Family-B G-protein-coupled receptors

Protein family review

Family-B G-protein-coupled receptors

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Summary

All G-protein-coupled receptors (GPCRs) share a common molecular architecture (with seven putative transmembrane segments) and a common signaling mechanism, in that they interact with G proteins (heterotrimeric GTPases) to regulate the synthesis of intracellular second messengers such as cyclic AMP, inositol phosphates, diacylglycerol and calcium ions. Historically, GPCRs have been classified into six families, which were thought to be unrelated; three of these are found in vertebrates. Recent work has identified several new GCPR families and suggested the possibility of a common evolutionary origin for all of them. Family B (the secretin-receptor family or 'family 2') of the GPCRs is a small but structurally and functionally diverse group of proteins that includes receptors for polypeptide hormones, molecules thought to mediate intercellular interactions at the plasma membrane and a group of *Drosophila* proteins that regulate stress responses and longevity. Family-B GPCRs have been found in all animal species investigated, including mammals, *Caenorhabditis elegans* and *Drosophila melanogaster*, but not in plants, fungi or prokaryotes. In this article, I describe the structures and functions of family-B GPCRs and propose a simplified nomenclature for these proteins.

All G-protein-coupled receptors (GPCRs) are thought to have the same molecular architecture, consisting of seven transmembrane domains (7TM), three extracellular loops (EC1, EC2, EC3), three intracellular loops (IC1, IC2, and IC3), an amino-terminal extracellular domain and an intracellular carboxyl terminus. This topology is predicted from the analysis of hydropathy profiles and from a limited amount of experimental evidence, most importantly from the crystal structure of the visual pigment rhodopsin [1], a GPCR for which the activating stimulus is light. GPCRs were classified by Kolakowski [2] into six families: the rhodopsin family (A), the secretin-receptor family (B), the metabotropic glutamate receptor family (C), fungal pheromone P- and α-factor receptors (D), fungal pheromone A- and M-factor receptors (E) and cyclic-AMP receptors from *Dictyostelium* (F). Note that families A-C are named after well-known members and contain other members that are not rhodopsins, secretin receptors or metabotropic glutamate receptors, respectively. The 7TM topology was thought to have evolved independently in each family, representing a "remarkable example of a molecular convergence" [3]. More recent work has identified several new families of putative GPCRs and provided evidence that some or all of these proteins may have a common evolutionary origin. Josefsson [4] analyzed distant sequence homologies between GPCR families and suggested that the GPCRs fall into three superfamilies. Graul and Sadée [5] confirmed and extended this analysis to propose a common evolutionary origin for most of the known GPCRs (Figure 1).

In 1991, Nagata's group reported the expression cloning of the receptor for the gut hormone secretin [6]. The secretin receptor was predicted to have the 7TM topology characteristic of GPCRs and was capable of regulating intracellular concentrations of cyclic AMP by coupling to adenylate cyclase. But the amino-acid sequence of the protein was only very distantly homologous to that of other known GPCRs and the secretin receptor became, thus, the prototype member of family B [2].

Gene organization and evolutionary history

Since 1991, at least 33 human genes encoding members of family B have been identified (Figure 2). Family-B GPCRs

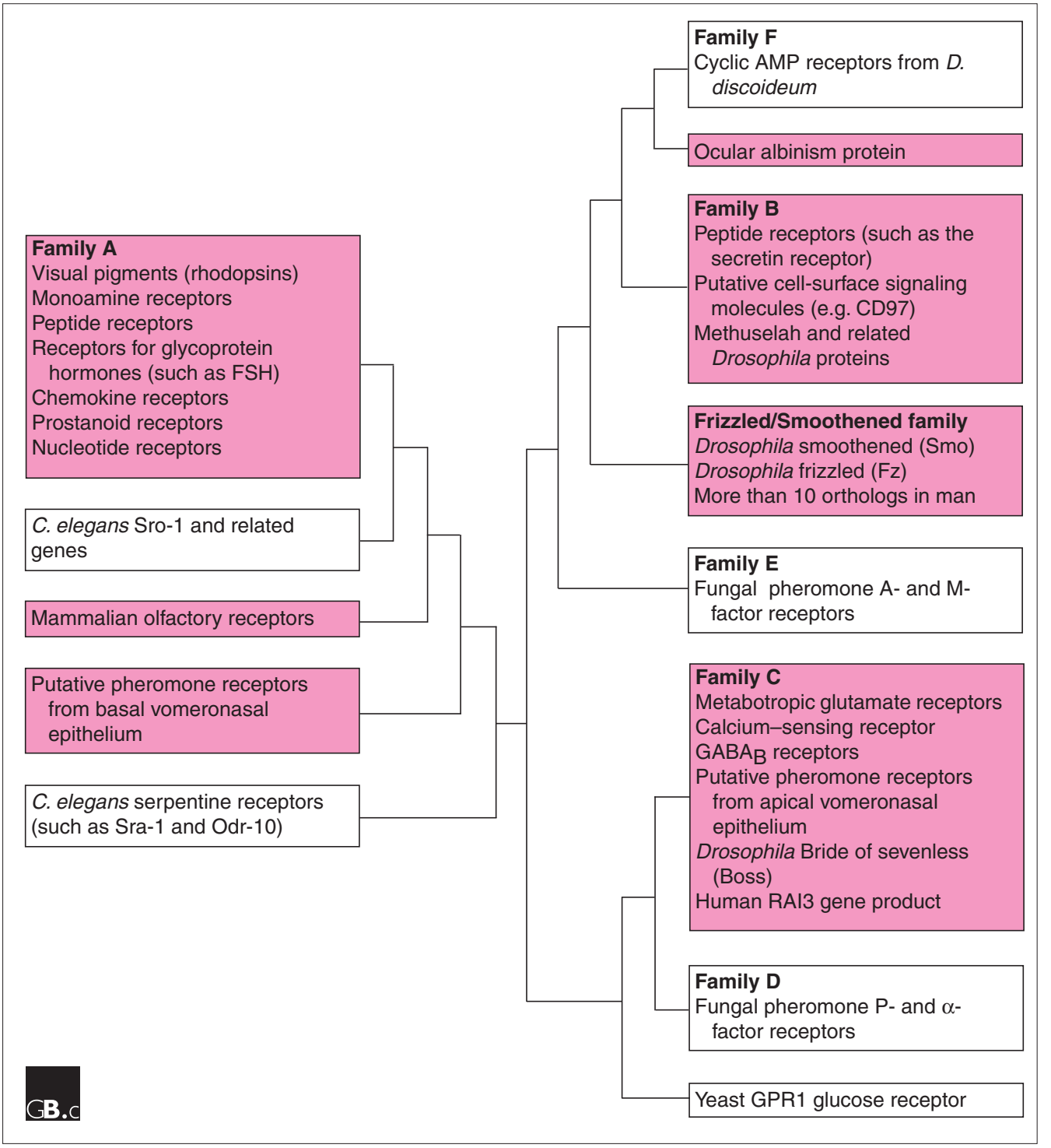

Figure 1
An overview of the relationships of GPCRs, based on the analysis reported by Graul and Sadée [5]. Families of GPCRs with representatives in mammals are highlighted in pink.

have been identified throughout the animal kingdom, but not in plants, in fungi or in prokaryotes. There are at least 18 genes encoding family-B GPCRs in *Drosophila* and six in *Caenorhabditis elegans*. Within family B, three distinct subfamilies of GPCRs can be identified. I propose here a simplified nomenclature - B1, B2 and B3 - for these subfamilies.

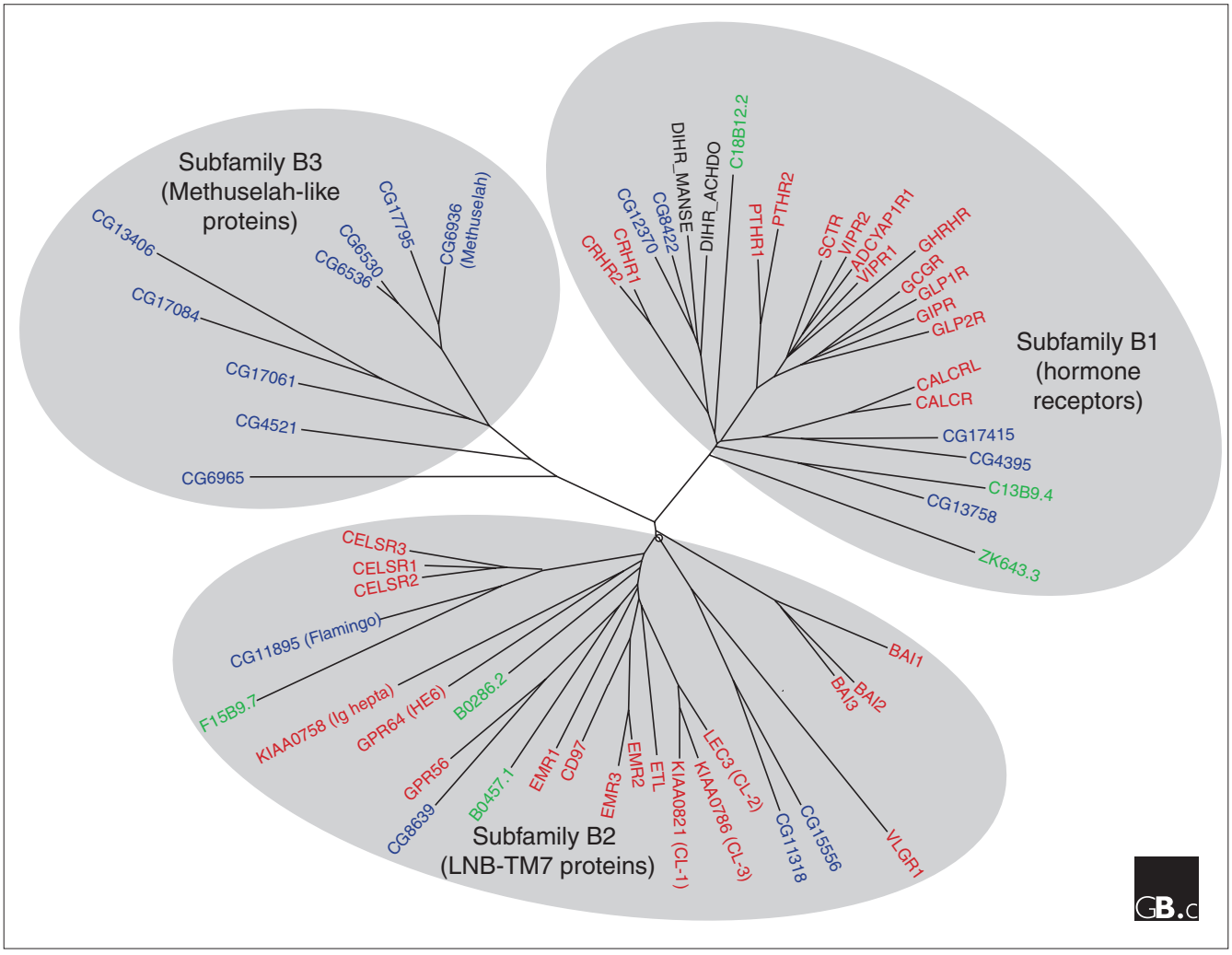

Figure 2
A phylogenetic tree of family-B GPCRs. Human (red), *Drosophila* (blue) and *C. elegans* (green) genes are named according to the HUGO human gene nomenclature database [45], the Celera Publication Site [46] and WormBase [47], respectively. The phylogenetic tree was constructed using an all-against-all comparison of the protein sequences [48].

Subfamily B1

Subfamily B1 consists of the classical hormone receptors, encoded by 15 genes in humans, with at least five putative members in *Drosophila* and three in *C. elegans*. The ligands for receptors in this family (see Table 1) are polypeptide hormones of 27–141 amino-acid residues; nine of the mammalian receptors respond to ligands that are structurally related to one another (glucagon, glucagon-like peptides (GLP-1, GLP-2), glucose-dependent insulinotropic polypeptide (GIP), secretin, vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and growth-hormone-releasing hormone (GHRH)). Three of the subfamily-B1 ligands (glucagon, GLP-1 and GLP-2) are synthesized by post-translational processing of a single polypeptide precursor, proglucagon. All members of this subfamily have been shown to be capable of regulating intracellular concentrations of cyclic AMP by coupling to adenylate cyclase through the stimulatory G protein G_S. Some members of the subfamily are capable of signaling through additional G-protein-coupled signaling pathways, for example though activation of phospholipase C.

Subfamily B2

Subfamily B2 consists of a large number of family-B GPCRs with long extracellular amino termini, containing diverse structural elements, linked to the core 7TM motif. Members of this subfamily have been given various names: EGF-TM7 receptors, to reflect the presence of epidermal growth factor (EGF) domains within the amino-terminal regions of many of these proteins [7]; LN-TM7 receptors, denoting seven-transmembrane proteins with a long amino terminus [8]; and LNB-TM7 receptors, to denote 'long amino terminus,

Table 1

Functions of hormone receptors in family B1

Gene name	Gene product	Ligand(s), with the name of the precursor gene in italics, where it is different	Principal functions controlled by the receptor
SCTR	Secretin receptor	Secretin (*SCT*)	Secretion of HCO_3^-, enzymes, and K^+ by the pancreas [41]
VIPR1	$VPAC_1$ receptor	VIP, PACAP (*ADCYAP1*)	Neuromodulation, T-cell differentiation
VIPR2	$VPAC_2$ receptor	VIP, PACAP (*ADCYAP1*)	Circadian rhythms [42]
ADCYAP1R1	PAC_1 receptor	PACAP (*ADCYAP1*)	Glucose homeostasis, nociception, learning and memory, circadian rhythms [29-33]
GCGR	Glucagon receptor	Glucagon (*GCG*)	Hepatic glycogenolysis and gluconeogenesis, pancreatic secretion of insulin [41]
GHRHR	Growth hormone releasing hormone (GHRH) receptor	GHRH	Release of pituitary growth hormone [41]
GLP1R	Glucagon-related peptide 1 (GLP-1) receptor	GLP-1 (*GCG*)	Pancreatic secretion of insulin and glucagon [41]
GLP2R	Glucagon-related peptide 2 (GLP-2) receptor	GLP-2 (*GCG*)	Proliferation of intestinal mucosal villus epithelium [41]
GIPR	Gastric inhibitory polypeptide (GIP) receptor	GIP	Pancreatic secretion of insulin [41]
CRHR1	corticotropin releasing factor 1 (CRF1) receptor	CRF (*CRH*), urocortin (*UCN*)	Secretion of ACTH [21]
CRHR2	Corticotropin releasing factor 2 (CRF2) receptor	Urocortin (*UCN*), urocortin II, urocortin III, perhaps CRF (*CRH*)	Stress-related autonomic, neuroendocrine, and behavioral function [21]
PTHR1	Parathyroid hormone 1 (PTH1) receptor	Parathyroid hormone, PTH-related peptide (*PTHLH*)	Calcium homeostasis in bone and kidney; skeletal, pancreatic, epidermal and mammary gland differentiation [43,44]
PTHR2	Parathyroid hormone 2 (PTH2) receptor	TIP39 [22]	Unknown
CALCRL	Calcitonin receptor-like receptor	Calcitonin gene related peptide (CGRP: *CALCA, CALCB*)*; adrenomedullin†‡(*ADM*)	Vascular tone
CALCR	Calcitonin receptor	Calcitonin†‡ (*CALCA*) amylin*‡ (*IAPP*) CGRP* (*CALCA, CALCB*)	Calcitonin: calcium homeostasis in bone and kidney; amylin: postprandial glucagon secretion, nutrient transit through the stomach

*When formed with accessory protein RAMP1 [23,24]; †when formed with accessory protein RAMP2 [23,24]; ‡when formed with accessory protein RAMP3 [23,24]. VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; ADCYAPR1R1, adenylate cyclase activating polypeptide 1 receptor 1.

family B' [9]. The prototype members of this subfamily were an EGF-module-containing, mucin-like hormone receptor (EMR1) isolated from a human neuroectodermal cDNA library [10] and the leukocyte cell-surface antigen CD97 [11]. EMR1 and CD97 are closely related in structure to two other human proteins (EMR2 and EMR3), and all members of this group are highly expressed in the immune system.

Subfamily B2 also includes the following subgroups. Firstly, the calcium-independent receptors for α-latrotoxin, a potent presynaptic neurotoxin from the venom of the black widow spider that stimulates massive neurotransmitter release leading to nerve-terminal degeneration. Three genes encoding calcium-independent latrotoxin receptors (CL-1 CL-2 and CL-3, also called lectomedin-2, lectomedin-3 and lectomedin-1) have been identified. Secondly, the brain-specific angiogenesis inhibitors 1, 2 and 3 (BAI1, BAI2, BAI3), a group of proteins that have been implicated in the vascularization of glioblastomas. Thirdly, the protein encoded by the *Drosophila* gene *flamingo*, also known as *starry night*, and its orthologs in humans (the cadherin EGF LAG seven-pass G-type receptors Celsr1, Celsr2 and Celsr3) and in *C. elegans* (F15B9.7). Finally, the subfamily includes a fourth, diverse, group of receptors that contain some motifs common to

receptors in subfamily B2 but are otherwise structurally unrelated (human epididymis 6 (HE6), EGF-TM7-latrophilin-related protein (ETL), the immunoglobulin-repeat-containing receptor Ig hepta, G-protein-coupled receptor 56 (GPR56) and very large G-protein-coupled receptor 1 (VLGR1)). Analysis of the sequenced human genome (1 April 2001 freeze [12]) indicates that there are at least 18 human genes encoding members of subfamily B2, and there are at least four in *Drosophila* and three in *C. elegans*. The structure and functions of members of subfamily B2 have been reviewed recently by Stacey *et al.* [9].

Subfamily B3

The prototype of a third group (subfamily B3) of family-B GPCRs is *methuselah* (*mth*), a gene isolated in a screen for single-gene mutations that extended average lifespan in *D. melanogaster* [13]. The gene encodes a polypeptide that displays sequence similarity to other family-B GPCRs solely within the TM7 region. A least eight paralogs of *methuselah* are encoded within the *Drosophila* genome sequence. There are no obvious homologs of *methuselah* within the sequences of the human or *C. elegans* genomes.

Family-B GPCRs are dispersed over 13 of the human chromosomes. A few of the genes are clustered in a manner that suggests that they may have arisen through ancestral gene duplications. Thus, genes encoding CD97, EMR2, EMR3 and the latrotoxin receptor CL-1 are found within an approximately 700 kilobase (kb) region on chromosome 19p13.13, and EMR1 lies about 9 megabases (Mb) telomeric to this. ETL and latrotoxin receptor CL-3 are closely linked on 1p31.1, and genes encoding the CRF2 receptor, the GHRH receptor and the PAC_1 receptor are clustered in an approximately 450 kb region on chromosome 7p14.3. The GHRH and PAC_1 receptors are very likely to have evolved as the result of gene duplication, as they are adjacent in the genome and there is evidence for a single precursor protein encoding the ligands GHRH and PACAP in lower vertebrates.

Characteristic structural features

The characteristic feature of all family-B GPCRs is the 7TM motif (Figure 3), which is distantly related to comparable regions of some other GPCR families but much more highly conserved within family B. Conserved cysteine residues within extracellular loops EC1 and EC2 probably form a disulphide bridge, by analogy with family-A GPCRs, in which this feature is also conserved [1]. In contrast to family-A GPCRs, however, many of which appear to rely on internal hydrophobic sequences for targeting to the plasma membrane, most family-B GPCRs appear to have an amino-terminal signal peptide. Studies using site-directed mutagenesis and the construction of chimeras between hormone receptors in family B have shown that the amino-terminal extracellular domain is essential for ligand binding but that the transmembrane domains and associated extracellular loop regions of the receptors provide critical information necessary for specific interaction with ligands. All of the hormone receptors in family B contain a conserved region within the amino-terminal extracellular domain close to TM1 that may play a role in ligand binding (Figure 4). This putative hormone-binding domain, which contains three or four conserved cysteine resides and two conserved tryptophan residues, also contains an aspartate, which may be critical for ligand binding in the hormone receptors (see the section on important mutants, below). Splice variation in this region of the PAC_1 receptor has been shown to influence ligand-binding specificity and affinity [14]. The putative hormone-binding domain is also present in over 50% of subfamily-B2 proteins, but the aspartate residue implicated in hormone binding is not conserved in these proteins.

As in family-A GPCRs, the intracellular loop IC3 of family-B GPCRs contains the major determinants required for specific G-protein coupling: splice variation in this region can give rise to receptors that differ in their ability to couple to different G proteins [15]. Alternative splicing in IC1 of the CRF1 [16] and calcitonin [17] receptors has also been reported to influence G-protein coupling. Many GPCRs in family B exhibit some agonist-independent (constitutive) activity in the wild-type form. In addition, naturally occurring point mutations of a histidine at the junction between IC1 and the TM2 and a threonine in TM6 of the human PTH1 receptor have been reported to be associated with constitutive activation of the PTH1 receptor in Jansen-type metaphyseal chondrodysplasia, a rare genetic form of short-limbed dwarfism caused by delayed endochondral bone formation that is associated with severe hypercalcemia and hypercalciuria [18]. Mutagenesis of the equivalent threonine in TM6 of the GIP receptor [19] and of the equivalent histidine at the junction between IC1 and TM2 of the $VPAC_1$ receptor [20] also leads to constitutive activity.

Almost all of the receptors in subfamily B2 contain two structural features in addition to the 7TM region shared by all members of family B: firstly, a mucin-like region rich in serine and threonine residues, and secondly, a conserved cysteine-rich proteolysis domain (Cys box), also referred to as the GPCR proteolysis site (GPS), that is known to be cleaved in latrotoxin receptor CL-1, in CD97 and in the ETL protein to generate a receptor with two subunits. It is possible that proteolytic cleavage may prove to be important for the function of many of the receptors in subfamily B2.

Receptors in subfamily B2 contain a variety of additional structural motifs in their large amino-terminal extracellular domains that suggest a role for this domain in cell-cell adhesion and signaling. These include EGF domains (in Celsr1, Celsr2, Celsr3, EMR1, EMR2, EMR3, CD97 and Flamingo), laminin and cadherin repeats (in Flamingo and its human orthologs Celsr1, Celsr2 and Celsr3), olfactomedin-like domains (in the latrotoxin receptors), thrombospondin type 1

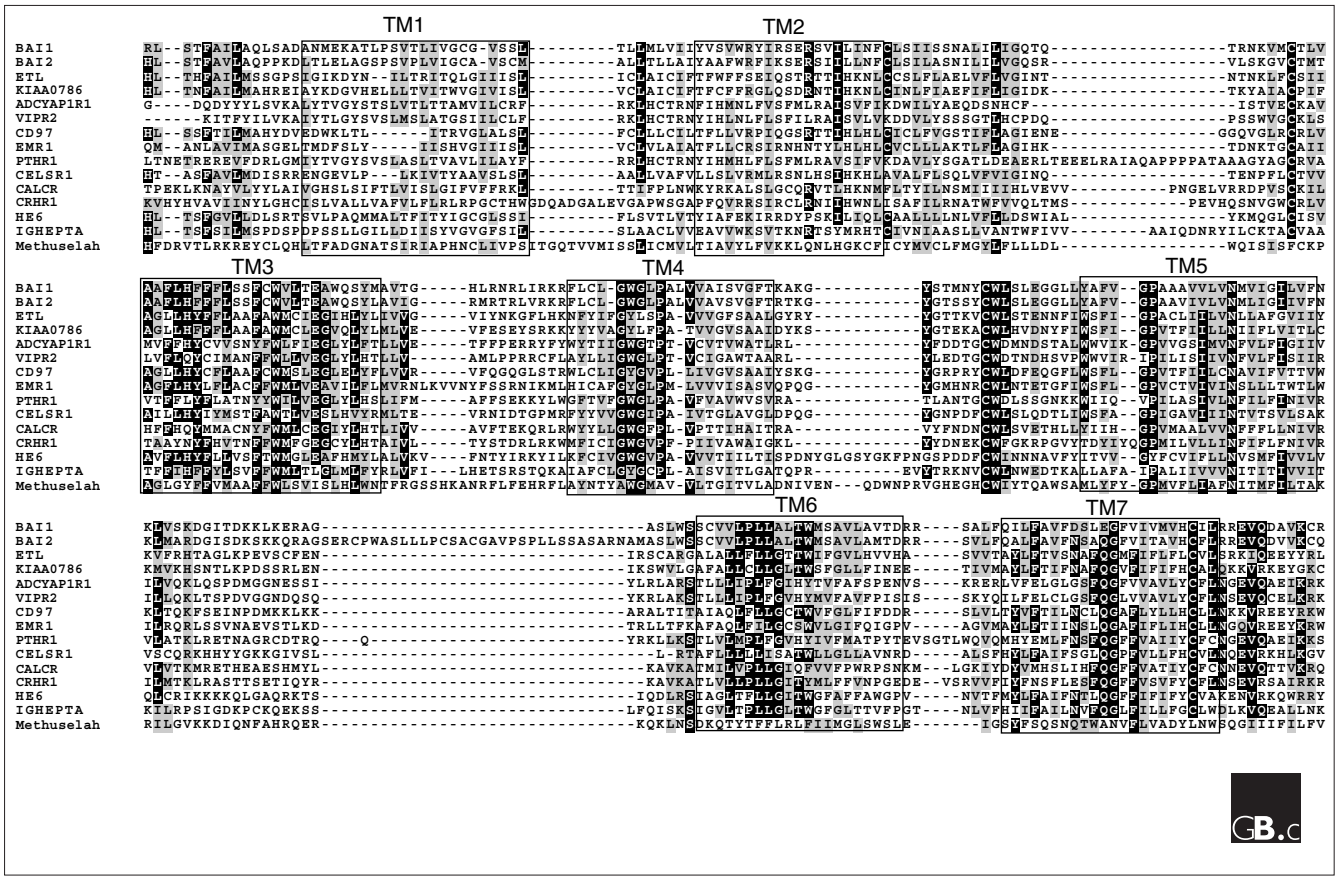

Figure 3
Alignment of the 7TM region of selected family-B GPCRs. The alignment was generated using the Multiple Alignment General Interface (MAGI) suite of software (alignment using ClustalW [49], display of results using Boxshade) at the UK Human Genome Mapping Project Resource Centre [37]. Black background indicates conserved residues; gray background indicates similar residues; TM1-TM7 indicate transmembrane domains. See text and Table 1 for abbreviations.

repeats (in BAI1, BAI2 and BAI3) and, in Ig hepta, an immunoglobulin C-2-type domain also found in fibroblast growth factor (FGF) receptor 2 and in the neural cell adhesion molecule L1. VLGR1 has two copies of a motif (Calx-beta) present in Na^+-Ca^{2+} exchangers and integrin subunit β4.

Localization and function

The names, ligands and functions of the 15 hormone receptors in family B1 are summarized in Table 1. As mentioned above, nine of the ligands are structurally related and three of the receptors respond selectively to different ligands that are all synthesized by post-translational processing of a single polypeptide precursor (proglucagon). The $VPAC_1$ and $VPAC_2$ receptors respond to physiological concentrations of two different ligands (PACAP and VIP), whereas the PAC_1 receptor responds selectively to PACAP. Until recently, there were thought to be two receptors for corticotropin-releasing factor (CRF). Recent studies suggest, however, that although CRF is a physiological ligand for the CRF1 receptor, the most potent ligands for the CRF2 receptor are the urocortins, a family of peptides structurally related to CRF but encoded by different genes with different expression patterns [21]. Likewise, two receptors originally described as receptors for parathyroid hormone (PTH1 and PTH2 receptors) appear to have different physiological ligands: PTH1 receptors respond to parathyroid hormone and parathyroid hormone-related protein (PTHrP), and PTH2 receptors respond to the 'tubero-infundibular peptide of 39 residues' (TIP39), a novel peptide isolated from bovine hypothalamus [22].

A family of three small accessory proteins called receptor activity-modifying proteins (RAMPs) play a role in determining the ligand-binding specificity of two hormone receptors in subfamily B1 in a manner that has not been described for any other GPCRs. Co-expression of the human calcitonin-receptor-like (CALCRL) gene with RAMP1 results in a receptor selective for calcitonin-gene-related peptide (CGRP), whereas RAMP2 and RAMP3 promote the expression of receptors selective for the 52-amino-acid peptide hormone adrenomedullin [23]. Likewise, expression of the calcitonin receptor (CALCR) gene with RAMP2 results in a

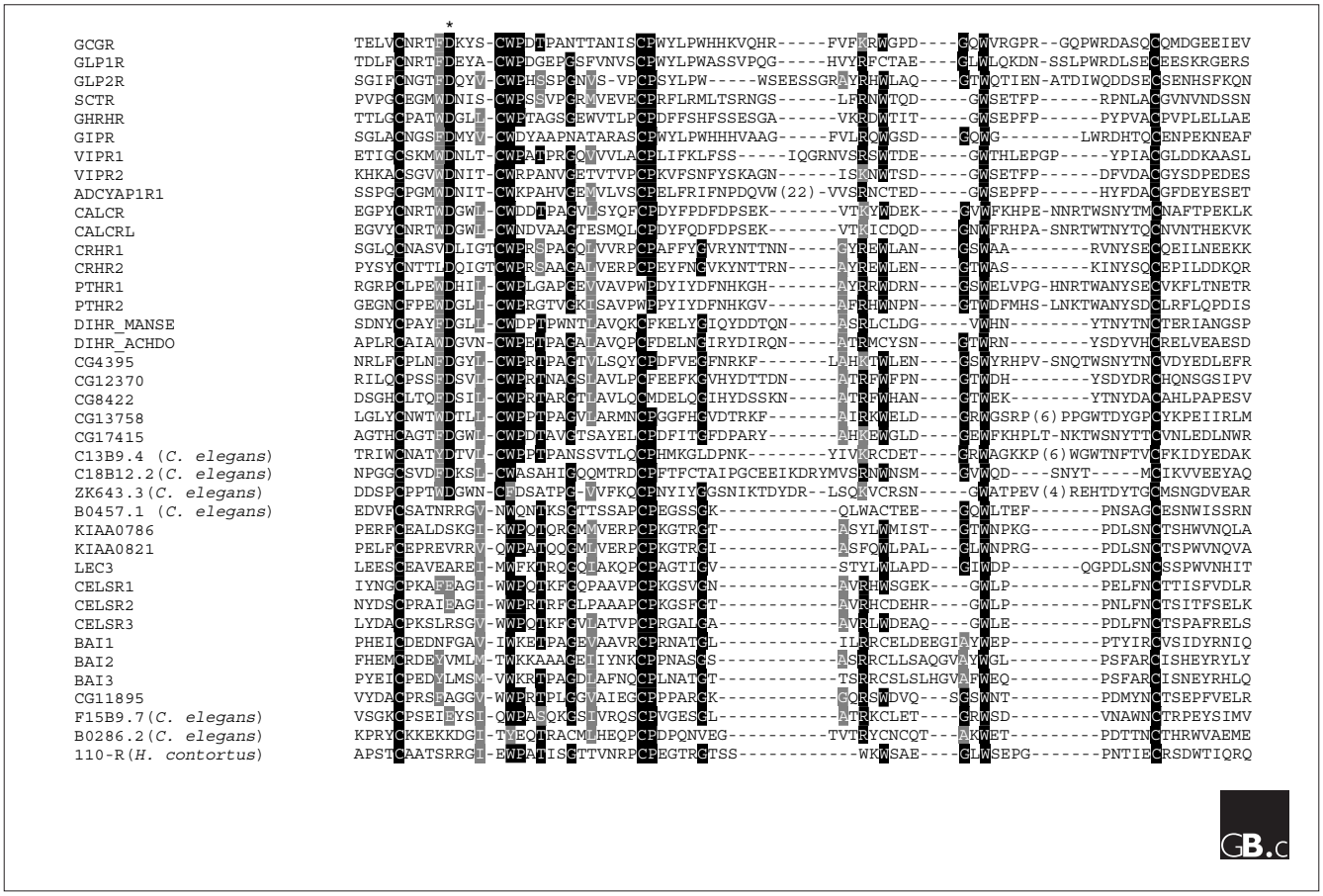

Figure 4
Alignment of a putative hormone-binding domain in the extracellular region of family-B GPCRs. A conserved asparagine thought to be important for hormone binding is indicated by an asterisk. See Figure 3 for details of the construction of the alignment.

receptor responding most potently to calcitonin, expression of CALCR with RAMP3 results in a receptor responding to both calcitonin and the pancreatic hormone amylin; and expression of CALCR with RAMP1 promoted the formation of a receptor responding most potently to amylin and CGRP [24]. Thus, the interaction of the products of two receptor genes with the three RAMPs is potentially capable of generating six pharmacologically distinct receptors. If this phenomenon proves to be more widespread than is known at present, it would change the way molecular pharmacologists view the function of GPCRs and destroy the dogma of 'one gene, one type of receptor pharmacology'.

The functions of the five putative hormone receptors identified in the *Drosophila* genome and the nature of their ligands is currently unknown. Two of them (CG4395 and CG17415) are most closely related to the mammalian receptors for calcitonin and CRF, however, and a further two receptors, encoded by *CG8422* and *CG12370*, are related to diuretic hormone receptors (peptide hormones involved in the regulation of fluid and ion secretion), which have been identified in other insects but have no known mammalian homologs [25].

The functions of most of the receptors in subfamily B2 are unknown, although their tissue distribution suggests that many of them may have important functions in the nervous system or in the immune system. CD97, a glycoprotein that is present on the surface of most activated leukocytes, has been shown to interact with CD55 (decay-accelerating factor), a glycosyl-phosphatidylinositol-linked cell-surface molecule that plays a role in regulating complement activation [26]. CD97 is the only receptor in subfamily B2 for which the ligand has so far been identified, although there is evidence for the existence of a ligand for EMR3, another member of subfamily B2 expressed in the immune system, on human macrophages and activated neutrophils [27]. The *Drosophila* gene *flamingo*/*starry night* is thought to function downstream of *frizzled*, which encodes a receptor for Wnt signaling proteins, and *dishevelled*, which encodes an intracellular transducer of the Wnt signal, in determining the tissue polarity of cuticular structures and, independent

of *frizzled,* in shaping the dendritic fields of developing *Drosophila* neurons [28]. Orthologs in humans (Celsr1, Celsr2 and Celsr3) and in *C. elegans* (F15B9.7) probably play similar roles in determining tissue polarity and in synaptogenesis. The receptors for latrotoxin may be involved in control of synaptic exocytosis, and BAI1, BAI2 and BAI3 are thought to play a role in controlling angiogenesis in the brain, but the nature of their ligands remains unknown.

Important mutants

Gene targeting has been used to create null mutants of many of the hormone receptors in subfamily B1 and in some of their ligands. Much of the information in Table 1 is inferred from studies of these mutations, many of which do not impair normal development but result in subtle but important phenotypes in a variety of body systems. For example, the PAC_1 receptor is widely distributed in the central and peripheral nervous systems, most abundantly in the dentate gyrus of the hippocampus and in the spinal cord, but is also expressed in pancreatic beta (insulin-secreting) cells. Consistent with the tissue distribution of this receptor, PAC_1-receptor-null mice displayed several independent abnormalities including a deficit in hippocampus-dependent associative learning accompanied by an impairment of mossy fiber long-term potentiation [29]; greater locomotor activity and less anxiety-like behavior than wild-type littermates [30]; abnormal phase-shifting of the circadian clock in response to light [31]; impaired responses to painful stimuli [32]; and reduced insulin secretion in response to glucose [33]. Mutations of other hormone receptors in family B can be expected to have similarly pleiotropic effects.

The *little* mouse, a dwarf mutant deficient in growth hormone secretion, has provided valuable insights into the signaling mechanisms of family B GPCRs. The phenotype of *little* mice is due to a nucleotide substitution that replaces the critical asparagine residue within the putative hormone-binding domain of the GHRH receptor with a glycine residue. The mutation abolishes the ability of the receptor to bind GHRH. In man, rare mutations leading to an inactive, truncated or mis-spliced product of the *GHRHR* gene have been shown to give rise to an equivalent phenotype. Mutagenesis of the equivalent residue of the human $VPAC_1$ receptor has been shown to abolish hormone binding.

Studies of PTH1 receptor knockout mice have revealed important roles for this receptor in bone growth and in the development of the teeth and the mammary gland: they have a defect in endochondral bone development that leads to skull deformities and disproportionately short limbs. Studies in these mice have played a key role in characterizing a negative feedback loop in which the morphogen Indian hedgehog, synthesized in chondrocytes that are in the process of differentiating into hypertrophic (non-proliferating) cells, stimulates parathyroid-hormone-related protein (PTHrP) production in the perichondrium, and PTHrP, in turn, inhibits proliferating chondrocytes from moving down the differentiation pathway. PTH1 receptor mutations leading to constitutive activity of the receptor are thought to be the cause of the Jansen type of metaphyseal chondrodysplasia [18]. Inactivating mutations in the PTH1 receptor gene give rise to Blomstrand chondrodysplasia, a condition characterized by premature endochondral bone maturation, abnormal tooth development and the absence of breast tissue [34].

Frontiers

Important insights into the physiological roles of some of the hormone receptors in family B have been obtained from analysis of knockout mice lacking the receptors or their ligands. Gene targeting will be a valuable tool to explore the functions of the remaining members of the family. The wide spectrum of abnormalities manifested in knockouts of some family-B receptors (such as the PAC_1 receptor) and the embryonic lethality of others (such as the PTH1 receptor) indicate that tissue-specific and/or temporally controlled gene targeting will be increasingly used. The creation of 'floxed' mice, in which expression of the PAC_1 receptor gene [29] or the PTH1 receptor ligand PTHrP [35] can be abolished selectively in tissues expressing Cre recombinase, have been reported recently.

The mechanisms by which proteins in subfamily B2 exert their actions remain unknown. It is not formally certain that G-protein coupling and the activation of intracellular second messenger synthesis is required for function. To address this, it will be necessary to identify the ligands for these receptors, which, like the prototype ligand CD55, are likely to be integral membrane proteins expressed on the cell surface. *In vitro* investigation of the role of G-protein coupling in the actions of proteins in subfamily B2 will probably require the characterization of soluble, biologically active fragments of the ligands. It will be important to establish whether proteolytic cleavage of the amino-terminal extracellular domain of proteins in subfamily B2 to generate receptors with two subunits, a process known to occur in CL-1, in CD97 and in ETL, is a requirement for the activity of other members of the B2 subfamily. It is possible that proteolysis might unmask a binding site for an exogenous ligand, or, alternatively, the soluble fragment of the extracellular domain liberated by proteolysis might itself be the physiological ligand, by analogy with the thrombin receptor and other protease-activated receptors in family A [36].

Finally, it will be important to investigate GPCRs of family B as candidate genes for human genetic disease. For example, loci conferring susceptibility to skin disorders, inflammatory bowel disease, cerebellar ataxia and deformities of the limbs, urogenital system and palate have been mapped close to the region of chromosome 19p13 that contains the cluster of genes encoding CD97, EMR2, EMR3 and CL-1.

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