Structural biology

First step of receptor switch-off visualized

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The structure of rhodopsin, an archetypal member of the G-protein-coupled family of receptors, in complex with its specific kinase enzyme, reveals the molecular mechanism of the first step of receptor inactivation. See p.600

Cell behaviour is regulated by myriad external stimuli that act through specialized receptor proteins at the cell surface. G-protein-coupled receptors (GPCRs) are the largest family of receptors and mediate cellular responses to hormones, neurotransmitter molecules, light and other stimuli by activating G proteins inside cells. This signalling is terminated by a two-step mechanism: G-protein-coupled receptor kinase (GRK) enzymes add phosphate chemical groups to an active receptor, and the active phosphorylated receptor is then bound by arrestin proteins, precluding its further coupling to G proteins. Chen *et al.*[1] report on page 600 the first structure of a GPCR–GRK complex: the light-responsive receptor rhodopsin bound to its specific kinase, GRK1 (also known as rhodopsin kinase).

Phosphorylation of GPCRs by GRKs is the crucial, rate-limiting step in switching off receptors, a process called desensitization. The receptor–GRK interaction determines the dynamics of G-protein-mediated signalling because it affects the subsequent recruitment of arrestins, which bind to most GPCRs with high affinity only after the receptor is phosphorylated. Receptor-bound arrestins shut down G-protein-dependent signalling and initiate signalling through other pathways, which are determined by the pattern of phosphate groups attached to a receptor by a GRK (ref. 2). The determination of the rhodopsin–GRK1 structure is an important step forward in understanding GPCR–GRK interactions that might transform our models of the regulation of GPCR signalling.

The structure presented by Chen and colleagues is a tour de force owing to its 4-ångström resolution and the biological insights it yields. GRKs bind to GPCRs only transiently, such that capturing a GPCR–GRK complex to determine its structure poses a considerable challenge. Two previous attempts at elucidating the structure of GPCR–GRK complexes[3,4] involved various biochemical and biophysical methods, combined with negative-stain electron microscopy and modelling. Because the proposed structures were deduced rather than determined, they were, naturally, unable to explain certain established biochemical findings.

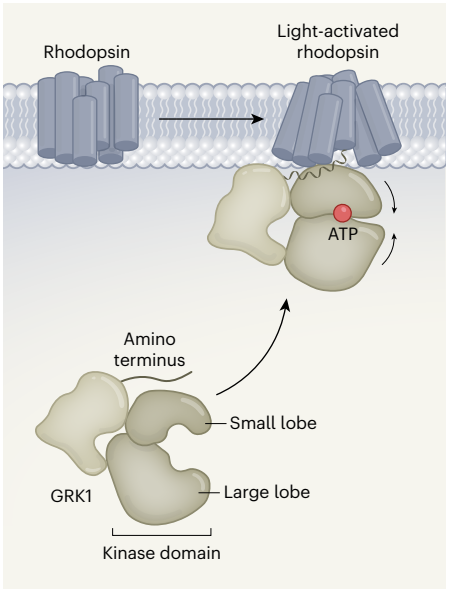

Figure 1 | A first step to switching off G-protein-coupled receptors. The switch-off (desensitization) of an active G-protein-coupled receptor protein (GPCR) is initiated when it binds to an enzyme called a G-protein-coupled receptor kinase (GRK). Chen *et al.*[1] used cryo-electron microscopy to determine the structure of the GPCR rhodopsin in complex with its GRK, GRK1. The inactive receptor does not attract GRK1 and, under baseline conditions, the small and large lobes of the kinase domain of GRK1 are misaligned, making it inactive. However, when rhodopsin is activated by a photon of light, the GRK amino terminus preferentially binds to active (rather than inactive) GPCRs by forming an α-helix and engaging the cavity that opens up between the membrane-spanning helices of the receptor. The receptor also binds to the kinase domain, 'pushing' its two lobes into alignment to create an active catalytic site that can bind to ATP molecules, which is necessary for transferring phosphates from them to the receptor.

In this context, the work by Chen *et al.*[1] presents the first real structure of a GPCR–GRK complex. To achieve this feat, the authors crosslinked the two proteins and used two different antibodies to make the complex stable for long enough to be characterized using cryo-electron microscopy. The resulting insights were certainly worth the effort.

Most GRKs, including GRK1, show a clear preference for activated GPCRs. Thus, conformational changes in receptors resulting from their activation must be recognized by GRKs to facilitate binding. The key question in GPCR biology is: what common features do active, as opposed to inactive, GPCRs have that 'attract' GRKs?

In the structure obtained by Chen *et al.*, the amino terminus of the GRK (which is specific to and shared by members of the GRK family) was found to insert into the cavity that opens up between the membrane-spanning helices of the receptor on GPCR activation[5]. This cavity is also engaged by other binding partners, such as transducin (the G protein activated by rhodopsin[6]) and visual arrestin-1 (ref. 7), that prefer active GPCRs over inactive ones. The N terminus of GRK1 forms an α-helix on binding to rhodopsin, as do the parts of transducin and arrestin-1 that engage the same receptor cavity (Fig. 1).

It was previously known that the basal phosphorylating activity of GRKs is low, but increases dramatically when GRK binds to a receptor. As such, the receptor itself activates the kinase, serving as both a substrate and an allosteric activator (that is, it activates the catalytic site of the kinase by binding to the enzyme at another site). The part of a GRK that is responsible for its phosphorylating activity — the kinase domain — consists of a small lobe and a large lobe. In the non-active GRK, the two lobes are misaligned, characteristic of inactive kinases generally[8]. For the receptor to activate GRKs[9], it must force the alignment of the two GRK lobes to create an active catalytic site. In the structure resolved by Chen *et al.*, the receptor interacts with the kinase domain, suggesting that the binding of the receptor to this domain pushes the lobes into alignment[1], thereby promoting the kinase activity (Fig. 1).

Naturally, many questions remain. First, rhodopsin is a specialized receptor that responds to light and is expressed almost exclusively in rod photoreceptor cells in the retina, enabling vision. Similarly, GRK1 is a specialized kinase with a high preference for rhodopsin over non-visual GPCRs. By contrast, five of the seven mammalian GRK subtypes show widespread expression in various cell types, and each interacts with diverse GPCRs. Thus, the structures of GRKs bound to different GPCRs are needed to identify common mechanisms of GPCR regulation.

Second, the structure of the GPCR–GRK complex described here represents the final

stage of the interaction between these proteins, and the process of binding requires further study. In most cases, GRKs need to attach more than one phosphate group to a receptor to enable tight arrestin binding. Therefore, a GRK probably has to bind to an active GPCR more than once to phosphorylate several amino-acid residues in the GPCR.

The dynamics of GRK binding to a GPCR, as well as those of GRK activation, remain to be determined, for example using biophysical methods. Characterizing the steps that the two proteins go through to achieve the complex revealed by this structure is crucial for the development of molecular tools to modify or control this process, which in turn could be used to channel GPCR signalling towards a desired direction.

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