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Structural mechanism of arrestin activation

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The large and multifunctional family of G protein-coupled receptors (GPCRs) are regulated by a small family of structurally conserved arrestin proteins. In order to bind an active GPCR, arrestin must first be activated by interaction with the phosphorylated receptor C-terminus. Recent years have witnessed major developments in high-resolution crystal structures of pre-active arrestins and arrestin or arrestin-derived peptides in complex with an active GPCR. Although each structure individually offers only a limited snapshot, taken together and interpreted in light of recent complementary functional data, they offer valuable insight into how arrestin is activated by and couples to a phosphorylated active GPCR.

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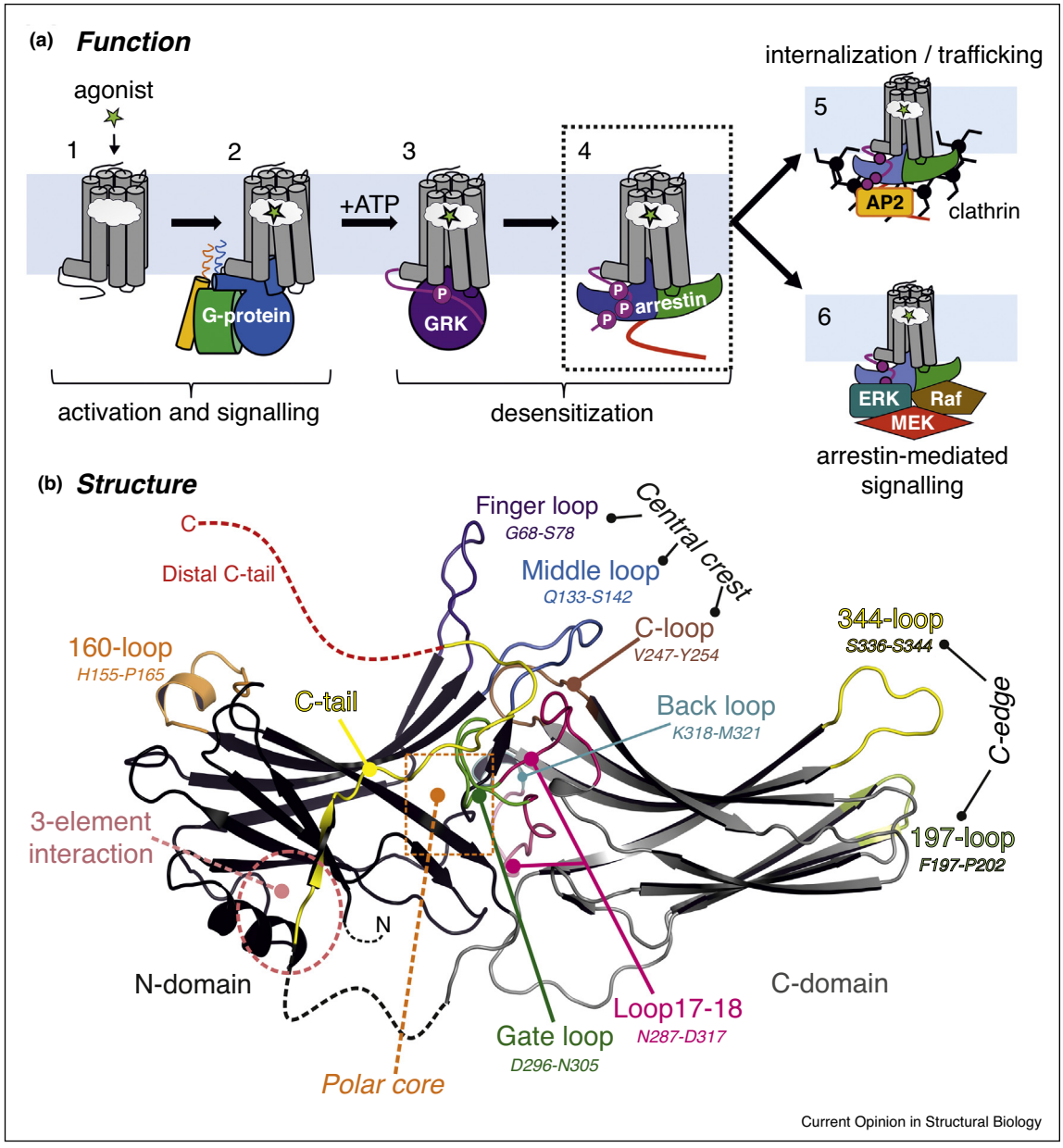
Despite the variety of ligands bound by different members of the large family of G protein-coupled receptors (GPCRs), all GPCRs share a common structure of seven transmembrane (TM) helices and a mostly conserved activation mechanism. Agonist (activating ligand) binding stabilizes an arrangement of the TM helices with an open cytoplasmic crevice that binds and activates specific G proteins [1–5]. Activated receptors (R*) are phosphorylated by specific kinases on multiple sites primarily within the C-terminus. Phosphorylated, active receptor (R*P) is bound by the protein arrestin, which physically blocks further G protein-coupling [6] (reviewed in Refs. [7,8]). Arrestins are versatile proteins that also mediate receptor internalization and trafficking, and initiate further rounds of signalling by scaffolding other signalling proteins (Figure 1a) (reviewed in Refs. [9,10]). In spite of this significant functional repertoire, the large GPCR family is regulated by only four structurally similar arrestins. Intriguingly, the pattern of phosphorylation within the R*-C-terminus [11•] and the conformation of R* [12,13] modulate the conformation of receptor-bound arrestin to bring about different cellular responses (reviewed in Refs. [14,15]).

Structurally arrestins consist of two crescent-shaped beta-sandwiches, termed the N- and C-domains (Figure 1b) [16–20]. The side-by-side placement of the domains gives rise to the *central crest*, composed of the *finger loop* (residues G68–S78),[3] a key receptor-binding element [21–23,24••], and the *middle loop* (residues Q133–S142) and the *C-loop* (residues V247–Y254), both stabilizing elements for inactive basal-state arrestin [25,26]. The interface of the N- and C-domains is stabilized by hydrophobic interactions and hydrogen bond networks including the well-described *polar core*, a network of buried charged residues [19,27]. The long C-terminal tail (*C-tail*) also stabilizes the basal structure of arrestin and is anchored to the N-domain at several points, including a hydrophobic *3-element interaction* [28] and a salt bridge to the central arginine (R175 in arrestin-1) of the *polar core* (Figure 2a). In the basal state, arrestins are unable to bind active receptors, and interaction with receptor-attached phosphates is critical for R* interaction [29]. Receptor-binding by arrestin is often discussed in terms of two distinct steps [30–32]. Firstly arrestin forms a low-affinity *pre-complex* with the receptor, in which the phosphorylated receptor C-terminus (*Rpp*) repels the acidic *C-tail* of arrestin and thereby gains access to the numerous basic residues in the N-domain [33,34]. *C-tail* displacement induces conformational changes and domain movements in arrestin that allow the second binding step, namely tight binding of the receptor and formation of a *high-affinity complex*.

Recent years have witnessed major developments in high-resolution crystal structures pertaining to the arrestin activation mechanism. These include the structure of a naturally occurring constitutively active form of arrestin-1 called **p44**, which lacks the regulatory *C-tail* [35••].[4] The p44 structure was published in the same journal issue as that of arrestin-2 bound to a peptide analogue of the phosphorylated C-terminus of the V2 vasopressin receptor (V2Rpp) [37••]. In the **arrestin-2–**

[3] Loop residue numbers refer to bovine arrestin-1.
[4] Note that another structure of p44 was reported by Granzin *et al.* in 2012 (PDB entry 3UGU [36]). In contrast to the structure discussed here (PDB entry 4J2Q), the 3UGU structure adopts a conformation very similar to basal-state arrestin [36]. This difference likely reflects the conformational equilibrium of p44.

Figure 1



***Arrestin structure and function*.(a)** Schematic of GPCR activation and desensitization. (1) The inactive receptor consists of a closed 7TM helical bundle. (2) Agonist binding stabilizes the active receptor, which couples to a heterotrimeric G protein. (3) The active receptor is also phosphorylated on its C-terminus by a GPCR kinase (GRK). (4) The phosphorylated, active receptor is bound by arrestin, which blocks further G protein coupling. Depending on the extent and pattern of receptor phosphorylation, arrestin binding can have different functional outcomes. (5) Receptors can be internalized into clathrin-coated pits. (6) Arrestin can induce G protein-independent signalling by scaffolding other signalling proteins. **(b)** Structure of basal arrestin. The N-domain is black and the C-domain is grey. Loops that are discussed in the text are indicated in different colours, and residue numbers refer to bovine arrestin-1. Parts of the structure not resolved in the crystal structure are indicated by dashed lines. The locations of the *polar core*, *3-element interaction*, *central crest* and *C-edge* are also shown. Structure model based on the crystal structure reported by Hirsch *et al.* (PDB entry 1CF1, molecule A) [19].

V2Rpp structure, the phosphopeptide binds within a positively charged crevice along the lateral side of the arrestin N-domain, which is occupied by the *C-tail* in basal arrestin. The remarkable similarity of the p44 and arrestin-2–V2Rpp structures validates the functional relevance of the observed conformational changes [38]. Similar but less dramatic conformational changes were also observed in the crystal structure of an arrestin-1