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Editorial

Protein Flexibility and Cellular Signaling

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Traditional view of cellular signaling was based on the assumption that each protein has only two states, active an inactive, like a light switch, that can be "on" or "off". This view was applied to cell surface receptors and downstream signaling proteins, including G protein-coupled receptors (GPCRs) and their immediate signal transducers, G proteins and arrestins. Arguably the most enduring model of GPCR signaling, extended ternary complex model [1] explicitly posited the existence of the receptor in two states, R (inactive) and R* (active). Based on this assumption, the model posited that G proteins interact only with the R* state, forming the signaling ternary complex agonist-R*-G protein [1]. As the signaling of prototypical GPCR, rhodopsin, was quenched by receptor phosphorylation followed by the binding of arrestin [2], the field assumed that G proteins, GPCR kinases (GRKs), that selectively phosphorylate active GPCRs (reviewed in [3]), and arrestins recognize the same active state of the receptor, so that GPCR desensitization can be explained by a simple competition between G proteins and arrestins for the active receptor [4,5]. This view was extrapolated to the whole GPCR family [6]. The first biophysical data appeared to support this simplistic model: GPCR activation was found to require a rigid body motion of the transmembrane helices [7]. Inter-helical distance measurements using pulse EPR technique double electron-electron resonance (DEER) in the inactive and light-activated rhodopsin generally agreed with this model [8], although close inspection of distance distributions shows multiple peaks in some cases, which suggests that things may not be that simple [8]. Crystal structures of inactive [9] and light-activated [10,11] rhodopsin, as well as the structures of a prototypical non-visual GPCR, β2-adrenergic receptor (β2AR), also in the inactive [12] and active agonist-liganded state [13] revealed the expected helix movement upon activation and appeared consistent with this two-state model.

Inherent constitutive activity of GPCRs and the action of activity-suppressing inverse agonists could also be interpreted in the context of the two-state model, although the first complications arose: the presence of the G protein greatly affected the spectrum of a conformationally sensitive fluorescent probe, in fact, to a greater extent than the ligand [14]. This could still be accounted for by the idea that the ligands and G protein simply change the fraction of the receptor population in each of the two states. However, the structure of the complex of the β2AR with its cognate G protein, Gs [15], was quite different from the structure of agonist-liganded receptor [13], suggesting that the receptor can assume more than one conformation in its active state. In case of the β2AR this issue was directly addressed by the application of several biophysical methods [16]. The data showed that the unliganded or antagonist-liganded receptor exists in equilibrium of several conformations, with at least two apparently inactive, whereas the agonist shifts this equilibrium towards presumably active states, of which there are also more than one [16]. In fact, the number of discernible active states depends on the method used to detect them [16], suggesting that even the most sensitive method yields the minimum number of distinct states, rather than revealing all states that are present. These data are consistent with the existence of multiple active and inactive states revealed by intra-rhodopsin distance measurements earlier [8]. Careful comparison of the conformations of light-activated rhodopsin in detergent and in a native-like lipid environment in nanodiscs revealed that while the receptor appeared to act as a simple on-off switch in the detergent, in lipid environment even spectroscopically homogeneous active Meta rhodopsin IIb+ state was represented by several conformations, more than one of which was selected by its cognate G protein transducin [17]. Thus, active GPCRs are by no means present in a single active conformation, but rather exist as a conformational ensemble, raising a possibility that distinct states might favor different signal transducers [17]. This is in line with the current understanding of biased GPCR signaling: different ligands, orthosteric and allosteric, selectively increase the abundance of distinct GPCR conformations, thereby directing the signaling to different transducers (reviewed in [18-20]). Importantly, conformationally encoded bias applies not only to the G protein-arrestin dichotomy, which is usually assumed. Many GPCRs couple to more than one type of G proteins. Recent finding that receptor-bound Gs and Gi assume different poses, and that Gi requires much smaller outward movement of the transmembrane helix VI [21], suggests that preferential coupling to an individual G protein subtype out of several possible can be also imparted by the agonist that shifts the receptor into a particular active conformation. Structures of the complexes of the same receptor with different G proteins are needed to definitively resolve this issue.

As far as conformational flexibility is concerned, GPCRs are not unique among signaling proteins. Two signal transducers, G proteins and arrestins, illustrate this point. While crystal [15,22] and cryo-EM [23,24] structures of the complexes of different GPCRs with Gs show one particular conformation of the bound G protein, biophysical methods suggest that receptor-associated G protein is quite flexible, existing in many conformations [25,26]. In particular, relative orientation of the helical and Ras-like domains of the G protein α-subunit does not appear to be fixed. Functional role of this flexibility remains to be elucidated. The inspection of the distribution of measured DEER distances within Gi α-subunit in all four functional states (inactive free, inactive in heterotrimeric complex with βγ-subunits, receptor-bound heterotrimer, and GTP-liganded active free α-subunit) shows multiple peaks [27], suggesting that Gi α-subunit remains very flexible in every state. As Gi α-subunit interacts with several partners, effectors as well as RGS proteins that facilitate its self-inactivation, it is entirely possible that distinct Gi α-subunit conformations have different functional preferences, selecting specific interaction partners. The structures of the complexes of the Gi α-subunit with effectors and RGS proteins are needed to test this idea.

The same is true for arrestins, that, in addition to their role in precluding G protein coupling to the receptor [2,4-6,28], were recognized as yet another class of signal transducers (reviewed in [29-31]). In their basal state, all four vertebrate arrestins have remarkably similar conformations [32-37]). The conformations of arrestin-1 [38], arrestin-2 [39], and arrestin-3 [40] activated by various means, as well as arrestin-1 in complex with rhodopsin [41-42] are also quite similar and clearly differ from the basal conformations. This can be interpreted as arrestins having only two conformational states, active and inactive. However, the distributions of distances between selected points in both free and receptor-bound arrestins, measured by DEER, are fairly wide [43-44], suggesting that arrestins are flexible in both states, exploring a pretty wide conformational space. This conclusion is also supported by the NMR data [45]. Some binding partners, such as ubiquitin ligases Mdm2 [46] and parkin [47], preferentially bind basal arrestins, others, like ERK1/2, prefer receptor-bound form [48], while some, like JNK3 or MEK1, effectively bind both [46,48]. Each non-visual arrestin was found to interact with more than 100 different non-receptor partners [49], which would be impossible without high conformational flexibility of these relatively small ~45 kDa proteins (reviewed in [50,51]). This notion is supported by the findings that separated arrestin peptides that do not have conformational constraints they would have in the full-length folded protein, bind known arrestin partners. The C-terminus of arrestin-2 binds two main components of the internalization machinery of the coated pit, clathrin and clathrin adaptor AP-2, thereby suppressing arrestin-dependent GPCR internalization in cells expressing receptors [52]. The N-terminus of arrestin-3 binds JNK3 and upstream kinases ASK1 and MKK4/7, facilitating JNK3 activation in cells [53]. Interestingly, careful inspection of all available structures shows that the transition from basal to active group of conformations induces significant changes in several arrestin elements, which identifies these parts as the most likely docking sites of proteins that preferentially bind inactive or active arrestins [54].

Arrestins apparently need three receptor-attached phosphates for high affinity binding [42,55,56]. Most GPCRs have a lot more than three phosphorylation sites, which suggested an idea that differential receptor phosphorylation can result in distinct conformations of receptor-bound arrestins that lead to distinct signaling capabilities of the complex [57,58]. While this tempting idea still needs to be confirmed experimentally, preferably by solving the structures of the complexes of the same arrestin with the same differentially phosphorylated receptor, numerous lines of indirect evidence suggest that it might be correct. Several distances between selected points in arrestin-1 and bound rhodopsin were measured by DEER [41,42]. In each case, complex distance distributions with multiple peaks were detected. While the most probable one invariably matched the crystal structure, the existence of alternative distances suggested that the complex likely has different "flavors", only one of which was captured in crystal. By the use of pairs of fluorescent substitutions in non-visual arrestins it was recently shown that in cells receptor-bound arrestins demonstrate distinct conformational signatures that correlate with their functions [59,60]. In agreement with these data, differentially phosphorylated receptor peptides were shown by NMR to induce distinct conformations of purified arrestin-2 [61]. In fact, the flexibility of the complex is the most likely reason for an apparent contradiction between the crystal structure of the arrestin-1-rhodopsin complex [41,42], where most of the concave side of the arrestin C-domain does not contact the receptor, and EPR evidence that many residues on this surface come into contact with the receptor in visual arrestin-1 [62] and non-visual arrestin-2 [63]. Moreover, numerous residues on the same concave side of the C-domain were found to determine receptor preference of arrestins [63-66], which suggests that they must contact GPCRs in the complex, even though they do not in the crystal structure.

Conclusion

In summary, the ability to assume numerous conformations is an inherent property of GPCRs, G proteins, and arrestins, and very likely all proteins involved in cellular signaling. Mutations that affect conformational flexibility of GPCRs [67,68] and arrestin-1 [69,70] were shown to underlie congenital disorders in humans. The fact that signaling proteins do not function as on-off switches, existing in only two (active and inactive) conformations, should always be taken into account. Conformational flexibility is the key to understanding protein function, and it is particularly important for the signaling proteins pharmacology deals with.

Footnote

[1]Here we use the systematic names of arrestins, 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).

Bibliography

1. Samama P., *et al.* "A mutation-induced activated state of the beta 2-adrenergic receptor. Extending the ternary complex model". *The Journal of Biological Chemistry* 268.7 (1993): 4625-4636.

2. Wilden U., *et al*. "Phosphodiesterase activation by photoexcited rhodopsin is quenched when rhodopsin is phosphorylated and binds the intrinsic 48-kDa protein of rod outer segments". *Proceedings of the National Academy of Sciences* 83.5 (1986): 1174-1178.

3. Gurevich E V., *et al.* "G protein-coupled receptor kinases: more than just kinases and not only for GPCRs". *Pharmacology and Therapeutics* 133.1 (2012): 40-46.

4. 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* 34.4 (1995): 1446-1454.

5. Krupnick J G., *et al.* "Mechanism of quenching of phototransduction. Binding competition between arrestin and transducin for phosphorhodopsin". *The Journal of Biological Chemistry* 272.29 (1997): 18125-18131.

6. Carman C V and Benovic J. L. "G-protein-coupled receptors: turn-ons and turn-offs". *Current Opinion In Neurobiology* 8.3 (1998): 335-344.

7. Farrens D L., *et al*. "Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin". *Science* 274.5288 (1996): 768-770.

8. Altenbach C., *et al.* "High-resolution distance mapping in rhodopsin reveals the pattern of helix movement due to activation". *Proceedings of the National Academy of Sciences of the United States of America* 105.21 (2008): 7439-7444.

9. Palczewski K., *et al.* "Crystal structure of rhodopsin: A G protein-coupled receptor". *Science* 289.5480 (2000): 739-745.

10. Scheerer P., *et al.* "Crystal structure of opsin in its G-protein-interacting conformation". *Nature* 455 (2008): 497-502.

11. Choe H W., *et al.* "Crystal structure of metarhodopsin II". *Nature* 471 (2011): 651-655.

12. Rasmussen S G., *et al.* "Crystal structure of the human beta2 adrenergic G-protein-coupled receptor". *Nature* 450.7168 (2007): 383-387.

13. Rosenbaum D M., *et al.* "Structure and function of an irreversible agonist-β(2) adrenoceptor complex". *Nature* 469.7329 (2011): 236-240.

14. Yao X J., *et al.* "The effect of ligand efficacy on the formation and stability of a GPCR-G protein complex". *Proceedings of the National Academy of Sciences of the United States of America* 106.23 (2009): 9501-9506.

15. Rasmussen S G., *et al.* "Crystal structure of the β2 adrenergic receptor-Gs protein complex". *Nature* 477 (2011): 549-555.

16. Manglik A., *et al.* "Structural Insights into the Dynamic Process of beta2-Adrenergic Receptor Signaling". *Cell* 161.5 (2015): 1101-1111.

17. Van Eps N., *et al*. "Conformational equilibria of light-activated rhodopsin in nanodiscs". *Proceedings of the National Academy of Sciences of the United States of America* A 114.16 (2017): E3268-E3275.

18. Gurevich V V and Gurevich E V. "Molecular Mechanisms of GPCR Signaling: A Structural Perspective". *International Journal of Molecular Sciences* 18.12 (2017): E2519.

19. Wisler J W., *et al*. "Recent developments in biased agonism". *Current Opinion in Cell Biology* 27(2014): 18-24.

20. Luttrell L M and Kenakin T P. "Refining efficacy: allosterism and bias in G protein-coupled receptor signaling". *Methods in Molecular Biology* 756 (2011): 3-35.

21. Van Eps N., *et al.* "Gi- and Gs-coupled GPCRs show different modes of G-protein binding". *Proceedings of the National Academy of Sciences of the United States of America* 115 (2018): 2383-2388.

22. Carpenter B., *et al.* "Structure of the adenosine A(2A) receptor bound to an engineered G protein". *Nature* 536.7614 (2016): 104-107.

23. Liang Y L., *et al.* "Phase-plate cryo-EM structure of a class B GPCR-G-protein complex". *Nature* 546.7656 (2017): 118-123.

24. Zhang Y., *et al.* "Cryo-EM structure of the activated GLP-1 receptor in complex with a G protein". *Nature* 546.7657 (2017): 248-253.

25. Westfield G H., *et al.* "Structural flexibility of the G alpha s alpha-helical domain in the beta2-adrenoceptor Gs complex". *Proceedings of the National Academy of Sciences of the United States of America* 108.38 (2011): 16086-16091.

26. Oldham W M., *et al.* "Mapping allosteric connections from the receptor to the nucleotide-binding pocket of heterotrimeric G proteins". *Proceedings of the National Academy of Sciences of the United States of America* 104.19 (2007): 7927-7932.

27. Van Eps N., *et al.* "Interaction of a G protein with an activated receptor opens the interdomain interface in the alpha subunit". *Proceedings of the National Academy of Sciences of the United States of America* 108.23 (2011): 9420-9424.

28. Gurevich V V., *et al.* "The functional cycle of visual arrestins in photoreceptor cells". *Progress in Retinal and Eye Research* 30.6 (2011): 405-430.

29. Gurevich V V and Gurevich E V. "The structural basis of arrestin-mediated regulation of G protein-coupled receptors". *Pharmacology and Therapeutics* 110.3 (2006): 465-502.

30. Peterson Y K and Luttrell L M. "The Diverse Roles of Arrestin Scaffolds in G Protein-Coupled Receptor Signaling". *Pharmacological Reviews* 69.3 (2017): 256-297.

31. Gurevich E V and Gurevich V V. "Arrestins are ubiquitous regulators of cellular signaling pathways". *Genome Biology* 7.9 (2006): 236.

32. Hirsch J A., *et al.* "The 2.8 A crystal structure of visual arrestin: a model for arrestin's regulation". *Cell* 97.2 (1999): 257-269.

33. Han M., *et al*. "Crystal structure of beta-arrestin at 1.9 A: possible mechanism of receptor binding and membrane translocation". *Structure* 9.9 (2001): 869-880.

34. Granzin J., *et al.* "X-ray crystal structure of arrestin from bovine rod outer segments". *Nature* 391.6670 (1998): 918-921.

35. Milano S K., *et al.* "Scaffolding functions of arrestin-2 revealed by crystal structure and mutagenesis". *Biochemistry* 41.10 (2002): 3321-3328.

36. Sutton R B., *et al.* "Crystal Structure of Cone Arrestin at 2.3Å: Evolution of Receptor Specificity". *Journal of Molecular Biology* 354.5 (2005): 1069-1080.

37. Zhan X., *et al. "*Crystal structure of arrestin-3 reveals the basis of the difference in receptor binding between two non-visual arrestins". *Journal of Molecular Biology* 406.3 (2011): 467-478.

38. Kim Y J., *et al.* "Crystal structure of pre-activated arrestin p44". *Nature* 497 (2013): 142-146.

39. Shukla A K., *et al*. "Structure of active beta-arrestin-1 bound to a G-protein-coupled receptor phosphopeptide". *Nature* 497.7447 (2013): 137-141.

40. Chen Q., *et al.* "Structural basis of arrestin-3 activation and signaling". *Nature Communications* 8 (2017): 1427.

41. Kang Y., *et al*. "Crystal structure of rhodopsin bound to arrestin determined by femtosecond X-ray laser". *Nature* 523.7562 (2015): 561-567.

42. Zhou X E., *et al*. "Structural Identification of Phosphorylation Codes for Arrestin Recruitment by G protein-Coupled Receptors". *Cell* 170.3 (2017): 457-469.

43. Kim M., *et al*. "Conformation of receptor-bound visual arrestin". Proceedings of the National Academy of Sciences USA 109.45 (2012): 18407-18412.

44. Zhuo Y., *et al*. "Identification of receptor binding-induced conformational changes in non-visual arrestins". *The Journal of Biological Chemistry* 289.30 (2014): 20991-21002.

45. Zhuang T., *et al*. "Involvement of Distinct Arrestin-1 Elements in Binding to Different Functional Forms of Rhodopsin". *Proceedings of the National Academy of Science USA* 110.3 (2013): 942-947.

46. Song X., *et al*. "Visual and both non-visual arrestins in their "inactive" conformation bind JNK3 and Mdm2 and relocalize them from the nucleus to the cytoplasm". *The Journal of Biological Chemistry* 281.30 (2006): 21491-21499.

47. Ahmed M., *et al*. "Ubiquitin ligase parkin promotes Mdm2-arrestin interaction but inhibits arrestin ubiquitination". *Biochemistry* 50.18 (2011): 3749-3763.

48. Coffa S., *et al*. "The Effect of Arrestin Conformation on the Recruitment of c-Raf1, MEK1, and ERK1/2 Activation". *PLOS One* 6.12 (2011): e28723.

49. Xiao, K., *et al*. "Functional specialization of beta-arrestin interactions revealed by proteomic analysis". *Proceedings of the National Academy of Sciences USA* 104.29 (2007): 12011-12016.

50. Gurevich V., *et al*. "Extensive shape shifting underlies functional versatility of arrestins". *Current Opinion in Cell Biology* 27 (2014): 1-9.

51. Gurevich, V., *et al*. "Arrestins: structural disorder creates rich functionality". *Protein Cell in Press* (2018).

52. Orsini MJ and Benovic JL. "Characterization of dominant negative arrestins that inhibit beta2-adrenergic receptor internalization by distinct mechanisms". *The Journal of Biological Chemistry* 273.51 (1998): 34616-34622.

53. Zhan X., *et al*. "Peptide mini-scaffold facilitates JNK3 activation in cells". *Scientific Reports* 6 (2016): 21025

54. Chen, Q., *et al*. "Structural basis of arrestin-dependent signal transduction". Trends in Biochemical Sciences in Press (2018).

55. Vishnivetskiy SA., *et al*. "Regulation of arrestin binding by rhodopsin phosphorylation level". *The Journal of Biological Chemistry* 282.44 (2007): 32075-32083.

56. Mendez A., *et al*. "Rapid and reproducible deactivation of rhodopsin requires multiple phosphorylation sites". *Neuron* 28.1 (2000): 153-164.

57. Tobin AB., *et al*. "Location, location, location...site-specific GPCR phosphorylation offers a mechanism for cell-type-specific signaling". Trends in Pharmacological Sciences 29.8 (2008) 413-420.

58. Nobles KN., *et al*. "Distinct phosphorylation sites on the β(2)-adrenergic receptor establish a barcode that encodes differential functions of β-arrestin". *Science Signaling* 4.185 (2011): ra51.

59. Lee MH., *et al*. "The conformational signature of β-arrestin2 predicts its trafficking and signalling functions". *Nature* 531.7596 (2016): 665-668.

60. Nuber S., *et al*. "β-Arrestin biosensors reveal a rapid, receptor-dependent activation/deactivation cycle". *Nature* 531.7596 (2016): 661-664.

61. Yang F., *et al*. "Phospho-selective mechanisms of arrestin conformations and functions revealed by unnatural amino acid incorporation and (19)F-NMR". *Nature Communications* 6 (2015): 8202.

62. Hanson SM., "Differential interaction of spin-labeled arrestin with inactive and active phosphorhodopsin". Proceedings of the *National Academy of Sciences USA* 103.13 (2006): 4900-4905.

63. Vishnivetskiy SA., *et al*. "Few residues within an extensive binding interface drive receptor interaction and determine the specificity of arrestin proteins". *The Journal of Biological Chemistry* 286.27 (2011) 24288-24299.

64. Vishnivetskiy SA., *et al*. "Mapping the arrestin-receptor interface: structural elements responsible for receptor specificity of arrestin proteins". *The Journal of Biological Chemistry* 279.2 (2004): 1262-1268.

65. Gimenez, L. E., *et al*. "Mutations in arrestin-3 differentially affect binding to neuropeptide Y receptor subtypes". *Cellular Signalling* 26.7 (2014) 1523-1531.

66. Gimenez LE., *et al*. "Manipulation of very few receptor discriminator residues greatly enhances receptor specificity of non-visual arrestins". *The Journal of Biological Chemistry* 287.35 (2012): 29495-29505.

67. Schoneberg, T., *et al*. "Mutant G-protein-coupled receptors as a cause of human diseases". *Pharmacology and Therapeutics* 104.3 (2004) 173-206.

68. Stoy H and Gurevich VV. "How genetic errors in GPCRs affect their function: Possible therapeutic strategies". *Genes and Diseases* 2.2 (2015): 108-132.

69. Sullivan LS., *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". Investigative Ophthalmology and Visual Science 58.5 (2017): 2774-2784.

70. Vishnivetskiy SA., *et al*. "Molecular Defects of the Disease-Causing Human Arrestin-1 C147F Mutant". *Investigative Ophthalmology and Visual Science* 59.1 (2018): 13-20.

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