highly industrialized areas of the Northern Hemisphere, whereas CFC destruction in the stratosphere (in both hemispheres) acts as the sink. This distribution of sources and sinks leads to concentration differences between the Northern and Southern hemispheres, which get smaller over time after emissions cease. The rate of exchange of air between the stratosphere and the underlying troposphere, and between the Northern and Southern hemispheres, both have a crucial role in driving the concentrations of ozone-depleting substances.

Montzka *et al.* have made a rigorous attempt to take into account natural variability in transport between the different regions of the atmosphere to calculate how it might have generated the observed levels of CFC-11, both by using simple 'box' models and by performing 3D computational simulations using comprehensive climate models that take into account atmospheric chemistry. They conclude that variations in transport alone cannot explain the recent slowing in the rate of decline in CFCs (Fig. 1), but that new emissions must have contributed.

Further evidence in support of their hypothesis comes from their observation of an increase in the difference between mean concentrations of atmospheric CFC-11 in the Northern and Southern hemispheres over the past few years — that is, the excess of CFC-11 in the Northern Hemisphere became larger. Moreover, after 2012, they observed the emergence of a strong relationship between the atmospheric concentration of CFC-11 and the concentrations of other ozone-depleting substances emitted as a result of human activities. These multiple lines of evidence support their conclusion that changes in atmospheric dynamics, especially in the stratosphere, must be acting in concert with renewed CFC-11 emissions to produce the observed concentrations. Such a careful analysis is crucial, because any claim of renewed — and therefore illegal — emissions will have political implications.

By taking into account the flow of the atmosphere to the locations at which the CFC-11 measurements were taken, Montzka *et al.* attribute the renewed emissions to east Asia. They also estimate that these emissions amount to about 13 gigagrams of CFC-11 per year (an increase of 25%) since 2012. However, the uncertainty in the inferred magnitude of fresh emissions might be up to 50%, mainly because of the difficulty in working out how air is transported between the stratosphere and the troposphere.

One way to reduce the uncertainties in the estimates and in the probable sources of the renewed emissions would be to use a high-resolution inverse-modelling approach, such as the one that has been used[8] in regional studies to attribute sources for emissions of hydrofluorocarbons (the chemicals that replaced CFCs and that are potential greenhouse gases). However, such an approach would probably need a denser global network of CFC measurements than is currently available. Moreover, regional inverse models would have to be extended to become global, high-resolution inverse models that include a well-resolved stratosphere and inter-hemispheric transport — which is a tall order, because few such comprehensive modelling systems are currently available that come close to the needed resolution.

Montzka and colleagues' study highlights once more that environmental regulations cannot be taken for granted and must be safeguarded, and that monitoring is required to ensure compliance. Continuous observations of the environment are crucial: not only satellite measurements that yield global coverage, but also readings from measurement networks across the world that yield more-accurate *in situ* data. Taken together with models that encompass both the troposphere and the stratosphere, such data can be used to make defensible inferences about the sources of polluting chemicals. ■

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STRUCTURAL BIOLOGY

Arresting vistas of arrestin activation

Computational and biochemical studies have revealed the mechanisms by which arrestin proteins are activated by G-protein-coupled receptors — potentially opening up broad avenues for drug discovery. SEE ARTICLE P.381 & LETTER P.452

BRIAN KRUMM & BRYAN L. ROTH

The largest family of drug targets in humans, and the principal therapeutic targets for at least 30% of approved medications in the United States, are the G-protein-coupled receptors[1] (GPCRs). When these transmembrane proteins detect extracellular agonist molecules, they transmit signals to the cell interior through G proteins inside the cells. The receptor is then sequentially phosphorylated to attenuate further signalling. The phosphorylated GPCR binds to an arrestin protein, and both undergo conformational changes that lead to the activation of arrestin-dependent cellular processes. Two papers in *Nature*, by Eichel *et al.*[2] and Latorraca *et al.*[3], now provide fresh insights into the mechanisms of arrestin activation and its consequences. Given the enormous potential of drugs that selectively target either G-protein or arrestin signalling, these findings might accelerate the development of safer and more-effective medications for a wide range of conditions.

Arrestins were first discovered in the visual system, where they bind to and inactivate a light-sensitive GPCR called rhodopsin[4]. They are now known to be almost universal regulators of GPCR signalling[5]. The binding of arrestin to GPCRs is enhanced by phosphorylation of the cytoplasmic tail — the carboxy terminus — of the receptors[6], and many models for arrestin binding and activation highlight the interaction of the protein with this region of the receptor.

It has been known since the 1990s that arrestins also bind at additional intracellular sites of several GPCRs, including the intracellular loops[7,8] (GPCRs have three intracellular loops that connect adjacent transmembrane regions of the receptor). In the past few years, structural[9] and biophysical studies[10] of arrestin bound to rhodopsin have clearly shown that arrestin binds to phosphorylated residues in the C terminus, as well as to a receptor core domain that includes intracellular loops 2 (IL2) and 3 (IL3). How these interactions lead to activation of arrestin and subsequent signalling has been obscure.

Latorraca and colleagues now cast light on this issue. The authors began by performing extensive computational simulations of the molecular dynamics of arrestin, both alone and during its interaction with various regions of rhodopsin. Their results indicate that 'active' arrestin fluctuates between active and inactive states, and that the receptor core domain and the phosphorylated tail can individually stabilize arrestin's active state. Moreover, the active

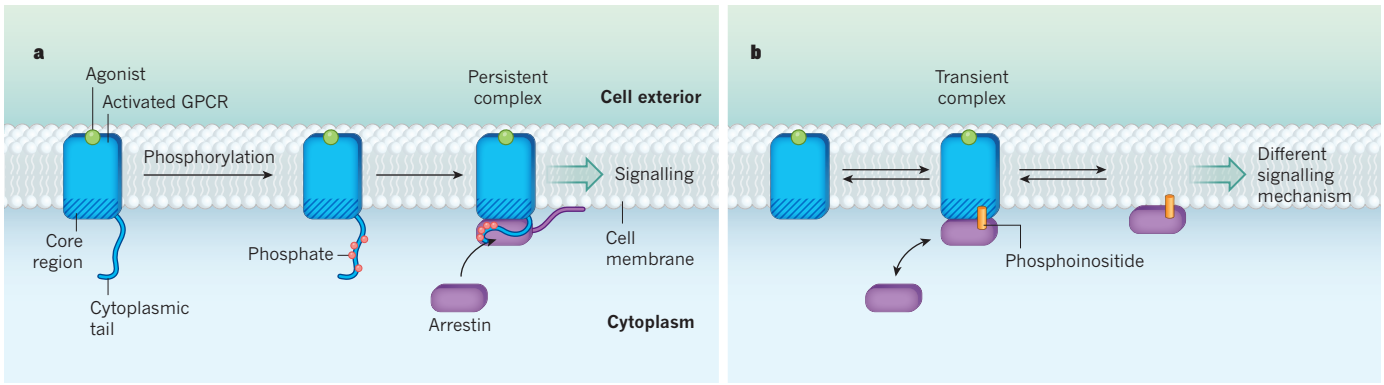

Figure 1 | Proposed mechanisms for arrestin activation. Arrestin proteins regulate the signalling of G-protein-coupled receptors (GPCRs) in cell membranes. Two papers[2,3] report computational and biochemical studies of GPCR–arrestin interactions, and propose mechanisms by which arrestin can be activated to redirect GPCR signalling. **a**, GPCRs are activated by the binding of agonist molecules. When the cytoplasmic tail of an activated GPCR is phosphorylated, arrestin is recruited to the receptor and interacts with both the cytoplasmic tail and the intracellular core region at the receptor's base. This activates arrestin, and leads to the formation of persistent GPCR–arrestin complexes that trigger arrestin-mediated signalling. A tail on arrestin that extends to proteins (not shown) in the cell membrane might also be needed for signalling. **b**, Alternatively, arrestin can interact transiently with the receptor core alone, in a process mediated by membrane-bound lipids known as phosphoinositides. The unbound, activated arrestin remains at the cell membrane, and goes on to trigger signalling through a different mechanism from that in **a**. (Figure adapted from Extended Data Fig. 9 in ref. 2.)

arrestin conformation is stabilized to an even greater extent when bound to both the core and the phosphorylated tail. The authors went on to perform further simulations of arrestin's interactions with the core domain. These suggest that a region of arrestin known as the finger loop stabilizes an interaction with the GPCR core domain, whereas interactions with IL2 and IL3 seem to trigger activation of arrestin.

A key insight from the molecular-dynamics simulations is that the two regions of arrestin to which the GPCR binds are allosterically coupled to each other: motions of the regions that bind the C terminus are coupled to motions of the regions that bind to the core, and vice versa. Importantly, the authors confirmed these computational findings directly in biophysical studies, using arrestin mutants tagged with fluorescent labels to monitor conformational changes at the protein surfaces that interact with the receptor's core and C terminus.

Latorraca and co-workers' simulations also suggest that the activated state of arrestin seems to persist even when the protein is not bound to the receptor. In their companion paper, Eichel *et al.* expand on and validate this prediction. The authors re-examined a phenomenon they had described previously[11], in which a GPCR known as the β1-adrenergic receptor (β1AR) interacts with arrestin only transiently when activated by an agonist. Arrestin then seems to be trafficked, independently of β1AR, to clathrin-coated endocytic structures (CCSs; vesicles that transport molecules into cells), where it can activate signalling proteins. This phenomenon is reminiscent of earlier findings that showed segregation of arrestins and GPCRs under some circumstances[12]. Such segregation was not anticipated by early models of GPCR–arrestin interactions, which posited that a stable GPCR–arrestin complex is essential for arrestin signalling[13].

Eichel and colleagues used a combination of microscopy techniques to show that transient engagement of the GPCR core, but not the C terminus, leads to prolonged accumulation of activated arrestin in CCSs. Such activation can be thought of as catalytic, because the GPCR activates arrestin but does not participate in subsequent downstream activation events — analogous to the way in which catalysts speed up reactions without directly taking part in them. The authors also demonstrated that the binding of phosphorylated lipids known as phosphoinositides at the cell membrane is essential for the capture of arrestin in CCSs after transient activation by GPCRs. The activated and GPCR-free arrestin can then activate downstream effectors such as ERKs.

The papers provide fresh insights that might accelerate the discovery and validation of biased GPCR agonists as therapeutic agents.

Taken together, these two papers open up a stunning new vista of GPCR–arrestin interactions in which an essential, bipartite interaction occurs through the receptor's core and phosphorylated C terminus (Fig. 1a). This engagement can lead to the formation of the persistent GPCR–arrestin 'scaffold' complexes that have frequently been observed and reported. An additional, transient form of this interaction leads to the catalytic activation of arrestin, which then accumulates in CCSs and triggers a specific form of arrestin signalling (Fig. 1b). It remains to be seen whether the mechanism of arrestin activation suggested by Latorraca and colleagues' molecular-dynamics simulations and by the structure of the arrestin–rhodopsin complex[9] is widely used by other arrestins and among GPCRs that do not undergo phosphorylation.

The idea of developing 'biased' GPCR agonists that selectively engage either G-protein or arrestin signalling[13] has tremendous potential for drug discovery. Such biased agonists have been proposed as safer and more-effective treatments for many disorders, including schizophrenia, chronic-pain conditions and heart disease[14,15]. By revealing cellular pathways for scaffold-based and catalytic arrestin activation, the current papers provide fresh insights that might accelerate the discovery and validation of biased GPCR agonists as therapeutic agents. ■

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