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Photoreceptor guanylate cyclase variants: cGMP production under control

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Abstract

Changes in the Ca^{2+} concentration are thought to affect many processes, including signal transduction in a vast number of biological systems. However, only in few cases the molecular mechanisms by which Ca^{2+} mediates its action are as well understood as in phototransduction. In dark-adapted photoreceptor cells, the equilibrium level of cGMP is maintained by two opposing activities, such as phosphodiesterase (PDE) and guanylate cyclase (GC). Upon absorption of photons, rhodopsin-G-protein-mediated activation of PDE leads to a transient decrease in [cGMP] and subsequently to lowering of [Ca^{2+}]. In turn, lower [Ca^{2+}] increases net production of cGMP by stimulation of GC until dark conditions are re-established. This activation of GC is mediated by Ca^{2+}-free forms of Ca^{2+}-binding proteins termed GC-activating proteins (GCAPs). The last decade brought the molecular identification of GCs and GCAPs in the visual system. Recent efforts have been directed toward understanding the properties of GC at the physiological and structural levels. Here, we summarize the recent progress and present a list of topics of ongoing research.

Keywords

retina; photoreceptor cells; guanylate cyclase; rhodopsin; Ca^{2+}-binding proteins; guanylate cyclase-activating protein

Abbreviations

AC, adenylate cyclase; ANP, atrial natriuretic peptide; CaM, calmodulin; CD, catalytic domain; DD, dimerization domain; ECD, extracellular domain; GC, guanylate cyclase; GCAP, guanylate cyclase-activating protein; Gt, rod photoreceptor G protein; ICD, intracellular domain; KHD, kinase-homology domain; Meta II (or R*), metarhodopsin II (photoactivated rhodopsin); NPR, natriuretic peptide receptor; PDB, Protein Data Bank; RMSD, root-mean-square deviation; PDE, phosphodiesterase; ROS, rod outer segments; STa, heat-stable enterotoxin; TM, transmembrane region

Among cyclic nucleotides, the utilization of cGMP is not understoodas well as that of cAMP. cGMP activates cGMP-dependent protein kinases, opens cGMP-gated cation channels, or regulates phosphodiesterases (PDEs) (Wong & Garbers, 1992). Guanylate cyclases (GCs) are

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enzymes that catalyze the conversion of GTP to cGMP, while specific subtypes of PDEs are involved in the hydrolysis of cGMP to GMP (Soderling & Beavo, 2000). GMP is recycled back to GTP by guanylate kinase and nucleoside diphosphokinase.

GCs come in two varieties: soluble and membrane-bound GCs with multiple isoenzymes of both forms being expressed ubiquitously (Drewett & Garbers, 1994; Kobialka & Gorczyca, 2000; Gorczyca *et al.*, 2003). The membrane-bound GCs display similar topologies and belong to a family of single trans-membrane-spanning signaling receptors (Singh *et al.*, 1988) (Fig. 1A). They are composed of extracellular domain (ECD), single-spanning transmembrane region (TM), and intracellular domain (ICD) which is further subdivided into kinase-homology domain (KHD) and catalytic domain (CD) (Garbers, 1989). In mammals, the family of membrane-bound GCs includes receptors for natriuretic peptides (NPRs), GC-A (NPR-A) and GC-B (NPR-B) (Garbers, 1989) (Fig. 1B). An intestinal peptide-binding receptor, GC-C belongs to the second group of the membrane-bound GCs (Fig. 1B) and is also the receptor for heat stable enterotoxin (STa). Four other GCs, GC-D (Fulle *et al.*, 1995; Juilfs *et al.*, 1997), GC-E, GC-F (discussed below) and GC-G (Schulz *et al.*, 1998), expressed in sensory and peripheral tissues, are considered orphan receptors because they display the membrane-bound GC topology, but the putative ligand for these cyclases has not been identified (Fig. 1B). This small number of GCs is in contrast with more than 29 GCs identified in *Caenorhabditis elegans* (Baude *et al.*, 1997; Marchese *et al.*, 1998). It was speculated that the large number of GCs could complement G protein-coupled receptors (GPCRs) in the olfaction system (Yu *et al.*, 1997). The interplay between olfactory GCs and olfactory GPCRs is unclear.

Phototransduction in the rod photoreceptor cell employs cGMP as a second messenger that couples absorption of light to changes in conductivity of cation channels in the plasma membrane (Yau & Baylor, 1989; Polans *et al.*, 1996; Baylor & Burns, 1998; Arshavsky *et al.*, 2002). Phototransduction events are initiated when a photon strikes rhodopsin causing photoisomerization of the chromophore 11-*cis*-retinal (Okada *et al.*, 2001; Filipek *et al.*, 2003). The photoisomerized chromophore induces a sequence of conformational changes in rhodopsin that culminates in the formation of Meta II, which catalyses the exchange of GDP to GTP in hundreds of Gt molecules (Leskov *et al.*, 2000; Heck & Hofmann, 2001) before it is phosphorylated (Kuhn & Dreyer, 1972; Bownds & Brodie, 1975; Frank & Buzney, 1975; Miller *et al.*, 1975; Kuhn & Bader, 1976; Palczewski, 1997; Palczewski & Benovic, 1991; Maeda *et al.*, 2003). Continuous Gt activation is prevented by the binding of arrestin to phosphorylated Meta II (Kuhn *et al.*, 1984; Wilden *et al.*, 1986) (reviewed by Okada *et al.*, 2001; Filipek *et al.*, 2003). Phototransduction proceeds with the GTP-α-subunit of Gt activating PDE (Stryer, 1983), and cGMP is hydrolyzed faster than it is replenished by GC. GC-E and GC-F were proposed to be involved in phototransduction. GC-E (also known as GC1 or retGC1) was cloned in 1992 (Shyjan *et al.*, 1992). A frameshift error was corrected soon after (Lowe *et al.*, 1995). GC-F (GC2 or retGC2) was cloned from retinal cDNA libraries (Lowe *et al.*, 1995; Yang *et al.*, 1995). Both isoenzymes are expressed in rod and cone photoreceptor cells (Dizhoor *et al.*, 1994; Yang & Garbers, 1997; Duda *et al.*, 2002; Imanishi *et al.*, 2002). GC-E was also found in the pineal gland, an organ developmentally related to the retina (Venkataraman *et al.*, 2000).

Reduced concentrations of cGMP result in the closing of the plasma membrane cGMP-gated cation channels (Fesenko *et al.*, 1985), and hyperpolarization of the cell. The Na^+/Ca^{2+}-K^+ exchanger (NCKX) removes Ca^{2+} from ROS, leading to lower levels of [Ca^{2+}] that in turn trigger a feedback mechanism of the enhancing photoreceptor GC activity through Ca^{2+}-binding protein GCAPs and restoring the dark levels of cGMP (reviewed in Polans *et al.*, 1996) (Fig. 2). The molecular identity of GCAP, whose presence was suspected from previous studies (Lolley & Racz, 1982; Koch & Stryer, 1988), was advanced in our laboratory by the original work of Dr. W. Gorczyca, who isolated the first form of the activator from

photoreceptor cells (Gorczyca *et al.*, 1994). The second GCAP2, was isolated independently by Gorczyca and Dizhoor (Dizhoor *et al.*, 1995; Gorczyca *et al.*, 1995), and the GCAP3 was cloned by us (Haeseleer *et al.*, 1999). Importantly, our progress on studies of the GC regulation also benefited from a close collaboration of our laboratory with Dr. W. Baehr (University of Utah, U.S.A.).

STRUCTURE OF PHOTORECEPTOR GUANYLATE CYCLASES AND THEIR RELATIONSHIP TO OTHER FAMILY MEMBERS

The basic topologies of orphan receptors consist of about 500-amino acid-long ECD, 23-residue hydrophobic TM and about 500–600 amino acid-long ICD that contains the signature domains of membrane GCs (KHD and CD) (Singh *et al.*, 1988; Drewett & Garbers, 1994; Potter & Hunter, 2001) (Figs. 1A and 3).

Signal peptide and extracellular domain (ECD)

All GCs contain a signal peptide sequence that targets the protein to the membranes. The N-terminal analysis of isolated GC-E reveals that the first 56 amino-acid residues are removed by signal peptide peptidase (Margulis *et al.*, 1993).

The ECDs are weakly related among the GC family (32–38%). The high-resolution structures of the ECDs were determined for GC-A (van den Akker *et al.*, 2000) (PDB ID: 1DP4) (Fig. 1C) and natriuretic “clearance” receptor (NPR-C) (He *et al.*, 2001) (PDB ID: 1JDN). The structure of the ECD of GC-A resembles an ancient class of proteins termed the bacterial type I periplasmic solute-binding proteins that bind small molecules between two structurally independent sub-domains. The ECD forms a dimer, and each monomer is dumbbell-shaped, with each domain consisting of a central sheet surrounded by helices. The arrangement of the monomers of ECD in the crystal is different than the model generated from the biochemical studies for GC-A (Rondeau *et al.*, 1995; De Lean *et al.*, 2003) and perhaps induced by the crystallization conditions. The ECD also contains a Cl^- ion that is essential for high affinity binding of ANP (Misono, 2000). The second high-resolution structure of the ECD was determined for the NPR-C with and without ligand (He *et al.*, 2001). The RMSD between ECDs of GC-A and NPR-C is about 2.5 Å. In the ligand-bound complex, a single natriuretic peptide molecule is bound in the interface of the NPR-C dimer, in agreement with the biochemical data. Hormone binding induces a 20 Å closure between the membrane-proximal domains of the dimer, suggesting conformation rearrangement with the ECD which induces changes that are propagated into the intracellular domain; thus ultimately enhanced the GC activity. From both structures, two disulfide bridges are demonstrated in identical positions (Fig. 3); however, the two Asn glycosylation sites are not conserved (Fig. 3), suggesting that these modifications are involved in the overall stability of the proteins rather than their having any functional significance. These modifications may also protect the receptor against proteolysis in the native tissue or assist folding by interacting with the ER chaperons like calnexin and calreticulin. As both crystallized fragments of the enzymes are produced in the heterologous expression systems, we need proofs that these sites are also utilized by the GCs in the native tissues. The NPR-C structure also contains two Cl^--binding sites which are believed to be important for the integral stability of the protein (He *et al.*, 2001), and not for ligand binding, as the position of this anion is distal from the ligand binding site and unchanged in the ligand-bound and free forms of the receptor.

No extracellular ligands have been found for GC-D, GC-E or GC-F, and these cyclases do not respond to any peptides that regulate activity of NPRs (Shyjan *et al.*, 1992; Yang *et al.*, 1995). Sequence analysis of the ECDs of orphan receptors suggests that they may fold into a somewhat different structure than those of GC-A and NPR-C. The ECD of GC-E, GC-F and

GC-D isoforms contain conserved Cys residues with high homology to other membrane-bound GCs (Fulle *et al.,* 1995), but the proven disulfide bridges are not conserved (Fig. 3). There is only the weak conservation throughout these ECDs, mostly among a few hydrophobic residues. Based on the sequence analysis of the ECDs, one N-linked glycosylation sites was predicted in GC-E, while more than one were in GC-D, but not in GC-F (Fulle *et al.,* 1995; Yang *et al.,* 1995). N-linked glycosylation was proven experimentally using bovine ROS as a source of GC-E and appears to be different than that of GC-A (Koch *et al.,* 1994). Additional studies are needed in the analysis of the glycosylation site and the composition of the sugar moieties attached to photoreceptor GCs.

An important issue for phototransduction is the question of putative ligands of GC-E and GC-F. The ligand would exert another level of regulation of GC that is important during dark- and light-adaptation (Fain *et al.,* 2001). The ligand for GC-E or GC-F should be diffusible; however, ECDs of these GCs are, in large part, sequestered in the lumen of disks of rod photoreceptor cells, or inaccessible within highly folded cone outer segment disks. If a ligand did exist, on the time scale of visual processes, renewal and re-synthesis of the ligand would be an unlikely process. However, the ligand could be a permanent subunit of the GC-E or GC-F. Because the GC-E and GC-F fragments lacking the ECD are highly active in the presence of GCAPs (Duda *et al.,* 1996; Laura *et al.,* 1996; Sokal *et al.,* 2002), this putative ligand is not essential for GC activity.

Exposure of membrane preparations containing GC-C to its ligand prior to addition of GTP resulted in dramatic inactivation and desensitization of the enzyme. GC-C inactivation could be a consequence of the conformational alterations induced by ligand binding (Bakre *et al.,* 2000). The nature of this desensitization and its general usage among all sensory GCs requires more experimental evidence.

Transmembrane domain (TM)

The function of the transmembrane segment of GCs is to transmit the signal from the ligand-binding site in the ECD to the ICD. This portion of the receptors allows a single passage through the membrane bilayer. The α-helix in the TM creates a rigid, hydrophobic region, which slips into membrane lipids. The GC-A and GC-C fragments containing ECD and TM were still capable of forming dimers (Chinkers & Wilson, 1992), suggesting that in addition to ICD, ECD are also in the dimeric form. The mechanism by which receptors with a single TM transduce extracellular signals into intracellular conformation changes is still unknown. However, based on the differences between the crystal structure of the ligand-bound and ligand-free ECD of NPR-C, the C-terminal region of this domain undergoes conformational changes (He *et al.,* 2001). Such remodeling of the ECD would affect the transmembrane organization and induce changes within the ICD. A soluble GC-E mutant lacking the ECD and TM showed typical Ca^{2+}-dependent stimulation by GCAP that was further enhanced by ATP (Sokal *et al.,* 2002). These *in vitro* experiments demonstrate that the TM is not essential for the activity.

Kinase-homology domain (KHD)

To attain maximal GC activity, GC-A requires the natriuretic peptide, and ATP or a non-hydrolizable ATP analog to relieve the CD inhibition (Chinkers & Garbers, 1989). This observation can be reconciled with the fact that GCs, as is true for most single-transmembrane spanning receptors, contain the ATP-binding domain homologous to protein kinases termed KHD. For example, mutations in GC-B within the Gly motif (GxxxG) of the KHD, which is critical for the formation of the ATP-binding pocket, decreased hormone dependent activity (Potter, 1998). Similar to other membrane-bound GCs, the ICDs of the sensory GCs also contain the KHD. Comparable motifs, $G^{617}xxxG^{621}$, $G^{502}xxx\text{-}G^{506}$, and $G^{471}xxxG^{475}$ are present in GC-D, GC-E and GC-F, respectively (Fig. 3) (Kobialka & Gorczyca, 2000).

The aligned sequence of the KHD with Ser/Thr kinases and Tyr kinases shows that 24 of the 33 highly conserved amino acids, important for proper structure and function (Sefton, 1989), are present in the photo-receptor KHDs. The stimulating effect of ATP could suggest that GC-E is phosphorylated; however, the activation by non-hydrolyzable ATP analogs excludes this possibility (Gorczyca *et al.,* 1994). In contrast, Aparicio and Applebury (1996) provided biochemical evidence that a member of the membrane receptor GC family (GC-E) possesses protein kinase activity. The authors suggested the existence of a single ATP-binding site within the KHD that both stimulates GC activity and catalyzes the transfer of the phosphate group in the Mg^{2+}-dependent manner. The substrate for the phosphorylation was the cyclase itself (autophosphorylation) or some exogenous substrates. This kinase activity had properties distinct from other Ser/Thr protein kinases identified in ROS, including protein kinase A, protein kinase C, and rhodopsin kinase. Comparison of the sequence of GC-E with sequences of members of the protein kinase family shows that most of the amino acids essential for ATP binding and kinase activity are conserved in the KHD of GC-E. A notable exception is Asp166 residue which is proposed to be a catalytic base for the transfer of the phosphate group in protein kinases (Sefton, 1989). This amino-acid residue is replaced in all membrane-bound GCs by Ser, Arg or Asn, and suggests that GCs may not display kinase activity. Therefore, protein kinase activity of GC-E remains an open question.

Dimerization domain (DD)

A short region between the KHD and CD has been proposed to contribute to ligand-independent dimerization of GC-A (Wilson & Chinkers, 1995). It appears that the DD is also necessary for GC activity based on deletion mutagenesis (Wilson & Chinkers, 1995). However, the CD fragments of GC-A form a homodimers that are enzymatically active (Thorpe *et al.,* 1991). Because the mechanism of the GC catalyzed reaction requires two subunits (reviewed by Hurley, 1998), in addition to the ECD, KHD, TM and DD, CD also contributes to the GC oligomerization.

Catalytic domain (CD)

The CD of orphan GCs closely resembles that of adenylyl cyclase (AC) type II. The crystal structure of the C2 domain of AC type II was solved (Tesmer *et al.,* 1997; Zhang *et al.,* 1997) and used to generate a model of the CD of bovine GC-E. In the homo-dimer of AC, Lys, Asp and Gln residues, which interact with a purine ring, determine substrate specificity. The Lys residue corresponds to E925 of GC-E (Tesmer *et al.,* 1997). Replacement of E925 by Lys and C995 by Asp in GC-E changes substrate specificity of the mutant from GTP to ATP (Tesmer *et al.,* 1997; Tucker *et al.,* 1998). Similar results were obtained for mutagenesis studies involving soluble GC and AC (Sunahara *et al.,* 1998).

The catalytic site is located in the cleft between two domains in the homo-dimer of CDs in the current model of GC (Fig. 1D). Thus, each domain contributes in forming two catalytic sites, where an Asp from one domain is a general base in the cyclization reaction, and the transition state is stabilized by a conserved Asn-Arg pair on the other domain (Hurley, 1998;Tucker *et al.,* 1998). An essential cofactor of GTP cyclization is a divalent metal ion (Mg^{2+} or Mn^{2+}), which forms a complex with GTP, where the metal ion is coordinated to the β- and γ-phosphate of this nucleotide as shown for GC-E (Koch *et al.,* 1990).

Carboxy terminal tail

Similar to GC-C, sensory GCs have a 40–60 amino acid-long extended C-terminal regions. This C-terminal extension is not found in NPRs. The C-terminal tail may be involved in the interaction with cytoskeletal proteins (Lucas *et al.,* 2000). In photoreceptor cells, tubulin associates tightly with GC-E (Schrem *et al.,* 1999). In addition, IKEPP (intestinal and kidney-enriched PDZ protein), associates with the C-terminal region of GC-C (Scott *et al.,* 2002). The

association with IKEPP significantly inhibits STa-mediated activation of GC-C. Extension of the C-terminus of the GC-E by GCAP1 eliminated enzyme activity (Sokal *et al.*, 2002), suggesting a unique function of the short C-terminal region in photoreceptor GCs. Structural studies will determine the role of this region on the mechanistic level.

REGULATION OF PHOTORECEPTOR GUANYLATE CYCLASES

Regulation of GCs by GCAPs

In the mammalian retina, three GCAPs (GCAP1, GCAP2 and GCAP3) have been identified (Gorczyca *et al.*, 1994; 1995; Palczewski *et al.*, 1994; Dizhoor *et al.*, 1995; Haeseleer *et al.*, 1999; Kobialka & Gorczyca, 2000; Imanishi *et al.*, 2002; Gorczyca *et al.*, 2003) that regulate the activity of photoreceptor GCs in Ca^{2+}-dependent manners. GCAPs belong to the family of recoverin-like proteins with limited homology to CaM, and they are myristoylated at the N-terminus (Palczewski *et al.*, 1994). Members of the family have similar molecular masses and three functional EF-hand motifs for Ca^{2+} coordination. Several extensive reviews cover the properties of this subfamily and photoreceptor GCs (Polans *et al.*, 1996Polans *et al.*, 1997; Pugh *et al.*, 1999; Dizhoor, 2000; Palczewski *et al.*, 2000; Koch *et al.*, 2002), therefore, in the remaining part of this review, we will focus on the selected aspects the function/structure relationship of GCAPs.

The structure of unmyristoylated GCAP2 in the Ca^{2+}-bound form has been revealed by NMR (Ames *et al.*, 1999). The overall shape of the molecule resembles that of recoverin (Flaherty *et al.*, 1993; Ames *et al.*, 1994), neurocalcin (Vijay-Kumar & Kumar, 1999), and frequenin (Bourne *et al.*, 2001). The RMSD of the main chain atoms between the GCAP2 structure and recoverin is 2.2 Å and between GCAP2 and neurocalcin is 2.0 Å within the EF-hand motifs.

GCAP2 is a compact protein consisting of two regions separated by a flexible linker (Figs. 4A and 4B, helix 6) (Ames *et al.*, 1999). Similar to CaM, the N- and C-terminal domains contain a pair of EF-hands (Haeseleer *et al.*, 2002), the helix-loop-helix motifs (Fig. 4A, gray). EF-hand 1 is non-functional due to a lack of amino-acid residues essential for Ca^{2+} coordination. The linker between the two regions forms a U-shape, bringing together on one side all four EF-hands in a compact tandem array. This structure is different from the arrangement of EF-hand motifs in other CaM-like Ca^{2+}-binding proteins (see for comparison in (Haeseleer *et al.*, 2002)). Within this central region (helix 6), a key Y99 residue plays a critical role in the stabilization of the inactive form. When Y99 is changed in GCAP1, the mutant protein switches to the active conformation (Dizhoor *et al.*, 1998; Payne *et al.*, 1998; Sokal *et al.*, 1998). The high-resolution structures of myristoylated Ca^{2+}-free and -bound forms are critical for further understanding of how the Ca^{2+} signal is translated into a conformational change within this protein.

In the ROS membranes, GC activity increases in the presence of GCAPs when intracellular $[Ca^{2+}]_{free}$ drops below 100 nM and decreases when $[Ca^{2+}]_{free}$ is elevated (Gorczyca *et al.*, 1994; 1995; Palczewski *et al.*, 1994; Dizhoor *et al.*, 1995; Koch & Stryer, 1988; Haeseleer *et al.*, 1999; Imanishi *et al.*, 2002) (Fig. 5). Several models of the GCAP-mediated activation of photoreceptor GCs have been proposed (Hurley & Dizhoor, 2000; Koch, 2002; Koch *et al.*, 2002; Olshevskaya *et al.*, 2002).

Two properties of GC-GCAP appear to be consistent with most of the experimental data. First, GCAPs bind to GCs in a different manner than CaM does with its targets (Haeseleer *et al.*, 2002). Second, the interaction of GCAPs occurs by a multi-point attachment with the ICD of GCs and is stable in all ranges of $[Ca^{2+}]$ (Gorczyca *et al.*, 1994). Deletion of the ECD and the TM in GC-E has little effect on the interaction with GCAPs (Duda *et al.*, 1996; Laura *et al.*, 1996; Sokal *et al.*, 2002). The most critical part of GCAP1 for this interaction is the N-terminal

region (Palczewski *et al.,* 1994; Otto-Bruc *et al.,* 1997; Krylov *et al.,* 1999; Li *et al.,* 2001). Based on fluorescence methods (Sokal *et al.,* 1999b), proteolytic experiments (Rudnicka-Nawrot *et al.,* 1998), as well as chemical modification and modeling studies (Sokal *et al.,* 2001), we concluded that GCAP1 undergoes Ca^{2+}-dependent reorientation of helices at the interface of its N- and C-terminal regions. Such a rotation causes exposure of hydrophobic residues around central helix 6 (EF-hand 3 area) that ultimately leads to changes in the catalytic site of photoreceptors GCs (Sokal *et al.,* 1999b; 2001). GCAPs can modulate the catalytic activity of GC by lowering the activation energy of the GC-GTP transition state (Sokal *et al.,* 1999a). Based on the crystal structure of the C2 domain of AC, a contact region that is critical for the stimulation by $G_s\alpha$ was identified (Skiba & Hamm, 1998). In GC-E as in AC, a corresponding region is likely to form a loop between α-helix 3 and β-strand 4. When this region was replaced by the corresponding sequence of GCAP-insensitive GC-A, GCAPs did not stimulate the mutant (Sokal *et al.,* 1999a). In contrast to recoverin (Zozulya & Stryer, 1992; Ames *et al.,* 1997), it appears that GCAPs do not undergo the so called Ca^{2+}-myristoyl switch (Hughes *et al.,* 1995). However, removal of the N-terminal part (Fig. 4B) changed the Ca^{2+} inhibition profile of GCAP1 (Otto-Bruc *et al.,* 1997). This property is different for GCAP2, which is less affected by the mutation, deletion, or lack of a myristoylated group in the N-terminal region (Olshevskaya *et al.,* 1997; Hwang & Koch, 2002a; Hwang & Koch, 2002b).

S100 and GC

Sitaramayya and colleagues discovered that GC-E is also activated by S100 protein (Margulis *et al.,* 1996) (reviewed in Sitaramayya *et al.,* 2000). The physiological significance of this regulation awaits confirmation *in vivo*.

Phosphorylation/dephosphorylation

First, it was shown that cyclic-AMP-dependent protein kinase has an inhibitory effect on GC activity in rat cerebellum extracts (Kumakura *et al.,* 1978) and it was attributed to cyclase phosphorylation (Zwiller *et al.,* 1981). GC-A and -B are constitutively phosphorylated in heterologous expression systems (reviewed see Potter & Hunter, 2001). Because phosphorylation is essential for the receptor activity, the phosphate group(s) may have a role in the catalytic process or stabilize the active conformation of the enzyme. Ser^{497}, Thr^{500}, Ser^{502}, Ser^{506}, Ser^{510}, and Thr^{513} residues, and Ser^{513}, Thr^{516}, Ser^{518}, Ser^{523}, and Ser^{526} were identified as the major phosphorylation sites for GC-A and GC-B, respectively (Potter & Hunter, 1998a; 1998b; 1999) (Fig. 3). These residues are located within a 17-amino acid stretch of the KHD. Dephosphorylation of only a subset of these sites is proposed to be responsible for the desensitization of GCs (reviewed Potter & Hunter, 2001). It is critical to correlate the phosphorylation/dephosphorylation of these sites and to identify physiologically relevant protein kinases and phosphatases in selected tissues, rather than when the GC is over-expressed in the heterologous system. The basic properties of phosphatases involved in this process have been described (Bryan & Potter, 2002). The C-terminal fragment of GC-C contains a protein kinase C phosphorylation site, and GCs without C-terminal tail lose the ability to respond to ligand (Wada *et al.,* 1996; Deshmane *et al.,* 1997).

Studies on kinases that phosphorylated photoreceptor GCs are even less advanced. In addition to the mentioned work on autophosphorylation of GC-E (Aparicio & Applebury, 1996), GC-E appears to be modulated by protein kinase A and C (Wolbring & Schnetkamp, 1995). Advanced protein chemistry on photoreceptor GCs is needed for *in vitro* analysis of post-translational modifications to identification of changes that take place in photoreceptor GCs *in vivo* upon light stimulation.

DISEASES LINKED TO DEFECTS IN PHOTORECEPTOR GUANYLATE CYCLASE-E AND GUANYLATE CYCLASE-ACTIVATING PROTEIN 1

The link between mutations in photoreceptor GC and GCAP is outside of the scope of this review. Briefly, mutations within the GC-E gene are responsible for Leber's congenital amaurosis type 1 (LCA1) and specific cone-rod dystrophy type 6 (CRD or CORD6) (Perrault *et al.*, 1996; 1998) (reviewed in Duda & Koch, 2002; Newbold *et al.*, 2002; Perrault *et al.*, 1996). So far, no disease causing mutation has been found in the second photoreceptor specific GC-F. Mutations in GCAP1 are associated with autosomal dominant cone dystrophy (Payne *et al.*, 1998) and are reviewed extensively elsewhere (Palczewski *et al.*, 2000; Sokal *et al.*, 2000; Newbold *et al.*, 2002). So far, no mutation causing disease has been identified in the GCAP2 gene (Payne *et al.*, 1999).

FUNCTION OF PHOTORECEPTOR GUANYLATE CYCLASES AND GUANYLATE CYCLASE-ACTIVATING PROTEINS AS REVEALED BY GENETIC APPROACHES

The function of GCAPs and GCs becomes clearly delineated from the analysis of transgenic animals and phenotypes of human retinal diseases related to mutations of GCAP/GC. GC-E is not essential for photoreceptor development, but in the rd (retina degeneration) chicken model for human Leber's congenital amaurosis (Perrault *et al.*, 1999), the absence of GC-E prevents phototransduction and affects survival of rods and cones, similar to the human phenotype (Semple-Rowland *et al.*, 1998). In mice, disruption of the GC-E gene leads to cone-specific dystrophy, underscoring the species differences in GCAP/GC system (Yang *et al.*, 1999). Mouse photoreceptors with a disrupted GCAP1/GCAP2 gene array showed no Ca^{2+} dependent regulation of GC (Mendez *et al.*, 2001). The lack of Ca^{2+} sensitivity of GC activity indicates that S100 proteins have no role in regulation of GC in ROS. GCAP1 and not GCAP2 rescued normal photoreceptor responses in mice of the GCAP1/GCAP2 null background (Howes *et al.*, 2002; Pennesi *et al.*, 2003). Constitutive activation of GCAP1 causes autosomal dominant cone dystrophy (Dizhoor *et al.*, 1998; Payne *et al.*, 1998; Sokal *et al.*, 1998; 2000). It is unclear why rods are not affected. Other combinations of GCs and GCAPs are awaiting biochemical and physiological evaluations.

In summary, each protein that is involved in phototransduction is related to every other protein in more ways than is currently understood. Unraveling these complex interactions for the key components of phototransduction, GC and GCAPs, is the next challenge. Although the understanding of the GC-GCAP systems is quite advanced, clearly missed are structural studies that would allow us to verify several hypotheses at the molecular level. A new, promising approach is to employ soluble fusion forms of GCs complexed with GCAPs for structural studies (Sokal *et al.*, 2002). There is also a need for the high-resolution structure of GCAP1 in Ca^{2+}-bound and free forms to fill the gap in a collection of high-resolution structures of proteins involved in phototransduction (Ridge *et al.*, 2003).

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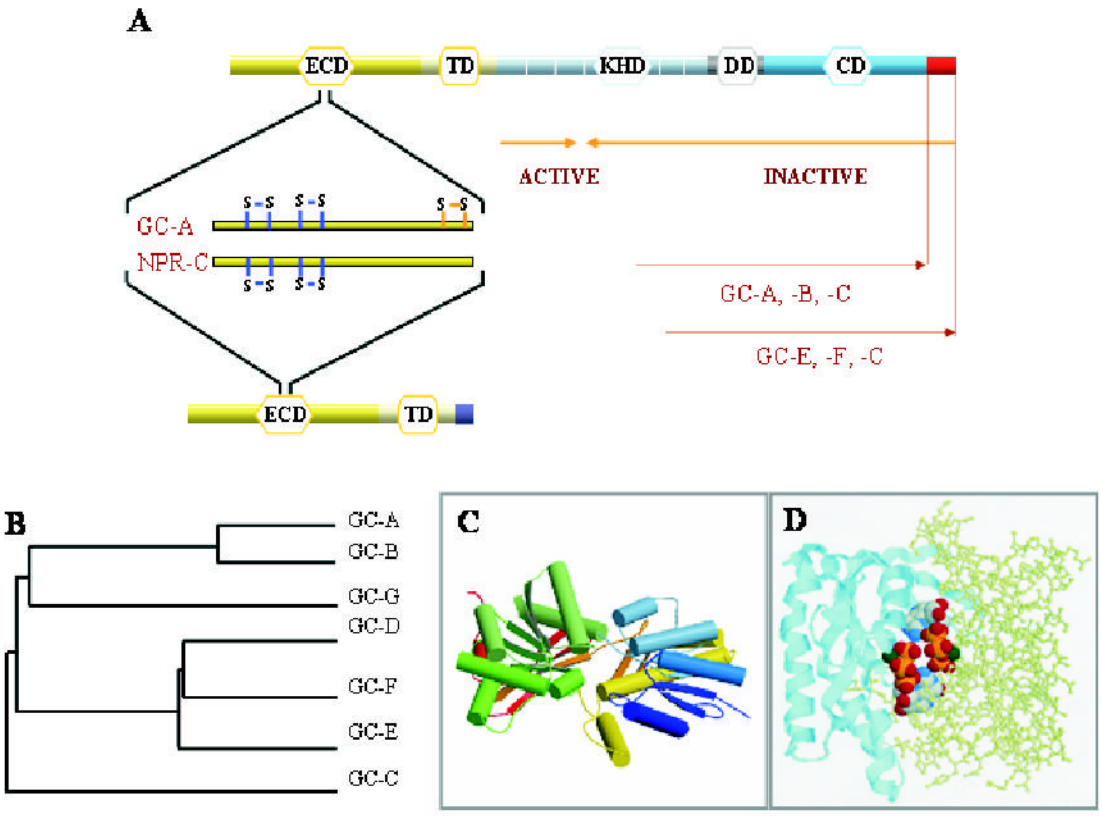


Figure 1. Guanylate cyclases and natriuretic peptide receptor C.

A. Topological representation of GC domains and NPR-C. ECD (extracellular domain), TM (transmembrane domain), KHD (kinase-homology domain), DD (dimerization domain), and CD (catalytic domain) are indicated. Blue lines indicates the positions of disulfide bridges as inferred from crystallographic studies in the ECD, and orange lines mark one disulfide bridge identified based on biochemical studies. Orange arrows mark the minimal truncated GC-E sequence needed for GC activity. The unique C-terminal fragment present in GC-E, -F and -C is shown as a red square. **B**. A phylogenetic tree calculated from the amino-acid sequences of mammalian GC is represented as the function of similarity between GCs. Analysis was performed using ClustalW program. **C**. Three-dimensional model of the ECD of NPR-C receptor (PDB ID: 1JDN) (He *et al.*, 2001). Arrows represent β-strands, cylinders are α-helices. The figure was drawn by using Molscript (Kraulis, 1991) and Roster3d (Merritt & Bacon, 1997) programs. **D**. Model of the CD from GC-E (1AWL) (Tucker *et al.*, 1998). Two subunits are shown in two different representation styles and two GTP molecules are shown in the default atom colors. Note that the active site is composed of residues from both subunits, and two active sites are present for the dimer. The figure was generated using the RasMol (Sayle & Milner-White, 1995).

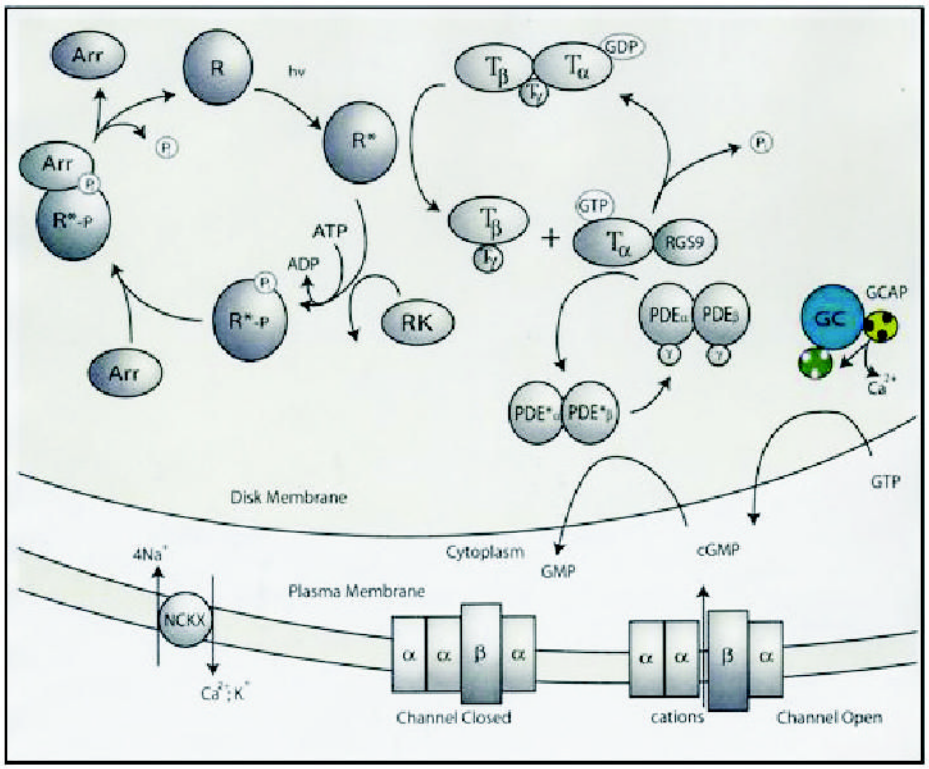
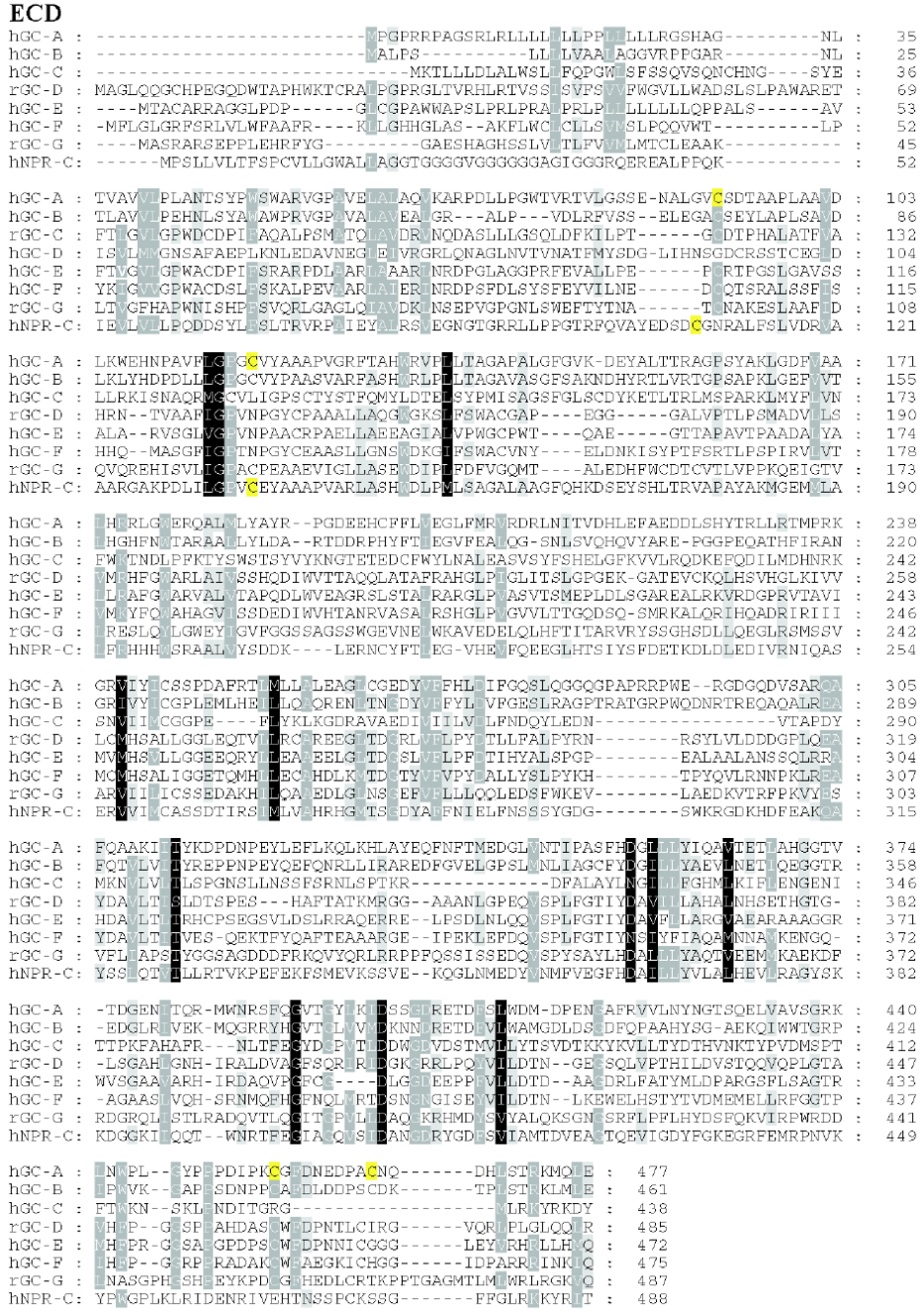


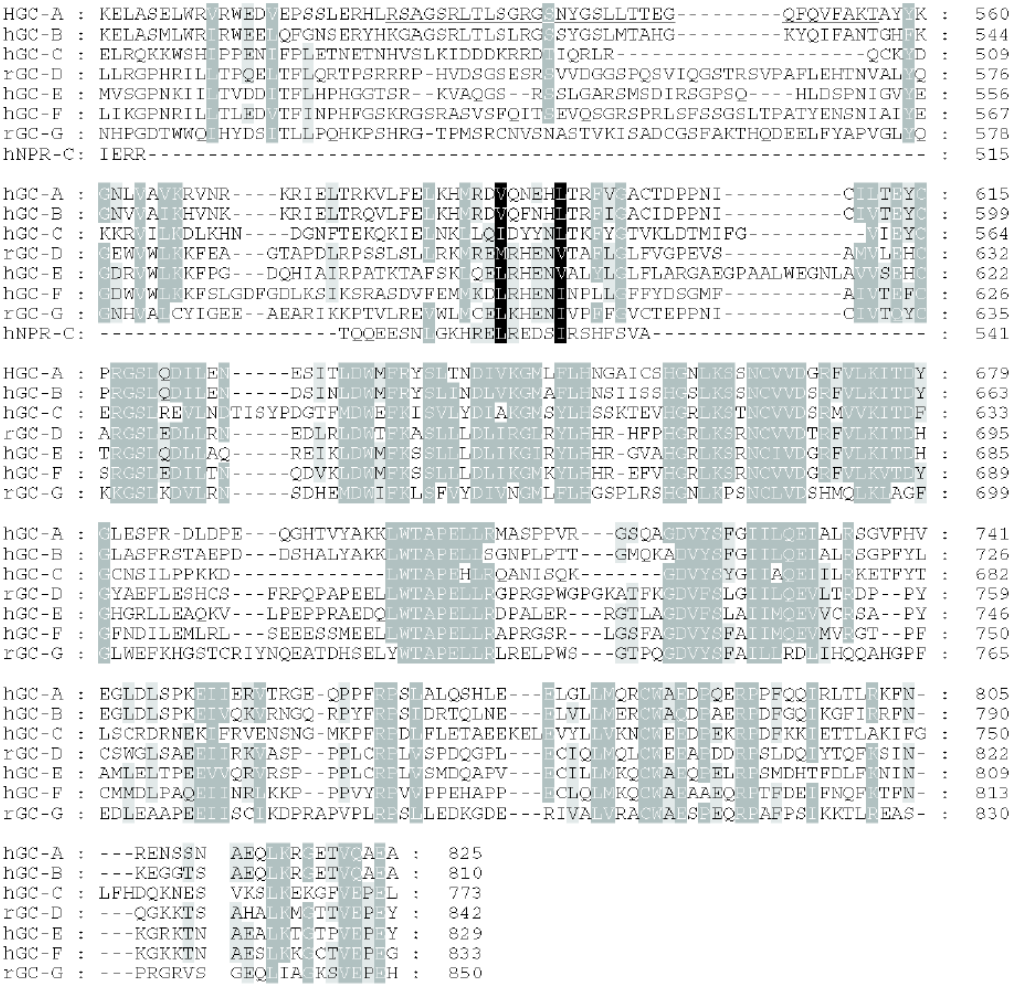
Figure 2. Phototransduction.

Absorption of light by rhodopsin (R) leads to transient conformation change and formation of the active form of this receptor, Meta II (or R*). In the dark, trimeric G-protein transducin (T) has bound GDP, which is replaced by GTP upon interaction with Meta II, and in turn transducin splits into α-subunit-GTP and βγ-subunits. The α-subunit-GTP activates PDE, which hydrolyses cGMP. Lowering [cGMP] causes closure of the cGMP-gated cation channel and hyperpolarization of the plasma membrane. Lower conductance of the cation through the channel also lowers [Ca^{2+}], because the ion is removed continuously by the Na^+/Ca^{2+}-K^+ exchanger. Dissociation of Ca^{2+} from GCAP1 leads to a conformation change in this Ca^{2+}-binding protein and activation of photoreceptors GCs (GC-GCAP shown in colors) and enhanced synthesis of cGMP. The restoration of the dark conditions requires inactivation of Meta II, hydrolysis of GTP by α-subunit of transducin, and inactivation of PDE (for details see Palczewski *et al.*, 2000; Polans *et al.*, 1996). The RGS9 complex consists of RGS9 protein, β5-subunit of G-protein and a membrane anchor, R9AP protein. Abbreviations used: R, rhodopsin; R*, Meta II (photoactivated rhodopsin); RGS, regulator of G-protein signaling; RK, rhodopsin kinase; Arr, arrestin; T, transducin; NCKX, Na^+/Ca^{2+}-K^+ exchanger.



TM

KHD



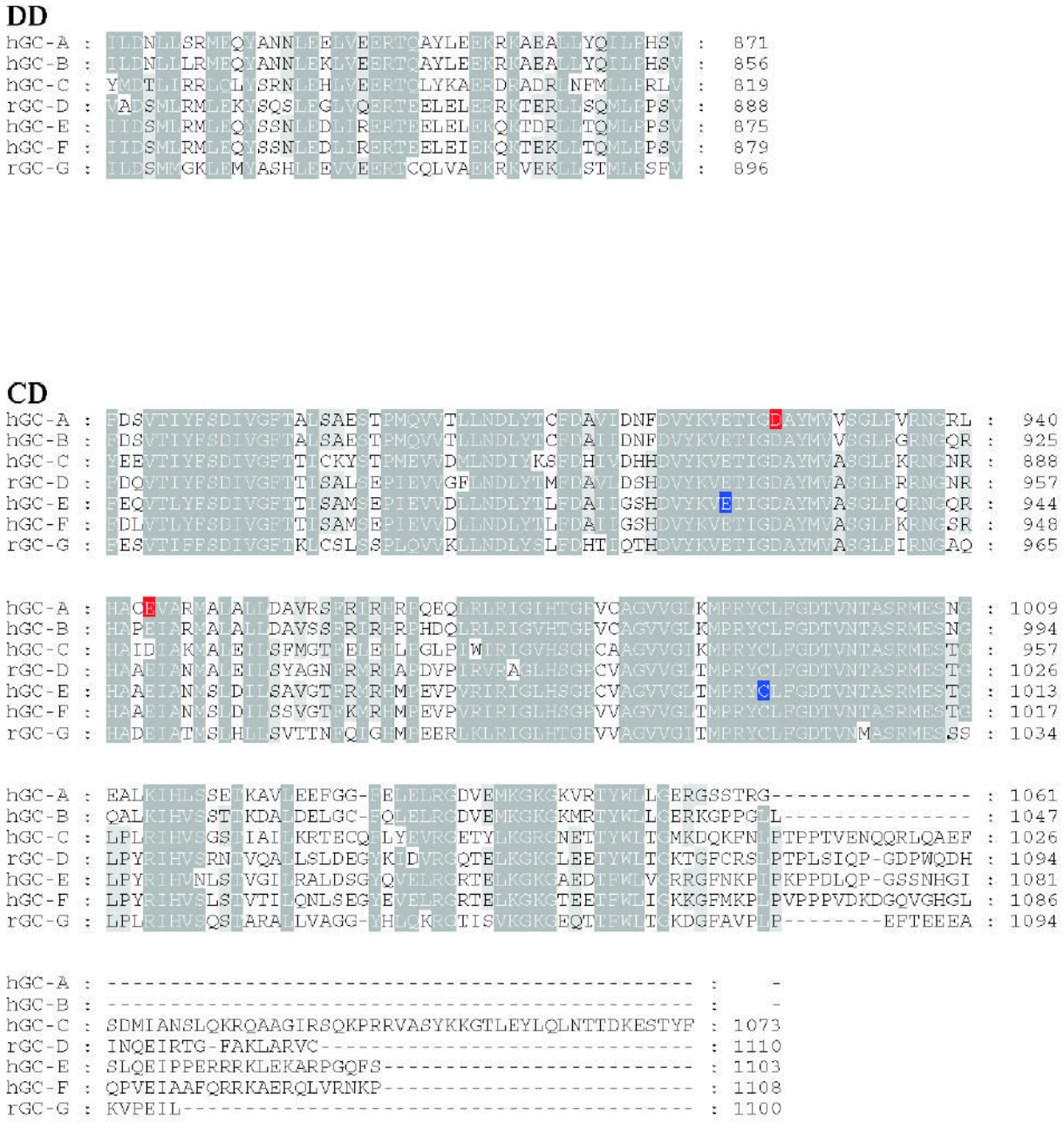


Figure 3. Sequence alignment of membrane-bound guanylate cyclases.

Residues that are 80–100% identical or highly homologous are in white letters on the black background, residues that are 60–80% homologous are in white letters on the gray background and residues that are 40–60% homologous on the gray background. For GC-F, the first 56 amino-acid residues (bovine) is the cleaved signal peptide. The cleavage site is showed in bold font and underlined letter (Margulis *et al.*, 1993). Two residues (marked on the blue background) in the catalytic site when mutated from E925K and C995D are sufficient to alter the nucleotide specificity from GTP to ATP (Tucker *et al.*, 1998). The highly phosphorylated region in GC-A within KHD is underlined. Note that this region is poorly conserved among photoreceptors GC. In red are residues that lead to full inactivation of GC-A (D to A) or

hyperactivity (E to A) (Thompson & Garbers, 1995). The sequences were downloaded from the ExPASy Molecular Biology Server (Swiss Protein Data).

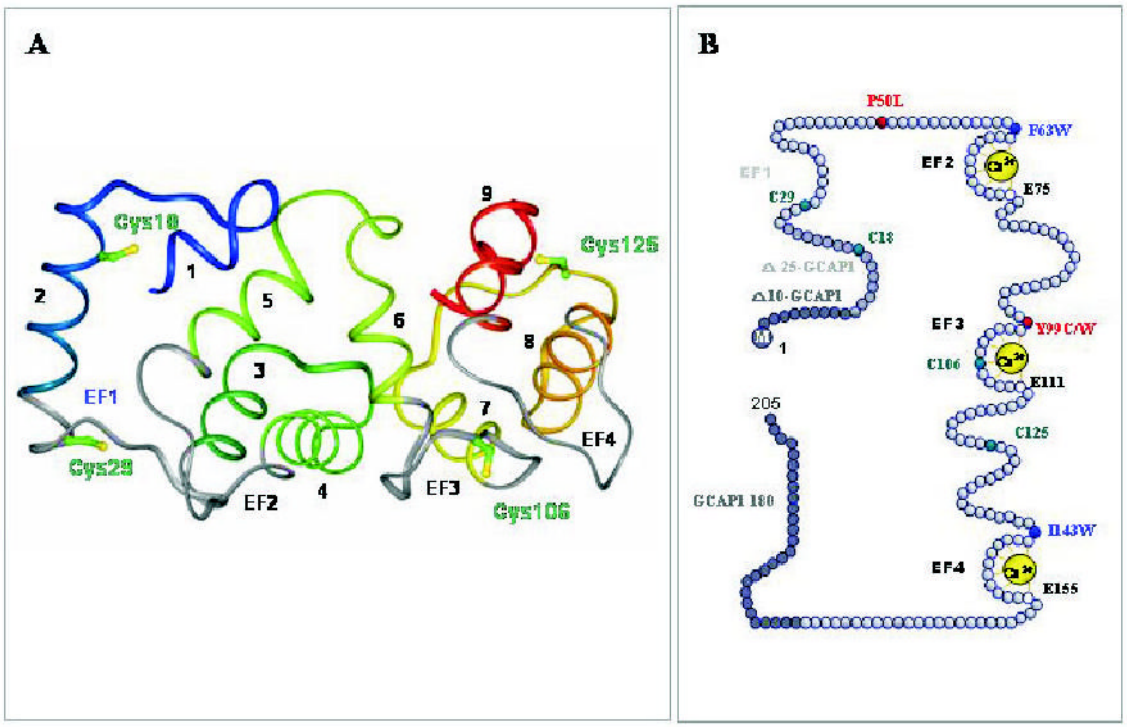


Figure 4. Structure of guanylate cyclase-activating protein 1.

A. A three dimensional model of GCAP1 based on the structure of GCAP2 (Ames *et al.*, 1999). The helices are numbered and the EF-hand motifs are shown in gray. The central helix 6 is bridging two halves of GCAP to form a compact structure. Note that EF-hand 1 is nonfunctional and the model lacks the N-terminal myristoylation group not present in the original structure of GCAP2. Cys residues are marked in the GCAP1 three-dimensional model. This figure is reproduced from (Sokal *et al.*, 2001) with permission from the American Society for Biochemistry and Molecular Biology. **B**. A model of the primary structure of GCAP1 and its mutants. Tyr and Pro residues mutated to Cys and Leu, respectively that are associated with autosomal dominant come dystrophy are shown in red. The native four Cys residues are represented in green, while hydrophobic residues, present in the front of Ca^{2+} binding loops, were used to identify conformational changes in GCAP1 (Sokal *et al.*, 1999b), are shown in blue. M indicates myristoylation of GCAP1. Active GCAP1-180 is a deletion mutant lacking the C-terminal region. Further C-terminus truncation renders GCAP1 inactive. Active Δ10-GCAP1 and inactive Δ25-GCAP1 are deletion mutants lacking the N-terminal region, thus identifying the minimal essential region of GCAP1 necessary for the stimulation of ROS GC (Otto-Bruc *et al.*, 1997). To disable the Ca^{2+} binding to EF2-, EF3- and EF4-hand motifs, Glu residues E^{75}, E^{111} and E^{125} (residues marked by white circles) were changed to Asp (D). Inactivation of EF-2 maintained Ca^{2+}-sensitivity of GCAP1, while inactivation of EF-hand 3 and EF-hand 4 led to constitutive activity (Rudnicka-Nawrot *et al.*, 1998).

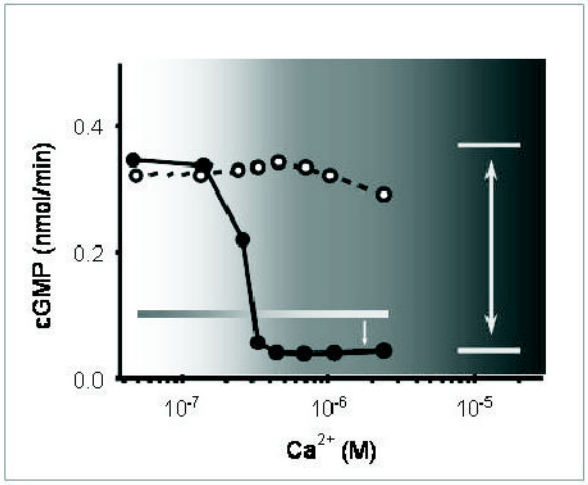


Figure 5. Ca^{2+}-dependent stimulation of GC activity by GCAP1 and Ca^{2+}-insensitivity of its triple mutant with disabled EF-hand loops.

ROS GC activity was reconstituted with GCAP1 (closed circles) or GCAP1-E75DE111DE155D mutant (open circles). Note that the mutant with disabled Ca^{2+} coordination is constitutively active in the tested range of $[Ca^{2+}]$. The shaded background shows the physiological range of Ca^{2+} changes in photoreceptor cells. The horizontal shaded box indicates the level of the basal cyclase activity and the double arrow indicates difference in activities between the mutant and GCAP1 as a function of $[Ca^{2+}]_{free}$, while the single arrow shows the difference in the level of suppression of basal activity by Ca^{2+}-bound GCAP1.