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Understanding GPCRs – from orphan receptors to novel drugs

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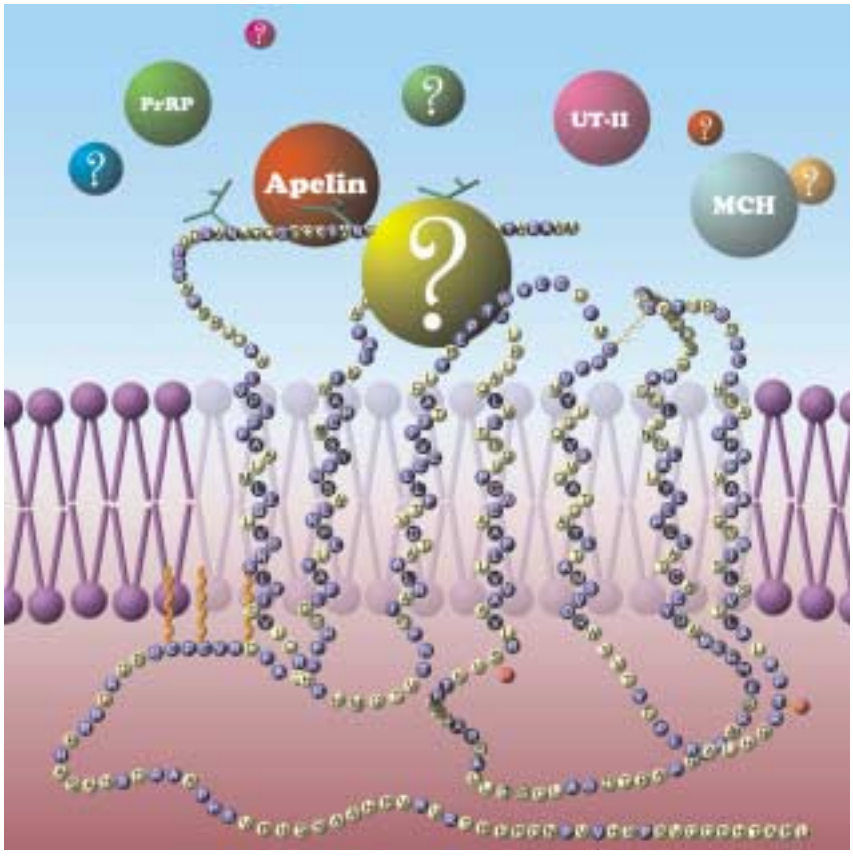
IBC's Sixth Annual International G-Protein-Coupled Receptor (GPCR) conference, *Taking Receptors from Orphans to Drugs* (11–13 June 2001, Litchfield, AZ, USA), saw the highest attendance yet in this series, with >160 delegates. This level of interest reflects how the near-completion of the Human Genome Project has defined what is required to understand the function of every GPCR, enabling many companies to contemplate orphan strategies. Identifying the genes is just the beginning; the big challenge is to attribute value to the receptors.

A major focus of the meeting was the use of high-throughput technologies to study receptors on a genome-wide scale for ligand identification and target validation. Recent advances in structural studies could provide new opportunities for rational ligand design. Another emerging trend is the discovery of receptor-interacting proteins, which complicate future considerations of GPCRs as drug targets.

Orphan strategies and target validation

A surprise from the human genome is that there are far fewer GPCRs than was previously predicted. Excluding sensory receptors, the total number of Family A, B and C GPCRs is ~400, of which only ~150 are 'orphans' for which the ligand is yet to be identified. The rationale for studying orphans is that, historically, GPCRs have proven to be excellent drug targets. Indeed, the list of recently liganded orphans, reviewed by Shelagh Wilson [GlaxoSmithKline (GSK), Harlow, UK], appears to justify the investment. Almost every case reveals an interesting biology, and clear new drug targets are emerging. For example, the newly identified receptors BLT2 and CysLT2, for the leukotrienes, LTD4 and LTB4, respectively, are potential targets for inflammation and asthma; the new purinergic P2T (P2Y12) receptor has an established role in platelet aggregation and thrombosis, and the two recently discovered neuropeptide FF receptors are targets for the treatment of pain. One of the most interesting recent pairings is the KiSS-1 metastasis-suppressor protein as a putative endogenous agonist of the Axor12/GPR54 orphan receptor. Links to cancer have been made for both ligand and receptor, and their coincident tissue distribution in placenta suggests a possible role in invasive growth.



Schematic representation of a G-protein-coupled receptor bound to an unknown ligand. Ligands shown include Apelin, melanin concentrating hormone (MCH), urotensin-II (UT-II) and prolactin-releasing peptide (PRP). Reproduced, with permission, from reference 2. Illustrators: Brett Clayton and Dennis Lee.

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Identification of a natural ligand is one of the fastest means to determine the biological role of a receptor, and companies such as Synaptic Pharmaceutical Corporation (Paramus, NJ, USA) and GSK have focused much of their efforts on this. Bioinformatics is the usual starting point, and many receptors have been 'deorphanized' solely through their homology to known receptors. Thus, MCH2 is identified as a new receptor for melanin-concentrating hormone, based on its close identity with SLC-1/MCH1. Edg-1, -3, -5 and -8 are all receptors for sphingosine-1-phosphate, and Edg-2, -4 and -7 are all receptors for lysophosphatidic acid. This year, six independent groups published the new histamine H4 receptor (identified through its similarity to H3), illustrating how competitive the orphan race has become. Receptor H4 is probably the eosinophil receptor described by Raible *et al.* in 1994 (Ref. 1), and is likely to trigger many new drug discovery projects.

With a good assay, fishing for ligands from small collections can be fruitful. Theresa Branchek (Synaptic) described the company's SNAP Discovery™ approach, in which orphans are subjected to a universal functional assay (UFA™) using the fluorescence imaging plate reader (FLIPR). Using this method, ligands have been found for 18 of 60 receptors, a remarkable success which Branchek puts down to the thoughtful composition of Synaptic's ligand bank. However, Vincent Dupriez (Euroscreen, Brussels, Belgium) made a case for using natural extracts as potential sources of ligands. Using Aequoscreen, a functional assay based on aequorin (a calcium-sensing protein from jellyfish) Euroscreen identified a fragment of hepatocellular carcinoma (HCC)-1 protein as a new high-affinity functional agonist of CC chemokine receptor-5 (CCR5). Dupriez emphasized that other approaches would not have achieved this identification.

In the absence of a ligand, value can be conferred on a receptor in various ways. Taqman (Applied BioSystems, Foster City, CA, USA), a high-throughput reverse transcription-polymerase chain reaction (RT-PCR) technique, enables quantitative expression profiling of GPCRs in different tissues. Incyte Genomics (Palo Alto, CA, USA) has created GPCR microarrays using combinations of synthetic polynucleotides, representing the largest number of GPCRs on glass, to date. These techniques allow comparative profiling of RNAs in, for instance, normal versus diseased tissue. Meanwhile, the resource held by Lifespan BioSciences (Seattle, WA, USA) centres on an impressive human-tissue bank of two million specimens covering every stage of all the major diseases. They are creating a proprietary database in which every GPCR is profiled in every cell type in normal and diseased tissue using immunohistochemistry. One of the most impressive companies is Deltagen (Menlo Park, CA, USA), whose high-throughput capacity to create knockout mice is currently running at one new knockout per day. All animals are subjected to a wide range of physiological tests and pathology and knocked out genes are replaced with *lacZ* to provide expression information, all of which (including pictures) is available through a versatile web interface.

Assay technologies for GPCR screening

Once receptors have been identified and prioritized, a central feature of most orphan programmes lies in the screening strategy. Newcomers to the field will be challenged by a burgeoning array of assay technologies, each claiming to be ideal for orphan receptors. The big decisions are whether to invest in an expensive piece of kit, or to put faith in an emerging technology that might not be fully validated.

According to Stephen Rees (GSK, Stevenage, UK), the ideal is a low cost, miniaturizable, generic, solvent-tolerant, non-radioactive single-step functional assay with low false-positive and negative hit rates. Unfortunately, such an assay doesn't exist yet! The FLIPR from Molecular Devices (Sunnyvale, CA, USA), as used to great effect by Synaptic and GSK, has the drawbacks of its expense and size. However, FLEXstation™, Molecular Devices' new bench-top alternative to FLIPR, is available at a fraction of the cost and produces excellent data that is ideal for assay development, lead optimization and academic research. The Aequoscreen (Euroscreen) gives an instant 'flash' response, and this also requires specialist detection equipment, such as the Hamamatsu FDSS6000 (Hamamatsu City, Japan), which allows plate additions directly on the detector. The versatile applications of this detector could make it a worthwhile purchase.

Many new techniques involve the use of fluorescent or luminescent fusion proteins. The BRET2™ (Biosignal Packard, Montréal, Québec, Canada) uses bio-fluorescence resonance-energy transfer from a luciferase to a green fluorescent protein (GFP), or similar fluorescent protein, to assay proximity between two proteins. Interactions between receptor–luciferase fusions and β-arrestin-GFP provide a moderately generic assay. Many variations on this theme are being presented, complicated by a profusion of alternative coloured fluorescent proteins and luciferases, many of which are not fully evaluated. For instance, data from Graeme Milligan (University of Glasgow, Glasgow, UK) indicated that some of the different coloured fluorescent proteins marketed by Clontech (Palo Alto, CA, USA) have a tendency to aggregate, making interpretation of receptor behaviour difficult.

Similarly, Biosignal's proprietary DeepBlueC coelenterazine (a new luciferase substrate) gives improved peak separation, but poor quantum efficiency. Assays based on fluorescent fusion-proteins are attractive in that they can provide additional information about a target, such as movement within a cell. They also require investment in new

equipment that might be expensive. The impressive LeadSeeker™ Cell Analysis System (Nycomed-Amersham, Little Chalfont, UK) is top of the price list for new equipment, and features a high-resolution confocal-based system that can handle ~30,000 compounds a day. On-line image analysis enables rapid conversion of images into data. Cheaper machines are available, such as the Arrayscan™ (Cellomics, Pittsburgh, PA, USA), with each one suited to different related applications. Time will tell which approaches provide the best advantages in this crowded and competitive area.

New trends in GPCR research

Besides the human genome, the most significant breakthrough in GPCR research in the past year was the 2.8 Å resolution crystal structure of bovine rhodopsin, presented by Ron Stenkamp (University of Washington, Seattle, WA, USA). The high B factor of the loop between the transmembrane helices V and VI suggests a site of dynamic movement. Philip Yeagle (University of Connecticut, Storrs, CT, USA) presented a novel alternative approach by solving the structures of synthetic peptides corresponding to each individual transmembrane and loop region of bacteriorhodopsin. The fragments were assembled into a three-dimensional model that superimposed rather well onto the bacteriorhodopsin X-ray crystal structure, and took only a few months to obtain.

The identification of novel receptor-interacting proteins is also challenging preconceptions regarding receptor behaviour. The interaction of transcription factors with GABA-B, identified by Julia White (GSK, Stevenage, UK), using yeast two-hybrid screens, suggests novel signalling mechanisms. Calcyon, a single transmembrane protein identified by Clare Bergson (Medical College of Georgia, Augusta, GA, USA), physically associates with dopamine D1 to stimulate intracellular calcium release. However, the blossoming literature on receptor dimerization was reviewed by Graeme Milligan with a note of caution. There is little doubt that many receptors can form homo- and/or heterodimers, however Milligan's group believes that some of the most popular techniques to show this, such as co-immunoprecipitation, can lead to artefactual results. In short, 'if you look hard enough, everything comes down with everything!' As time goes on, clear evidence of functional significance will be required for a dimerization event to be credible.

The meeting was one of the most stimulating IBC GPCR conferences thanks to the good attendance and the sense of an exploding global interest in orphan receptors. The race is clearly on to identify ligands and disease associations. The winners stand to gain intellectual property and the chance to develop the best and most novel drugs.

Reference

1 Raible, D.G. *et al.* (1994) Pharmacologic characterization of a novel histamine receptor on human eosinophils. *Am. J. Respir. Crit. Care Med.* 149, 1506–1511

2 Lee, D.K. *et al.* (2001) Orphan G-protein-coupled receptors in the CNS. *Curr. Opin. Pharmacol.* 1, 31–39