G-protein-coupled receptors at a glance

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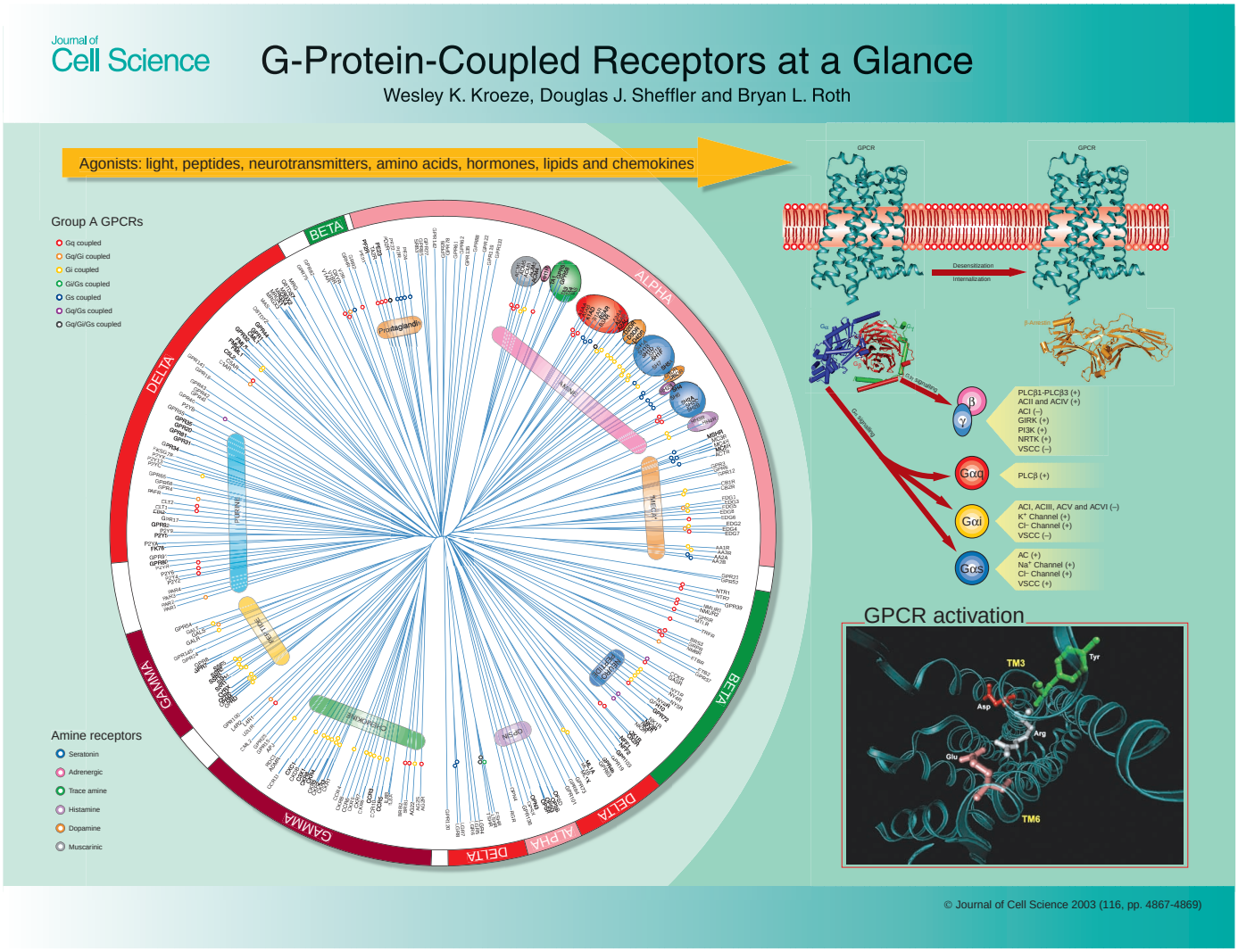
G-protein-coupled receptors (GPCRs) constitute a large and diverse family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. They are among the largest and most diverse protein families in mammalian genomes. On the basis of homology with rhodopsin, they are predicted to contain seven membrane-spanning helices, an extracellular N-terminus and an intracellular C-terminus. This gives rise to their other names, the 7-TM receptors or the heptahelical receptors. GPCRs transduce extracellular stimuli to give intracellular signals through interaction of their intracellular domains with heterotrimeric G proteins, and the crystal structure of one member of this group, bovine rhodopsin, has recently been solved (Palczewski et al., 2000).

The presence of GPCRs in the genomes of bacteria, yeast, plants, nematodes and other invertebrate groups argues in favor of a relatively early evolutionary origin of this group of molecules. The diversity of GPCRs is dictated both by the multiplicity of stimuli to which they respond, as well as by the variety of intracellular signalling pathways they activate. These include light, neurotransmitters, odorants, biogenic amines, lipids, proteins, amino acids, hormones, nucleotides, chemokines and, undoubtedly, many others. In addition, there are at least 18 different human Gα proteins to which GPCRs can be coupled (Hermans, 2003; Wong, 2003). These Gα proteins form heterotrimeric complexes with Gβ subunits, of which there are at least 5 types, and Gγ subunits, of which there are at least 11 types (Hermans, 2003).

Estimates of the number of GPCRs in the human genome vary widely. Based on their sequences, as well as on their known or suspected functions, there are estimated to be five or six major classes of GPCR. In a recent analysis of the



(See poster insert)

GPCRs in the human genome, more than 800 GPCRs were listed (Fredriksson et al., 2003). Of this total, 701 were in the rhodopsin family (type A) and, of these, 241 were non-olfactory (Fredriksson et al., 2003). According to this analysis, there are approximately 460 type A olfactory receptors, although estimates range from 322 (Glusman et al., 2001; Takeda et al., 2002) to 900 (Venter et al., 2001), of which 347 have already been cloned (Zozulya et al., 2001). This large number of olfactory receptors accounts for the ability of humans to detect a wide variety of exogenous (olfactory) ligands. A study similar to that of Fredriksson et al. (Fredriksson et al., 2003) identified 367 human endoGPCRs and 392 mouse endoGPCRs (Vassilatis et al., 2003); the term endoGPCR refers to GPCRs for endogenous (non-olfactory) ligands. In view of the known existence of alternatively spliced variants and editing isoforms of GPCRs, it is likely that the true number of GPCRs will never be known and is much higher than estimated.

The tree shown illustrates the relationships among the primary protein sequences of 274 type A rhodopsin-like GPCRs; for clarity, the secretin family receptors (of which there are 15), the adhesion receptor family (24), the glutamate receptor family (15) and the frizzled/taste2 receptor family (24) were not included. To construct this tree, the list of receptors used by Fredriksson et al. (Fredriksson et al., 2003) served as a starting point, and newly discovered 'orphan' receptors were added to the list (http://kidb.bioc.cwru.edu/rothlab/jalview/viewJalView.html).

The protein sequence of each receptor was obtained, and the sequences of the N- and C-termini, which are of variable length and show little similarity among the receptors, were trimmed manually. The protein sequences were then aligned and the tree was drawn using the ClustalW server (http://clustalw.genome.ad.jp). An alignment file is available at http://kidb.bioc.cwru.edu/rothlab/jalview/viewJalView.html and can be examined with a more viewer-friendly interface using the JalView applet at that site. The G-protein-coupling information in the poster is derived from the review by Wong (Wong, 2003).

The groupings of the receptors in the poster are thus similar, but not identical, to those of Fredriksson et al. (Fredriksson et al., 2003). For example, Fredriksson's α, β, γ and δ groups, which appear to be 'monophyletic' in their tree, were not monophyletic in ours; this is likely to be due to slight differences in the options used in the two alignments, and the relative imprecision of the location of the roots of the branches in both trees. Interestingly, the orphan receptors GPR57 and GPR58 were grouped with the trace amine receptors, and comparison of their sequences indicates that these orphans probably constitute the human equivalent of the type 2 trace amine receptors of rodents. Thus, trees of this type may serve to help in the process of 'de-orphanizing' receptors.

How do GPCRs work?

The first step in signal transduction is ligand binding. The nature of GPCR ligand-binding sites is best studied by a combination of site-directed mutagenesis, molecular modelling of the receptors and screening of large numbers of potential ligands. Our group curates the largest publicly accessible database of ligand affinities as part of the Psychoactive Drug Screening Program (http://pdsp.cwru.edu), while the most comprehensive database of the effects of mutations in GPCRs upon ligand binding can be found at http://www-grap.fagmed.uit.no. Agonist binding is followed by a change in the conformation of the receptor that may involve disruption of a strong ionic interaction between the third and sixth transmembrane helices (Ballesteros et al., 2001; Shapiro et al., 2002), which facilitates activation of the G-protein heterotrimer. Depending on the type of G protein to which the receptor is coupled, a variety of downstream signalling pathways can be activated (reviewed by Marinissen and Gutkind, 2001; Neves et al., 2002). Signalling is then attenuated (desensitized) by GPCR internalization, which is facilitated by arrestin binding (Ferguson, 2001). Signalling, desensitization and eventual resensitization are regulated by complex interactions of various intracellular domains of the GPCRs with numerous intracellular proteins (Hall and Lefkowitz, 2003; Bockaert et al., 2003).

Although many studies have used β-adrenergic receptors as prototypical GPCRs, it has become increasingly clear that much more can be learned by systematic study of other receptors. Our studies of the serotonin 5-HT2A receptor, for instance, showed that GPCR internalization and desensitization can occur by arrestin-independent pathways (Bhatnagar et al., 2001; Gray et al., 2003) and similar findings have been reported for other GPCRs (Lee et al., 1998). Interactions of GPCRs with other proteins, including cytoskeletal components such as PSD-95 (Hall and Lefkowitz, 2002; Xia et al., 2003), are increasingly being found to be important for regulating the activity, targeting and trafficking of GPCRs.

GPCRs are attractive targets for magic bullets

Although the biology of GPCRs is certainly intriguing, their ultimate importance is underscored by the fact that at least one third (Robas et al., 2003) and perhaps as many as half (Flower, 1999) of currently marketed drugs target GPCRs, although only 10% of GPCRs are known drug targets (Vassilatis et al., 2003). As new functions for GPCRs are discovered, especially for the orphan GPCRs for which no function is currently known, the number of drugs that target GPCRs can only be expected to increase. This is a focus of intense research effort, both in academia and in industry.

In addition to biological studies of the types summarized above, much excitement remains in the field because of the continuing de-orphanization of GPCRs and the subsequent elucidation of their pharmacology and physiology.

Once a large enough panel of GPCRs has been obtained and comprehensively characterized, a systematic analysis of the 'receptorome' (the portion of the proteome encoding receptors) can yield important discoveries. We have used such an approach to discover the molecular mechanisms responsible for serious drug side-effects – for example, phen/fen-induced heart disease

(Rothman et al., 2000) and weight gain associated with the use of atypical antipsychotics (Kroeze et al., 2003). Additionally, screening the receptorome has been used to elucidate the actions of natural compounds and to obtain validated molecular targets for drug discovery (e.g. Roth et al., 2002).

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