studies of the effect of network structure on a wide range of dynamic phenomena. Another paper was also pivotal: in 1999, Barabási and Albert proposed the 'preferential-attachment' network model[6], which highlighted that the probability distribution describing the number of connections that form between nodes in real-world networks is often characterized by 'heavy-tailed' distributions, instead of the Poisson distribution predicted by random networks. The broad spectrum of emergent behaviour and phase transitions encapsulated in networks that have clustered connectedness (as in Watts and Strogatz's model) and heterogeneous connectedness (as in the preferential-attachment model) attracted the attention of scientists from many fields.

A string of discoveries followed, highlighting how the complex structure of such networks underpins real-world systems, with implications for network robustness, the spreading of epidemics, information flow and the synchronization of collective behaviour across networks[7,8]. For example, the small-world connectivity pattern proved to be the key to understanding the structure of the World Wide Web[9] and how anatomical and functional areas of the brain communicate with each other[10]. Other structural properties of networks came under the microscope soon after[11–13], such as modularity and the concept of structural motifs, all of which helped scientists to characterize and understand the architecture of living and artificial systems, from subcellular networks to ecosystems and the Internet.

The current generation of network research cross-fertilizes areas that benefit from unprecedented computing power, big data sets and new computational modelling techniques, and thus provides a bridge between the dynamics of individual nodes and the emergent properties of macroscopic networks. But the immediacy and the simplicity of the small-world and preferential-attachment models still underpin our understanding of network topology. Indeed, the relevance of these models to different areas of science laid the foundation of the multidisciplinary field now known as network science.

Integrating knowledge and methodologies from fields as disparate as the social sciences, physics, biology, computer science and applied mathematics was not easy. It took several years to find common ground, agree on definitions and reconcile and appreciate the different approaches that each field had adopted to study networks. This is still a work in progress, presenting all the difficulties and traps inherent in interdisciplinary work. However, in the past 20 years a vibrant network-science community has emerged, with its own prestigious journals, research institutes and conferences attended by thousands of scientists.

By the 20th anniversary of the paper, more than 18,000 papers have cited the model, which is now considered to be one of the benchmark network topologies. Watts and Strogatz closed their paper by saying: "We hope that our work will stimulate further studies of small-world networks." Perhaps no statement has ever been more prophetic. ■

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STRUCTURAL BIOLOGY

A complex story of receptor signalling

G-protein–coupled receptors activate different G-protein types to trigger divergent signalling pathways. Four structures of receptor–G-protein complexes shed light on this selectivity. SEE ARTICLES P.547, P.553 & P.559 & LETTER P.620

MICHAEL J. CAPPER & DANIEL WACKER

About one-third of all drugs, including opioid painkillers, antihistamines and many antipsychotics, target members of a family of proteins called G-protein-coupled receptors (GPCRs)[1]. This reflects the fact that GPCRs are important in almost all aspects of human physiology, and suggests that many more of them will be promising drug targets for numerous diseases. GPCRs span the cell membrane and convert myriad extracellular signals, including neurotransmitter molecules, hormones, and even light, into a cellular response by activating cellular G proteins and other transducer proteins. Four papers[2–5] in this issue help to unravel the mystery of how GPCRs selectively activate a particular group of G proteins known as $G_{i/o}$, and provide clues that might aid the design of improved GPCR-targeting drugs.

Although more than 800 GPCRs are encoded in the human genome, they couple to only a small number of intracellular signal transducers, including 16 Gα proteins[6]. The latter proteins assemble with Gβ and Gγ proteins to form heterotrimeric G proteins. The G-protein complex disassembles on activation by GPCRs, whereupon the various subunits activate different signalling pathways. For instance, stimulatory Gα proteins (known as G_s) increase cellular levels of cyclic AMP molecules, which regulate various cellular processes. Structures of G_s-bound GPCRs have been reported[7,8] that have begun to elucidate the general activation mechanism of Gα proteins, and of G_s in particular. But much less is known about how GPCRs selectively activate inhibitory Gα proteins, which include G_{i1}, G_{i2}, G_{i3} and G_o, and are collectively known as $G_{i/o}$.

The four papers in this issue report structures of $G_{i/o}$-bound GPCRs obtained using cryo-electron microscopy: Koehl *et al.*[2] (page 547) report the structure of the μ-opioid receptor bound to G_{i1}; Draper-Joyce *et al.*[3] (page 559) describe the adenosine A_1 receptor in complex with G_{i2}; García-Nafría *et al.*[4] (page 620) report the $5HT_{1B}$ receptor bound to G_o; and Kang *et al.*[5] (page 553) reveal the structure of the light receptor rhodopsin in complex with G_{i1}. The G-protein activation cycle involves the binding and release of nucleotides to and from the G proteins, and all of the reported structures capture the receptors bound to the nucleotide-free state of their respective G proteins.

In some respects, the four structures are similar to those of the previously published GPCR–G_s complexes[7,8], probably because G_s- and $G_{i/o}$-containing complexes have the same overall conformation at the stage of the G-protein activation cycle captured by the structures. Nevertheless, the $G_{i/o}$-containing structures reveal striking differences at the receptor–G-protein interface when compared with the G_s-containing structures. For example, there are no interactions between the receptors and the Gβ subunits in the $G_{i/o}$-containing structures.

The four structures uncover several key interactions at the GPCR–$G_{i/o}$ interface mediated by the α5 helix — an α-helix structure in the carboxy terminus of Gα subunits. It is

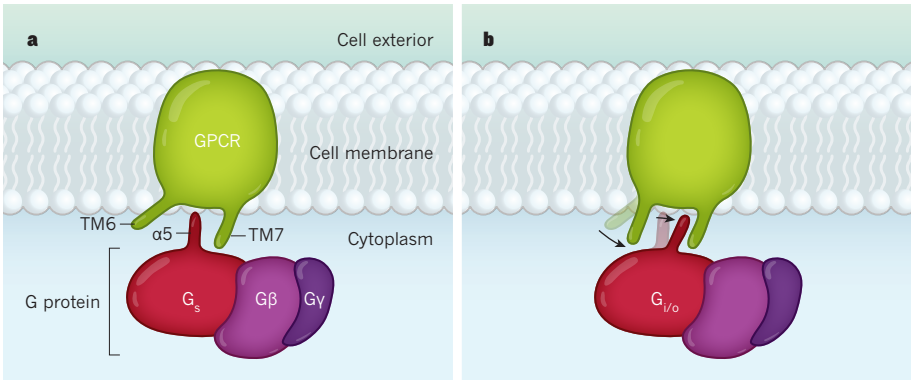

Figure 1 | Structural differences in complexes of G-protein-coupled receptors (GPCRs) with G proteins. GPCRs are transmembrane receptors that activate cellular signalling pathways by binding to G proteins, which have three subunits: α, β and γ. Stimulatory Gα proteins are known as G_s, whereas inhibitory Gα proteins (G_i and G_o proteins) are collectively known as $G_{i/o}$. Many GPCRs selectively bind to G_s or $G_{i/o}$, but the basis of this selectivity was unknown. **a**, This cartoon shows the positions of three α-helices in complexes of GPCRs with G_s-containing G proteins, based on previously reported structures[7,8]. TM6 and TM7 are transmembrane helices in the GPCR, whereas α5 is in the carboxy terminus region of G_s. **b**, Four papers[2–5] now report the structures of GPCRs in complex with $G_{i/o}$ proteins. Compared with **a**, the α5 helices are rotated and moved slightly towards TM7, and away from TM6. The outward displacement of TM6 is smaller than that in **a**. The smaller displacement of TM6 might block the binding of G_s proteins, thus explaining how GPCRs bind selectively to $G_{i/o}$.

known that the binding of this helix to the receptor's cytoplasmic site triggers conformational rearrangements in Gα that cause the release of a nucleotide (GDP) bound to Gα, initiating G-protein activation[9]. The positioning of the $G_{i/o}$ α5 helices in the new structures is different from that of the analogous helices in the GPCR–G_s complexes. Specifically, the $G_{i/o}$ α5 helices are rotated and translated slightly towards transmembrane helix (TM) 7 in the GPCR and away from TM6. Moreover, TM6 is displaced outwards from the receptor core by a smaller amount than occurs in the G_s-bound GPCRs (Fig. 1). The authors of all four papers therefore suggest that the smaller displacement of TM6 might preclude binding of G_s and help to explain how GPCRs can bind selectively to $G_{i/o}$ proteins.

The difference in the positioning of the α5 helices seems to be due to the G_s α5 helices containing bulkier amino-acid residues than those of the $G_{i/o}$ α5 helices. Moreover, Kang *et al.* analysed and compared the amino-acid sequences for TM6 in the G_s- and $G_{i/o}$-coupled receptors, and suggest that the different patterns of hydrophobic and hydrophilic residues observed in the two systems might affect the amount of displacement of TM6, and thus contribute to $G_{i/o}$ specificity.

Comparison of the four GPCR–$G_{i/o}$ structures reveals considerable structural plasticity at the interface. This is not surprising, given that $G_{i/o}$ proteins are engaged by hundreds of GPCRs that have diverse structures and sequences. Draper-Joyce *et al.* thus suggest that G-protein specificity is not necessarily encoded by evolutionarily conserved interactions between specific amino-acid residues, but might be based on "pocket complementarity", in which conformational rearrangements produce regions on the GPCR cytoplasmic site that are conducive to the binding of specific G proteins. Further evidence for this comes from the fact that all the structures of the GPCR–$G_{i/o}$ complexes display markedly smaller GPCR–G protein interfaces than do the structures of GPCR–G_s complexes. This is particularly pronounced for the 5-HT_{1B} receptor–G_o interface surface, which García-Nafría *et al.* report has an area of 822 square ångströms; this compares with 1,260 Å^2 and 1,135 Å^2 for the interfaces in the G_s-bound β_2-adrenergic[7] and adenosine A_{2A} receptors[8], respectively.

Finally, Koehl *et al.* report subtle, yet potentially crucial, differences in the conformations of G_{i1} and G_s that occur during the transition between the GDP-bound and the nucleotide-free states of the proteins. Given that GPCRs catalyse specific structural transitions in specific G-protein subtypes, it is tempting to speculate that the observed conformational differences might also contribute to the G-protein specificity of GPCRs.

"The G-protein specificity is not necessarily encoded by evolutionarily conserved interactions between specific amino-acid residues."

This body of work provides a key step towards delineating the molecular mechanisms by which GPCR conformations drive the activation of one signalling pathway in preference to another. Many more such structures are sure to follow, and will probably reveal structural hallmarks that drive GPCR coupling to other G proteins and signal transducers, such as arrestin proteins. However, as the authors of all four papers point out, these studies provide only snapshots of the G-protein activation pathway, and are thus incomplete. The coupling specificity of GPCRs depends on several factors not addressed by the new structures, including the pre-coupling of G proteins[10] (a preliminary step in which GPCRs and G proteins associate with each other, before actually coupling), and the binding of the GDP-bound form of G proteins[11]. The lifetimes of distinct receptor conformations can also determine the specificity of GPCRs for transducers[12,13], adding a kinetic dimension to GPCR signalling that needs to be considered.

A comprehensive molecular model of GPCR specificity for G proteins and transducers would not only improve our understanding of how GPCRs elicit complicated signals involving multiple, occasionally intersecting, pathways, but also facilitate the design of better drugs that target GPCRs. In particular, it could allow the structure-based design of drugs that selectively activate or inhibit particular signalling pathways, thereby making them safer and more effective than currently available therapeutics.

For example, the painkilling properties of opioid medications such as morphine are thought to arise from the activation of a G_i protein by the μ-opioid receptor, whereas coupling of the receptor to arrestin probably causes the drugs' addictive properties and the — often fatal — depression of respiratory functions. Much effort has thus been dedicated to designing opioid compounds that provide pain relief, but that reduce the risk of addiction or overdose. A flurry of structures of isolated GPCRs has already greatly facilitated the discovery of compounds that bind to the receptors, and that are useful tools for laboratory studies[14]. But it is the structures of GPCR signalling complexes that will allow the rational design of pathway-selective drugs. After all, GPCR signalling is, literally, a complex story. ■

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