurevich EV, Vishnivetskiy SA, Ahmed MR, Song X, Gurevich VV. Each rhodopsin molecule binds its own arrestin. Proc Natl Acad Sci USA 2007;104: 3125–3128. [PubMed: 17360618]
16. Song X, Vishnivetskiy SA, Seo J, Chen J, Gurevich EV, Gurevich VV. Arrestin-1 expression in rods: balancing functional performance and photoreceptor health. Neuroscience 2011;174:37–49. [PubMed: 21075174]
17. 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]
18. Barak LS, Ferguson SS, Zhang J, Caron MG. A beta-arrestin/green fluorescent protein biosensor for detecting G protein-coupled receptor activation. J Biol Chem 1997;272:27497–27500. [PubMed: 9346876]
19. 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]
20. Gurevich EV, Benovic JL, Gurevich VV. Arrestin2 and arrestin3 are differentially expressed in the rat brain during postnatal development. Neuroscience 2002; 109: 421–436. [PubMed: 11823056]
21. Gurevich EV, Benovic JL, Gurevich VV. Arrestin2 expression selectively increases during neural differentiation. J Neurochem 2004;91:1404–1416. [PubMed: 15584917]
22. 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]
23. 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]
24. 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]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

25. 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]
26. Krupnick JG, Gurevich VV, Benovic JL. Mechanism of quenching of photo-transduction. Binding competition between arrestin and transducin for phosphorhodopsin. J Biol Chem 1997;272:18125–18131. [PubMed: 9218446]
27. 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]
28. 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]
29. Laporte SA, Oakley RH, Zhang J, et al. The β2-adrenergic receptor/arrestin complex recruits the clathrin adaptor AP-2 during endocytosis. Proc Natl Acad Sci USA 1999;96:3712–3717. [PubMed: 10097102]
30. 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]
31. 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]
32. 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]
33. Luttrell LM, Roudabush FL, Choy EW, et al. Activation and targeting of extra-cellular signal-regulated kinases by beta-arrestin scaffolds. Proc Natl Acad Sci USA 2001;98:2449–2454. [PubMed: 11226259]
34. Bruchas MR, Macey TA, Lowe JD, Chavkin C. Kappa opioid receptor activation of p38 MAPK is GRK3- and arrestin-dependent in neurons and astrocytes. J Biol Chem 2006;281:18081–18089. [PubMed: 16648139]
35. Perry SJ, Baillie GS, Kohout TA, et al. Targeting of cyclic AMP degradation to beta 2-adrenergic receptors by beta-arrestins. Science 2002;298:834–836. [PubMed: 12399592]
36. 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]
37. Nair KS, Hanson SM, Kennedy MJ, Hurley JB, Gurevich VV, Slepak VZ. Direct binding of visual arrestin to microtubules determines the differential subcellular localization of its splice variants in rod photoreceptors. J Biol Chem 2004;279: 41240–41248. [PubMed: 15272005]
38. 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]
39. Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. Science 2001;294:1307–1313. [PubMed: 11588219]
40. Bhandari D, Trejo J, Benovic JL, Marchese A. Arrestin-2 interacts with the ubiquitin-protein isopeptide ligase atrophin-interacting protein 4 and mediates endosomal sorting of the chemokine receptor CXCR4. J Biol Chem 2007;282:36971–36979. [PubMed: 17947233]
41. Ahmed MR, Zhan X, Song X, Kook S, Gurevich VV, Gurevich EV. Ubiquitin ligase parkin promotes Mdm2-arrestin interaction but inhibits arrestin ubiquitination. Biochemistry 2011;50:3749–3763. [PubMed: 21466165]
42. Gurevich VV, Gurevich EV. The structural basis of arrestin-mediated regulation of G protein-coupled receptors. Pharmacol Ther 2006;110:465–502. [PubMed: 16460808]
43. Peterson YK, Luttrell LM. The diverse roles of arrestin scaffolds in G protein-coupled receptor signaling. Pharmacol Rev 2017;69:256–297. [PubMed: 28626043]
44. Xu J, Dodd RL, Makino CL, Simon MI, Baylor DA, Chen J. Prolonged photoresponses in transgenic mouse rods lacking arrestin. Nature 1997;389:505–509. [PubMed: 9333241]
45. Chen CK, Burns ME, Spencer M, et al. Abnormal photoresponses and light-induced apoptosis in rods lacking rhodopsin kinase. Proc Natl Acad Sci USA 1999;96: 3718–3722. [PubMed: 10097103]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

46. Chen J, Makino CL, Peachey NS, Baylor DA, Simon MI. Mechanisms of rhodopsin inactivation in vivo as revealed by a COOH-terminal truncation mutant. Science 1995;267:374–377. [PubMed: 7824934]
47. Mendez A, Burns ME, Roca A, et al. Rapid and reproducible deactivation of rhodopsin requires multiple phosphorylation sites. Neuron 2000;28:153–164. [PubMed: 11086991]
48. Fuchs S, Nakazawa M, Maw M, Tamai M. Oguchi Y, Gal A. A homozygous 1-base pair deletion in the arrestin gene is a frequent cause of Oguchi disease in Japanese. Nat Genet 1995;10:360–362. [PubMed: 7670478]
49. Nakazawa M, Wada Y, Fuchs S, Gal A, Tamai M. Oguchi disease: phenotypic characteristics of patients with the frequent 1147delA mutation in the arrestin gene. Retina 1997;17:17–22. [PubMed: 9051837]
50. Nakazawa M Wada Y, Tamai M. Arrestin gene mutations in autosomal recessive retinitis pigmentosa. Arch Ophthalmol 1998;16:498–501.
51. Hirsch JA, Schubert C, Gurevich VV, Sigler PB. The 2.8 A crystal structure of visual arrestin: a model for arrestin's regulation. Cell 1999;97:257–269. [PubMed: 10219246]
52. Frischmeyer PA, Dietz HC. Nonsense-mediated mRNA decay in health and disease. Hum Mol Genet 1999;8:1893–1900. [PubMed: 10469842]
53. Hilleren P, Parker R. Mechanisms of mRNA surveillance in eukaryotes. Annu Rev Genet 1999;33:229–260. [PubMed: 10690409]
54. Hentze MW, Kulozik AE. A perfect message: RNA surveillance and nonsense-mediated decay. Cell 1999;96:307–310. [PubMed: 10025395]
55. Berner N, Reutter KR, Wolf DH. Protein quality control of the endoplasmic reticulum and ubiquitin-proteasome-triggered degradation of aberrant proteins: yeast pioneers the path. Annu Rev Biochem 2018;87:751–782. [PubMed: 29394096]
56. Cleghorn WM, Tsakem EL, Song X, et al. Progressive reduction of its expression in rods reveals two pools of arrestin-1 in the outer segment with different roles in photoresponse recovery. PLoS One 2011;6, e22797. [PubMed: 21818392]
57. Sullivan LS, Bowne SJ, Koboldt DC, et al. A novel dominant mutation in SAG, the arrestin-1 gene, is a common cause of retinitis pigmentosa in Hispanic families in the Southwestern United States. Invest Ophthalmol Vis Sci 2017;58:2774–2784. [PubMed: 28549094]
58. Vishnivetskiy SA, Lee RJ, Zhou XE, et al. Functional role of the three conserved cysteines in the N domain of visual arrestin-1. J Biol Chem 2017;292:12496–12502. [PubMed: 28536260]
59. 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]
60. 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]
61. Vishnivetskiy SA, Sullivan LS, Bowne SJ, Daiger SP, Gurevich EV, Gurevich VV. Molecular defects of the disease-causing human arrestin-1 C147F mutant. Invest Ophthalmol Vis Sci 2018;59:13–20. [PubMed: 29305604]
62. Al-Ubaidi MR, Font RL, Quiambao AB, et al. Bilateral retinal and brain tumors in transgenic mice expressing simian virus 40 large T antigen under control of the human interphotoreceptor retinoid-binding protein promoter. J Cell Biol 1992;119:1681–1687. [PubMed: 1334963]
63. Kim R, Emi M, Tanabe K, Murakami S. Role of the unfolded protein response in cell death. Apoptosis 2006;11:5–13. [PubMed: 16374548]
64. Goldstein O, Jordan JA, Aguirre GD, Acland GM. A non-stop S-antigen gene mutation is associated with late onset hereditary retinal degeneration in dogs. Mol Vis 2013;19:1871–1884. [PubMed: 24019744]
65. Chang HS, Won ES, Lee HY, Ham BJ, Kim YG, Lee MS. Association of ARRB1 polymorphisms with the risk of major depressive disorder and with treatment response to mirtazapine. J Psychopharmacol 2015;29:615–622. [PubMed: 25294870]
66. Petit AC. El Asmar K, David DJ, et al. The association of β-arrestin2 polymorphisms with response to antidepressant treatment in depressed patients. Prog Neuropsychopharmacol Biol Psychiatry 2018;81:74–79. [PubMed: 29031912]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

67. Sun D, Ma JZ, Payne TJ, Li MD. Beta-arrestins 1 and 2 are associated with nicotine dependence in European American smokers. Mol Psychiatry 2008;13:398–406. [PubMed: 17579607]
68. Oneda B, Preisig M, Dobrinas M, Eap CB. Lack of association between genetic polymorphisms of ARRB2 and alcohol dependence in a Caucasian population. Alcohol Alcohol 2010;45:590–591. [PubMed: 20864483]
69. Björk K, Rimondini R, Hansson AC, et al. Modulation of voluntary ethanol consumption by beta-arrestin 2. FASEB J 2008;22:2552–2560. [PubMed: 18367649]
70. Ambrose-Lanci LM, Vaswani M, Clarke TK, et al. Association study of the β-arrestin 2 gene (ARRB2) with opioid and cocaine dependence in a European-American population. Psychiatr Genet 2012;22:141–145. [PubMed: 22472784]
71. Oneda B, Crettol S, Bochud M, et al. β-Arrestin2 influences the response to methadone in opioid-dependent patients. Pharm J 2010; 11:258.
72. Ikeda M, Ozaki N, Suzuki T, et al. Possible association of beta-arrestin 2 gene with methamphetamine use disorder, but not schizophrenia. Genes Brain Behav 2007;6: 107–112. [PubMed: 17233643]
73. Liou YJ, Wang YC, Chen JY, et al. The coding-synonymous polymorphism rs1045280 (Ser280Ser) in beta-arrestin 2 (ARRB2) gene is associated with tardive dyskinesia in Chinese patients with schizophrenia. Eur J Neurol 2008;15:1406–1408. [PubMed: 19049562]
74. Zhang X, He J-Q, Ding L, Paré PD, Sandford AJ. Promoter polymorphism and expression of β-arrestin 2 in neutrophils. Clin Chim Acta 2007;385:79–80. [PubMed: 17761157]
75. Conner DA, Mathier MA, Mortensen RM, et al. β-Arrestin l knockout mice appear normal but demonstrate altered cardiac responses to β-adrenergic stimulation. Circ Res 1997;81:1021–1026. [PubMed: 9400383]
76. Zurkovsky L, Sedaghat K, Ahmed MR, Gurevich VV, Gurevich EV. Arrestin-2 and arrestin-3 differentially modulate locomotor responses and sensitization to amphetamine. Neurophartnacology 2017;121:20–29.
77. Bohn LM, Lefkowitz RJ, Gainetdinov RR, Peppel K, Caron MG, Lin FT. Enhanced morphine analgesia in mice lacking beta-arrestin 2. Science 1999;286:2495–2498. [PubMed: 10617462]
78. Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. Cell 2005;122:261–273. [PubMed: 16051150]
79. Bohn LM, Gainetdinov RR, Sotnikova TD, et al. Enhanced rewarding properties of morphine, but not cocaine, in beta (arrestin)-2 knock-out mice. J Neurosci 2003;23: 10265–10273. [PubMed: 14614085]
80. Kohout TA, Lin FS, Perry SJ, Conner DA, Lefkowitz RJ. Beta-arrestin 1 and 2 differentially regulate heptahelical receptor signaling and trafficking. Proc Natl Acad Sci USA 2001;98:1601–1606. [PubMed: 11171997]
81. Zhang M, Liu X, Zhang Y, Zhao J. Loss of betaarrestin 1 and betaarrestin2 contributes to pulmonary hypoplasia and neonatal lethality in mice. Dev Biol 2010;339:407–417. [PubMed: 20060823]
82. Roman G, He J, Davis RL. kurtz, a novel nonvisual arrestin, is an essential neural gene in *Drosophila*. Gaieties 2000;155:1281–1295.
83. Tipping M, Kim Y, Kyriakakis P, Tong M. Shvartsman SY, Veraksa A. β-Arrestin Kurtz inhibits MAPK and Toll signalling in *Drosophila* development. EMBO J 2010;29:3222–3235. [PubMed: 20802461]
84. Coffa S, Breitman M, Spiller BW, Gurevich VV. A single mutation in arrestin-2 prevents ERK1/2 activation by reducing c-Rafl binding. Biochemistry 2011;50: 6951–6958. [PubMed: 21732673]
85. 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]
86. 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]
87. Seo J, Tsakem EL, Breitman M, Gurevich VV. Identification of arrestin-3-specific residues necessary for JNK3 kinase activation. J Biol Chem 2011;286:27894–27901. [PubMed: 21715332]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

88. Zhan X, Stoy H, Kaoud TS, et al. Peptide mini-scaffold facilitates JNK3 activation in cells. Sci Rep 2016;6:21025. [PubMed: 26868142]
89. 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]
90. Stoy H, Gurevich VV. How genetic errors in GPCRs affect their function: possible therapeutic strategies. Genes Dis 2015;2:108–132. [PubMed: 26229975]
91. Gurevich VV, Gurevich EV. The molecular acrobatics of arrestin activation. Trends Pharmacol Sci 2004;25:105–111. [PubMed: 15102497]
92. Samaranayake 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]
93. Vishnivetskiy SA, Chen Q, Palazzo MC, et al. Engineering visual arrestin-1 with special functional characteristics. J Biol Chem 2013;288:11741–11750. [PubMed: 23476014]
94. Kovoor 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]
95. Pan L, Gurevich EV, Gurevich VV. The nature of the arrestin x receptor complex determines the ultimate fate of the internalized receptor. J Biol Chem 2003;278: 11623–11632. [PubMed: 12525498]
96. 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]
97. Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS. Differential affinities of visual arrestin, βarrestinl, and βarrestm2 for G protein-coupled receptors delineate two major classes of receptors. J Biol Chem 2000;275:17201–17210. [PubMed: 10748214]
98. Hanson SM, Francis DJ, Vishnivetskiy SA, et al. Differential interaction of spinlabeled arrestin with inactive and active phosphorhodopsin. Proc Natl Acad Sci USA 2006;103:4900–4905. [PubMed: 16547131]
99. 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]
100. 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]
101. Chen Q, Perry NA, Vishnivetskiy SA. et al. Structural basis of arrestin-3 activation and signaling. Nat Commun 2017;8:1427. [PubMed: 29127291]
102. 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]
103. 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]

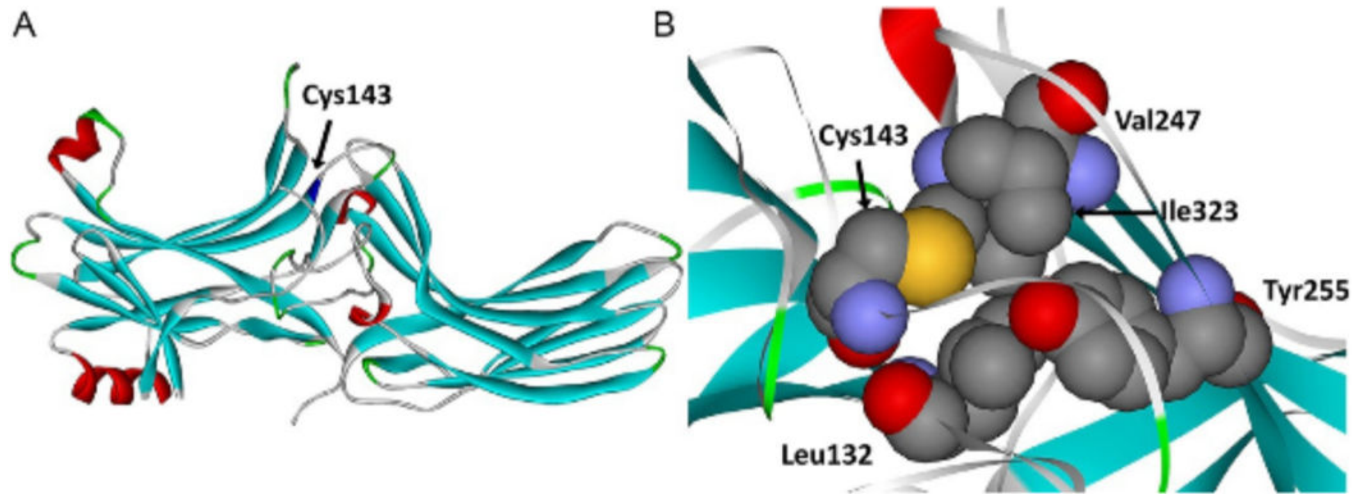

Fig. 1.

Dominant mutation in visual arrestin-1 causing retinal degeneration. (A) The position of Cys-147 in the arrestin-1 molecule (panels A and B are based on the crystal structure of bovine arrestin-1, PDB ID:1CF1,[51] so the homologous bovine Cys-143 is shown). (B) Cys-143 and its neighbors (indicated) are shown as CPK models, with the atoms colored, as follows: carbon, *gray;* oxygen, *red;* nitrogen, *blue;* sulfur, *yellow.* Note close packing, which would make the introduction of much bulkier side chain destabilizing.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

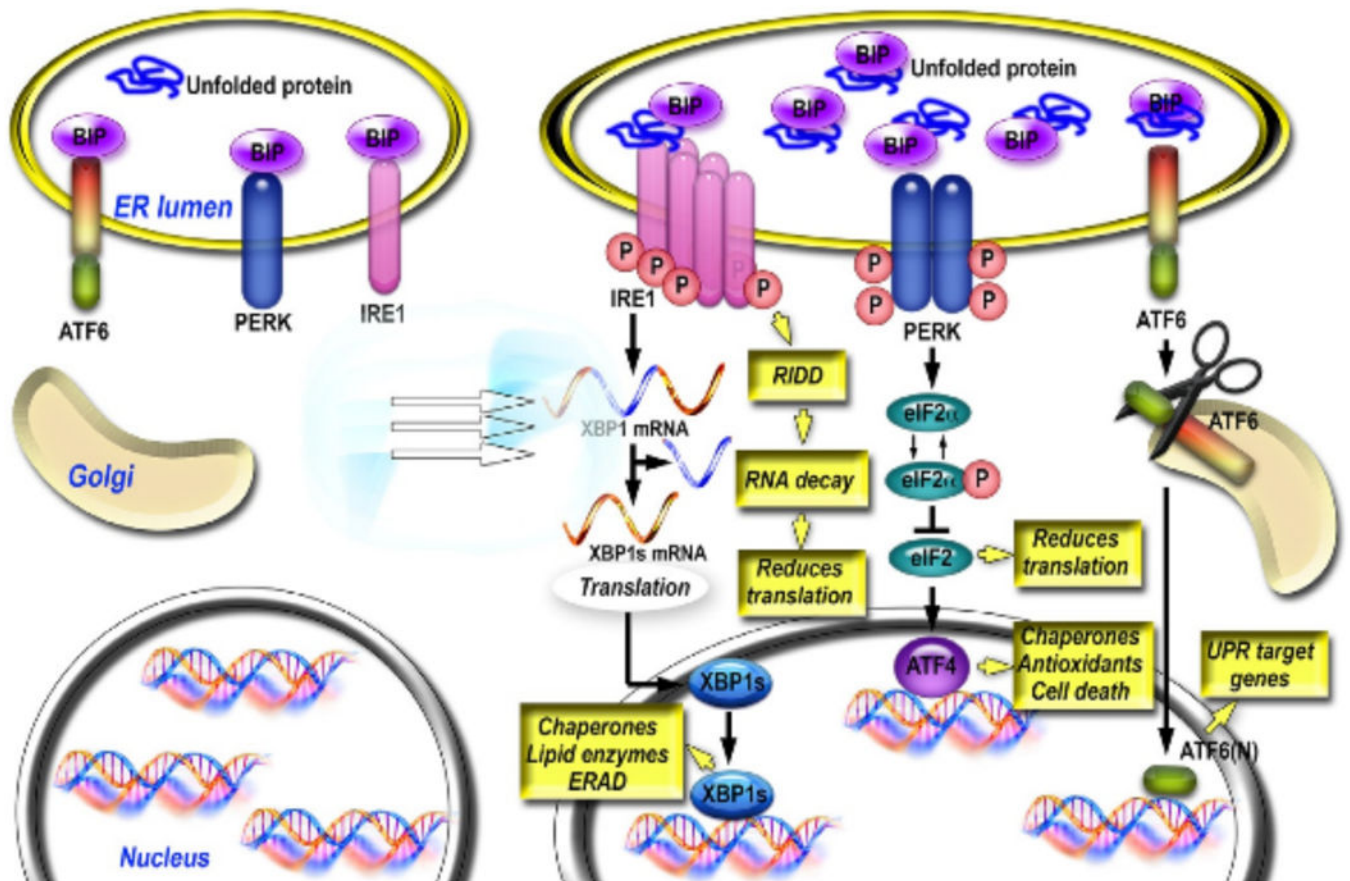

Fig. 2.

Mutant arrestin-1 induces unfolded protein response. Experimental data suggest that C147F mutant is significantly less stable than WT arrestin-1.[61] As arrestin-1 is the second most abundant protein in the rod photoreceptors after rhodopsin, its unfolding likely induces unfolded protein response, which eventually leads to rod death.[63] An increase in GRP78/BIP, which manifests the first step of the unfolded protein response, was detected in photoreceptor-derived 661W cells expressing this mutant, but not in cells expressing WT human arrestin-1.[61] The schematic is based on the mechanisms described earlier (see ref. 63 and references therein). Abbreviations: *ATF4,* activating transcription factor 4; *ATF6,* activating transcription factor 6; *BIP,* same as GPR78, a major ER chaperone, a.k.a. 78-kDa glucose-regulated protein; *elF2α,* eukaryotic translation initiation factor 2α; *ER,* endoplasmic reticulum; *ERAD,* ER-associated protein degradation; *IRE1,* inositol-requiring enzyme 1; *PERK,* PKR-like ER kinase; *RIDD,* regulated IRE1-dependent decay of mRNA; *UPR,* unfolded protein response; *XBP1,* X-box binding protein 1.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

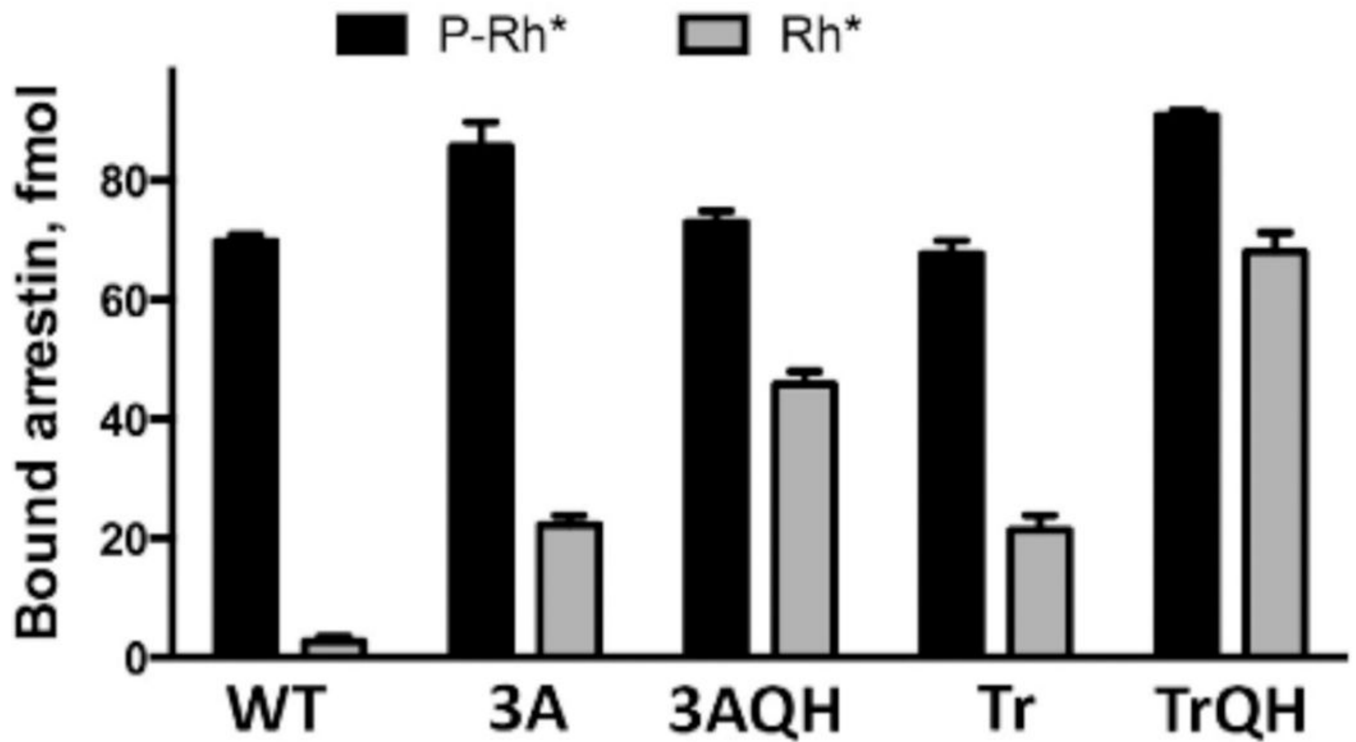

Fig. 3.

Phosphorylation dependence of the binding of WT arrestin-1 and its enhanced mutants. The binding in the in vitro assay of translated radiolabeled arrestins to purified phosphorylated (P-Rh*) and unphosphorylated (Rh*) light-activated rhodopsin (performed as described[17]) is shown.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

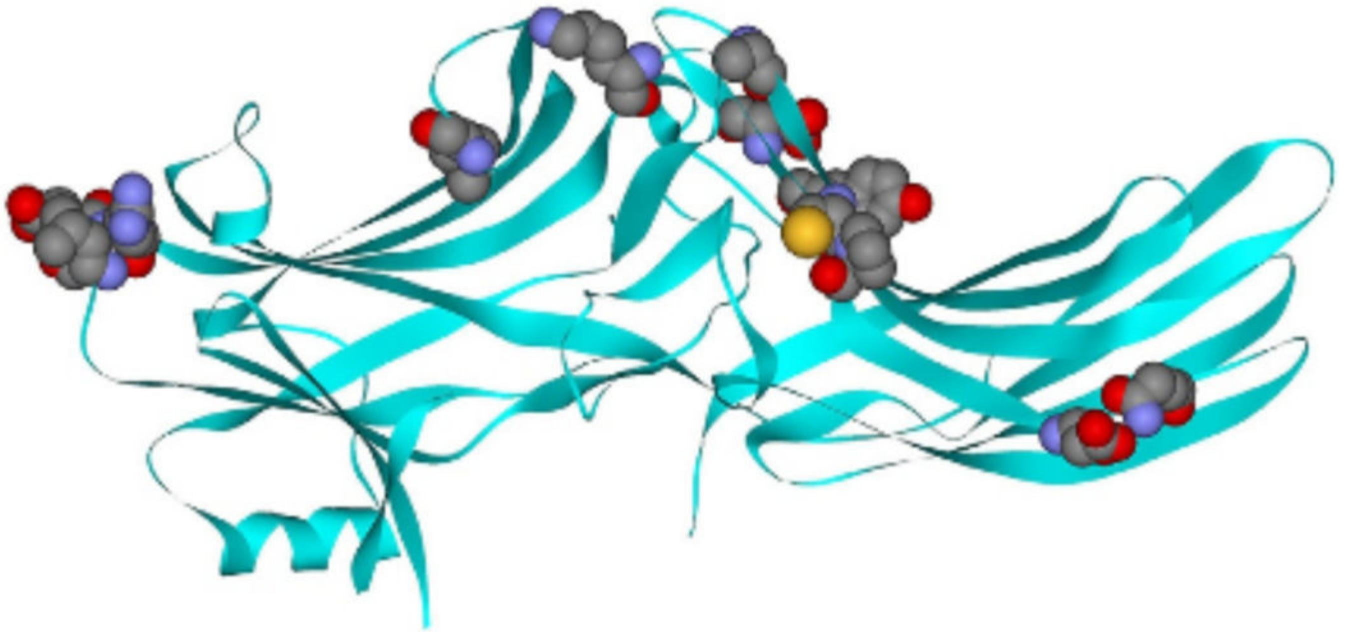

Fig. 4.

Arrestin-3 residues that determine receptor preference. The residues found to change receptor preference of nonvisual arrestin-3 in the in-cell BRET-based assay[19,101–103] are shown as CPK models on the crystal structure of the basal state of bovine arrestin-3 (PDB ID: **3P2D**[85]**),** with the atoms colored, as follows: *gray,* carbon; *red,* oxygen; *blue,* nitrogen; *yellow,* sulfur. Note the localization of these residues on the concave sides of both arrestin domains, with clustering on the central crest of the receptor-binding side of the molecule.