G PROTEIN-COUPLED RECEPTORS

Spinning a native GPCR structure

An NMR spectroscopy technique has enabled the determination of the full-length structure of CXC chemokine receptor 1 (CXCR1) in its membrane-bound native state — the first time a G protein-coupled receptor (GPCR) structure has been determined without modification of its amino acid sequence and under physiological conditions.

CORBIS

The solid-state NMR technique used in this study (named rotationally aligned solid-state NMR) harnesses elements of two established strategies to improve the resolution of NMR signals from unoriented solid samples with 'magic angle spinning' and the physical or magnetic alignment of oriented samples. It was developed by the authors to cater for the unique properties of membrane proteins in liquid crystalline phospholipid bilayers, which include rapid molecular rotation about the bilayer normal.

To determine the structure of CXCR1, the native sequence of the receptor (residues 1–350) was uniformly labelled with ^{13}C and ^{15}N, purified and then refolded in proteoliposomes. The conditions used for NMR analysis were such that CXCR1 was able to bind to its endogenous ligand (interleukin-8) and couple with its G protein. High-resolution spectra obtained enabled the assignment of 97% of the backbone resonances (residues 20–325), but neither the carboxyl terminus nor the amino terminus was detectable. The spectral data, the amino acid sequence of CXCR1 and the helical framework of bovine rhodopsin (the prototypical GPCR) were then fed into various software programs (Chemical Shift-Rosetta, Rosetta and Xplor-NIH) to generate the three-dimensional structure of CXCR1.

CXCR1 has all the classical elements of a GPCR: namely, seven transmembrane helices, three extracellular loops and three intracellular loops. The data also reveal that CXCR1 has an eighth helix that lies along the membrane surface, which the authors suggest may have a role in stabilizing its conformation. By comparing the structure of CXCR1 with CXCR4 — the only other chemokine receptor determined so far (by the thermostabilization and X-ray crystallography technique) — it was shown that the structure of CXCR1 shares many features with the structure of CXCR4. For example, the presence of two disulphide bonds and the location of charged residues are similar in both structures, highlighting their importance in ligand binding and receptor activation. However, a key advantage of this NMR technique is that the intracellular loops of CXCR1 were preserved, thereby allowing full functionality of the receptor with regard to interacting with its G protein. By contrast, the modifications that were necessary to crystallize CXCR4 rendered this functionality defunct.

Together, the near-native conditions in which the CXCR1 structure has been determined have revealed important features of GPCR activation and signal transduction that will facilitate studies aimed at probing the molecular interactions between GPCRs and their G proteins, as well as between GPCRs and their ligands (be they endogenous or drugs).

Man Tsuey Tse

ORIGINAL RESEARCH PAPER Park, S. H. *et al.* Structure of the chemokine receptor CXCR1 in phospholipid bilayers. *Nature* 21 Oct 2012 (doi:10.1038/nature11580)