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Review

Particulate guanylyl cyclases: multiple mechanisms of activation★✪

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Cyclic GMP (cGMP), a key messenger in several signal transduction pathways, is synthesized from GTP by a family of enzymes termed guanylyl cyclases, which are found in two forms: cytosolic (soluble) and membrane-bound (particulate). The past decade has brought significant progress in understanding the molecular mechanisms that underlie the regulation of particulate guanylyl cyclases and new members of their family have been identified. It has become more evident that the basic mechanism of catalysis of guanylyl cyclases is analogous to that recognized in adenylyl cyclases. Here we review the known basic mechanisms that contribute to the regulation of particulate guanylyl cyclases.

Discovered almost fourty years ago, cGMP for a long time has been considered as a "younger and poorer brother" of cAMP. Although now its role is appreciated and largely

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Abbreviations: AC, adenylyl cyclase; ANF, atrial natriuretic factor; ANP, atrial natriuretic peptide; ARM, ATP-regulatory module; BNP, brain natriuretic peptide; CD, catalytic domain; CNP, natriuretic peptide type C; DD, dimerisation domain; ECD, extracellular domain; GC, guanylyl cyclase; GCAP, guanylyl cyclase activating protein; pGC, particulate GC; sGC, soluble GC; GC-A to GC-G, isoforms of pGCs; KLD, kinase-like domain; NCS, neuronal calcium sensor; PDE, phosphodiesterase; PKA, protein kinase A; ROS, rod outer segment; TMD, transmembrane domain.

studied in such important processes as visual signal transduction, maintenance of blood pressure, aggregation of platelets, function of kidney and heart, it is still unclear in many intracellular mechanisms. Cyclic GMP may serve as a subtle switch between different signal transduction pathways. The nucleotide is an activator of protein kinases (Lohman *et al.,* 1997), can activate or inhibit phosphodiesterases of cAMP (Beavo, 1995) and directly regulates the opening of cationic channels (Finn *et al.,* 1996). The intracellular level of the nucleotide is controlled by the activity of opposite enzymes: synthesizing guanylyl cyclases (GCs) and hydrolyzing phosphodiesterases (PDEs). In vertebrates, guanylyl cyclases are found in two forms: cytosolic (soluble, sGCs) and membrane bound (particulate, pGCs). Both forms of the GCs synthesize cGMP in a similar way using GTP as a substrate and Mg^{2+} or Mn^{2+} as cofactors in the reaction that involves release of inorganic pyrophosphate and inversion of configuration at the α phosphorus atom (Senter *et al.,* 1983; Koch *et al.,* 1990). This mechanism demonstrates substrate stereospecificty and is similar to that recognized in adenylyl cyclases (Eckstein *et al.,* 1981). The stereochemical properties of catalysis were utilized in developing new assays of guanylyl cyclase activity (Gorczyca *et al.,* 1994b; Gorczyca, 2000). Soluble and particulate cyclases have different structures and are activated in different ways. Soluble GCs are heterodimers of α and β polypeptide chains, contain heme prosthetic group and can be activated by NO or CO (Koesling, 1998; Schmidt *et al.,* 1993). The activity of particulate cyclases appears to be regulated in a more complex way. The past decade uncovered new molecular mechanisms that underlie these regulations and new isoenzymes were identified (Garbers & Lowe, 1994; Wedel & Garbers, 1998). Here we present a short review on known mechanisms that govern regulation of mammalian particulate guanylyl cyclases activity.

DISTRIBUTION of pGCs

Particulate cyclases have been found in almost all tissues and cell types, however, their distribution is isoform specific. Seven different isoforms of the enzyme, in rat termed GC-A through GC-G (Wedel & Garbers, 1998), were identified to date in vertebrates (Table 1). Among them, GC-A appears to be the most widely expressed throughout the body in different cell types and was found in cells of the nervous system, cells of the immune system, kidney, lung, intestine, testis, and heart (Chinkers *et al.,* 1989; Drewett & Garbers, 1994; Kiemer & Vollmar, 1998; O'Dorissio *et al.,* 1984). Cyclase GC-B is also expressed in different organs including brain, kidney, lung, spleen, testis, eye (Chen & Burnett, 1998; Drewett *et al.,* 1995; Duda *et al.,* 1993b). Interestingly, GC-B recently was reported to be very abundant GC in the pineal gland of the rat (Müller *et al.,* 2000). The first reports localized GC-C almost exclusively in the intestinal tract, mainly in the small intestine, but it is also present in the liver, uterus, and kidney (Bakre *et al.,* 2000; Khare *et al.,* 1994; Krause *et al.,* 1997; Schulz *et al.,* 1990). Expression of this particulate cyclase varies in different colorectal cancer cell lines and for that reason GC-C has been suggested as a diagnostic and prognostic marker in colorectal cancers (Waldman *et al.,* 1998 and references therein). Cyclase GC-D is expressed mainly in olfactory cells (Fülle *et al.,* 1995).

Particulate cyclases GC-E and GC-F have been found mainly in the photoreceptor cells of the vertebrate retina and they are also termed retinal GCs (RetGC1 and RetGC2 in humans) or photoreceptor-specific GCs (Goraczniak *et al.,* 1994; 1997; Koutalos *et al.,* 1995; Lowe *et al.,* 1995; Shyjan *et al.,* 1992; Yang *et al.,* 1995; Yang & Garbers, 1997). Cyclase GC-E was also detected in the pineal gland of rat, bovine and chick (Venkataraman *et al.,* 1998; 2000; Yang *et al.,* 1995; Semple-Rowland *et al.,* 1999). The mRNA for

the last discovered particulate cyclase, namely GC-G, was found in lung, intestine, and skeletal muscle (Schulz *et al.,* 1998).

STRUCTURE OF pGCs

The first data concerning the structure of particulate GCs have been available since 1988 when guanylyl cyclase from the sea urchin sperm plasma membrane was cloned (Singh *et al.,* 1988). Soon, three different mammalian membrane-bound GCs were cloned (Chinkers *et al.,* 1989; Schulz *et al.,* 1989; 1990). The analysis of sequences revealed the same structural topology of all cloned pGCs: (1) a large N-terminal extracellular domain (ECD), which consists of about 500 amino-acid residues with several cysteines in conserved positions, (2) a single membrane-spanning domain (TMD, transmembrane domain) of about 25 amino acids in length, and (3) a large (500–600 amino-acid residues) intracellular domain. In the intracellular part of the enzymes, three structurally and functionally different regions are distinguished: (1) about 250 amino-acid residue domain containing sequences similar to sequences found in protein kinases, which is referred to as kinase-like domain (KLD), (2) about 250 amino-acid residue C-terminal region that has been recognized as the catalytic domain (CD), and (3) located between KLD and CD as about 50 amino-acid residue domain that is suggested to be a dimerization domain (DD) (Wilson & Chinkers, 1995). This structural organization is common for all particulate cyclases cloned to date in vertebrates

Table 1. Family of particulate cyclases

Isoenzyme	Ligand(s)	Other regulators of activity	Localization	Putative site of ATP binding (ARM)
GC-A (NPR-A, ANF-RGC)*	ANP>BNP**	ATP	Lung, kidney, brain, thymus, smooth muscle, retina, macrophages, neutrophiles, thymocytes, T lymphocytes	$G^{503}xG^{505}xxxG^{509}$ (Goraczniak *et al.*, 1992)
GC-B (NPR-B, CNP-RGC)	CNP	ATP	Lung, kidney, brain, thymus, smooth muscle, retina	$G^{499}xxxG^{503}$ (Duda *et al.*, 1993a)
GC-C (STa-R, STa-RGC)	Guanylin, uroguanylin, STa	ATP, PKC	Epithelial cells of the gastrointestinal tract (mainly small intestine), kidney, liver, uterus	Not present
GC-D	unknown	?	Olfactory cells	$G^{617}xxxG^{621}$ (Fülle *et al.,* 1995)
GC-E (RetGC1, ROS-GC1)	unknown	Ca^{2+}/GCAP1, Ca^{2+}/GCAP2, Ca^{2+}/GCAP3, Ca^{2+}/CD-GCAP, Ca^{2+}/neurocalcin, ATP	Retina (photoreceptors-ROS) pineal gland	$G^{502}xxxG^{506}$ (Goraczniak *et al.*, 1994; Lowe *et al.*, 1995; Shyjan *et al.*, 1992; Yang *et al.*, 1995)
GC-F (RetGC2, ROS-GC2)	unknown	Ca^{2+}/GCAP2, Ca^{2+}/GCAP3, Ca^{2+}/GCAP1 (?), ATP (?)	Retina (photoreceptors)	$G^{471}xxxG^{475}$ (Lowe *et al.*, 1995)
GC-G	unknown	?	Lung, intestine, sceletal muscle	$G^{575}xxxG^{579}$ (Schulz *et al.*, 1998)

*The names of human and bovine orthologs of rat pGCs are shown in parenthesis; **abbreviations are explained in the text

(Wedel & Garbers, 1998) and is presented schematically in Fig. 1.

Extracellular domains of cyclases GC-A, GC-B, and GC-C are the sites of peptide hormones binding. Intracellular domains are the sites of interaction with identified (in the case of GC-E and GC-F) and probably with so far unidentified proteins, supposedly kinases and/or phosphatases, which in turn regulate the activity of the enzyme. The similarity among different cyclases is, as one could predict, the highest in their catalytic domains and the lowest in extracellular domains. Alignment of amino-acid sequences reveals that cyclases GC-E and GC-F are the most homologous and, with cyclase GC-D, form a subfamily. Similarly, cyclases GC-A, GC-B, and GC-G form a second subfamily. Cyclase GC-C reveals a low homology with other pGCs (Schulz *et al.,* 1998).

REGULATION OF ACTIVITY

As noted above, the activity of particulate cyclases appears to be regulated in many different ways, however, all these regulatory mechanisms lead to the stabilization or destabilization of the active catalytic centre(s) formed as a result of the interaction of at least two monomeric pGCs (Fig. 1) (Hurley, 1998). The activation mechanisms of pGCs include: extracellular ligand binding (cyclase A, B, C and possibly D), phosphorylation of KLD domain (GC-A, GC-B, GC-C), and intracellular interaction with calcium-binding proteins (GC-E, GC-F and possibly GC-D). The specific mechanisms of GC-G activation are unknown. The desensitization of the enzymes is still an unclear process but in the case of cyclases GC-A, GC-B, and GC-C it is promoted by extracellular ligand binding (Bakre *et al.,*

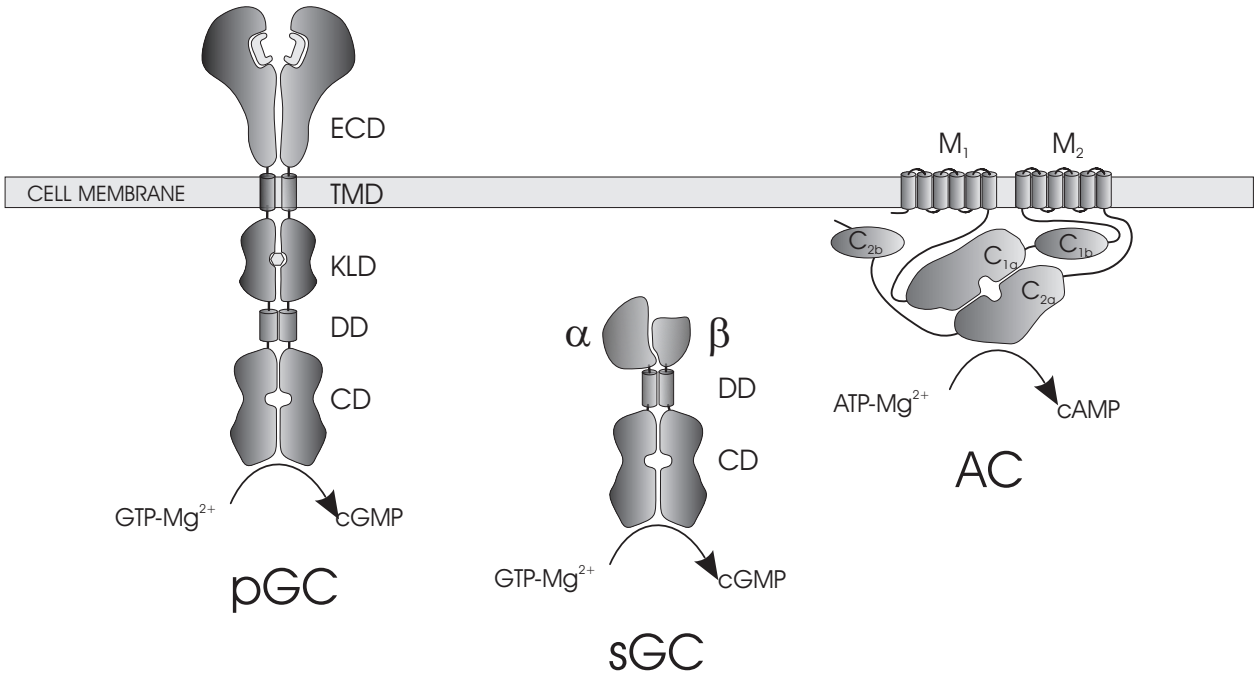

Figure 1. The domain structure of cyclases.

The single polypeptide chain of particulate cyclases (pGC) consists of: extracellular domain (ECD), single transmembrane domain (TMD), intracellular kinase-like domain (KLD), and single catalytic domain (CD). Particulate cyclases function as homodimers, whose formation is dependent on the existence of dimerization domain (DD). Soluble GCs (sGC) function as heterodimers of α and β polypeptide chains, each containing one catalytic domain. The single polypeptide chain of mammalian adenylyl cyclases (AC) contains: two transmembrane domains (M_1 and M_2) that contain six α-helices each, and two cytoplasmic regions C_1 and C_2 possesing homologous regions C_{1a} and C_{2a} that form together catalytic centre (Tang & Hurley, 1998). The catalytic domains of all known GCs are homologous to the C_{1a} and C_{2a} domains.

2000; Huo *et al.,* 1999; Labrecque *et al.,* 1999; Parkinson *et al.,* 1994) and it involves serine/threonine residues dephosphorylation of the KLD of GC-A and GC-B (Potter, 1998; Potter & Hunter, 1998). The activity of retinal cyclases GC-E and GC-F is supressed by specific calcium-binding proteins at high calcium ions concentration (Gorczyca, 1999; 2000).

Dimerization

A hypothesis that, to express their catalytic activity, pGCs need assembling to create at least dimeric form came from two observations: (1) adenylyl cyclases possess two putative catalytic domains and both of these domains are needed in an intact form for enzyme activity (Hurley, 1998; Tang & Hurley, 1998), and (2) soluble GCs were active only when their α and β subunits were coexpressed (Garbers & Lowe, 1994 and references therein). Reports of Lowe (1992) and later of Vaandrager *et al.* (1993, 1994) indicated that oligomeric forms of GC-A and GC-C preexist in the cells independently of ligand binding. It was also shown by Wilson & Chinkers (1995) and by Rudner *et al.* (1995) that oligomerization/dimerization is obligatory for enzyme activity and that the amphipatic region located between the KLD and CD, containing about 50 amino acids, is responsible for dimerization. Retinal cyclases have also been shown to form oligomeric/dimeric forms (Duda *et al.,* 1996; Tucker *et al.,* 1999; Yu *et al.,* 1999) with the homodimer form preferred (Yang & Garbers, 1997). Thus dimerization of pGCs seems to be obligatory for their activities. A recent report of Huo *et al.* (1999), however, suggests that dimerization depends only on ligand binding and preexisting oligomers are formed spontaneously only when pGCs are expressed in cells at high concentrations.

Peptide ligands

All particulate cyclases possess in their structures extracellular domains but only three of them are receptors for known extracellular ligands, while others are still orphan receptors (Table 1). Cyclase GC-A binds with high affinity to its extracellular domain a peptide hormone known as atrial natriuretic peptide (ANP) or atrial natriuretic factor (ANF) (Chinkers & Garbers, 1989). Also another peptide named brain natriuretic peptide (BNP) is able to bind to this cyclase receptor (Garbers & Lowe, 1994). Both peptides are able to stimulate the activity of cyclase independently. Cyclase GC-B is activated by a third natriuretic peptide, known as natriuretic peptide type C or CNP. All natriuretic peptides contain a common structural motif, which contains a ring formed by 17 amino-acid residues, 10 of which are conserved in each peptide (Chen & Burnett, 1998; Rosenzweig & Seidman, 1991). Cyclase GC-C is activated by the endogenous peptides guanylin or uroguanylin and can also be activated by exogenous bacterial thermostable enterotoxins (for example thermostable enterotoxin from *Escherichia coli*, STa), which are not related to natriuretic peptides (Krause *et al.,* 1997; Waldman *et al.,* 1998). Cyclases GC-D through GC-G are not activated by known ligands for other cyclases. Alignments of sequences of extracellular domains of these cyclases reveal a similarity to metabotropic glutamate receptors and it is speculated that ligands of these cyclases may not have peptide character (Wedel & Garbers, 1998). Some indications suggest that non-mammalian cyclases of GC-D type might be receptors for different odorants (Yu *et al.,* 1997; Dittman *et al.,* 1997).

Adenine nucleotides

Many years before the structure of pGCs were determined, adenine nucleotides had been known to enhance the activity of membrane-bound cyclases. ATP and its non-hydrolysable analogues were able to stimulate the activity of the cyclase that responded to ANP and was present in different rat organs

(Kurose *et al.,* 1987). The effect of adenine nucleotides was synergistic with ANP. The agents only slightly activated the enzyme when used separately but high activation of GC was observed in the presence of both. An important observation of these authors was that the effect of ATP was only observed when Mg^{2+} was used as a cofactor. In the presence of Mn^{2+}, the effect of ATP was insignificant. Later studies have shown that kinase-like domain in cyclase GC-A is responsible for the ATP effect (Chinkers & Garbers, 1989). Deletion of this domain resulted in permanent activity of GC-A independently of the presence of ANP or ATP. Thus, the kinase-like domain was suggested to be an inhibitory domain in GC-A, important for the transmission of the extracellular signal from ECD to CD, and a putative site of the direct ATP binding (Chinkers & Garbers, 1989; Chinkers *et al.,* 1991; Goraczniak *et al.,* 1992). The sites responsible for the ATP effect were recognized in GC-A and GC-B and it was proposed that the sequences GlyxGlyxxxGly in GC-A and GlyxxxGly in the GC-B, named the ATP-regulatory module (ARM), were responsible for the effect (Goraczniak *et al.,* 1992; Duda *et al.,* 1993a). The adenine nucleotide stimulatory properties, however, were observed for most pGCs (Chinkers *et al.,* 1991; Duda *et al.,* 1993b; Gazzano *et al.,* 1991a; 1991b; Gorczyca *et al.,* 1994b; Parkinson *et al.*, 1994; Tucker *et al.,* 1997; Vaandrager *et al.,* 1993), even those not bearing the ARM in their structures (Table 1).

Calcium and calcium-binding proteins

Photoreceptor specific guanylyl cyclase, also termed retinal GC (RetGC), belongs to the group of particulate guanylyl cyclases sharing their overall structure (Shyjan *et al.,* 1992). The mechanism of activation, however, appears to be quite different for RetGC than for other members of the family. RetGC is regulated in a Ca^{2+}-dependent manner and is insensitive to peptide ligands that activate other particulate cyclases. It has been known for a long time that guanylyl cyclase activity in rod outer segments (ROS) of the photoreceptor cells was negatively regulated by Ca^{2+} (Lolley & Racz, 1982). The enzyme was active at low (below 100 nM) and inactive at high (over 500 nM) concentrations of Ca^{2+}. Koch & Stryer (1988) were the first who postulated the participation of a soluble factor, possibly a protein, in this Ca^{2+}-dependent regulation of the enzyme activity. The search for the putative regulator of RetGC resulted in the identification of two different calcium-binding proteins in the bovine retina, which activated the enzyme below a 100 nM concentration of Ca^{2+}. The first of them was isolated from the outer segments of the photoreceptors (Gorczyca *et al.,* 1994a), cloned and named GCAP (guanylyl cyclase activating protein) (Palczewski *et al.,* 1994). The second activator of retinal GC was purified from the whole retinas and originally named p24 because of its apparent electrophoretical mobility (Dizhoor *et al.,* 1994). Its sequence was found to be in 42% identical with GCAP (now termed GCAP1) and the protein was named GCAP2 (Dizhoor *et al.,* 1995; Gorczyca *et al.,* 1995). The third member of the GCAP subfamily, GCAP3, was recently cloned from the human retina (Haeseleer *et al.,* 1999). GCAPs are proteins primary expressed in the photoreceptor cells of the retina, however, they were also detected in the bovine (Venkataraman *et al.,* 2000) and rat (Dejda & Gorczyca, unpublished) pineal glands. The expression of genes encoding GCAP1 and GCAP2 were also detected in the chicken pineal gland (Semple-Rowland *et al.,* 1999). Two isoforms of retina-specific GCs (GC-E and GC-F) were identified in mammalian retina to date (Goraczniak *et al.,* 1994, 1997; Lowe *et al.,* 1995; Shyjan *et al.,* 1992; Yang *et al.,* 1995). Both are regulated in a calcium-dependent manner by GCAPs, however, only GCAP2 and GCAP3 were shown to activate human RetGC1 and RetGC2, while GCAP1 activated only RetGC1 (Haeseleer *et al.,* 1999). The mechanism of regulation of RetGCs activity by GCAPs is unknown but it

probably involves their direct interaction with intracellular domains (KLD, CD) of the enzyme and dimerization (Duda *et al.,* 1996; Laura *et al.,* 1996; Laura & Hurley, 1998; Olshevskaya *et al.,* 1999b; Sokal *et al.,* 1999; Tucker *et al.,* 1997; 1999; Yu *et al.,* 1999). The sites that apparently are involved in regulation of effector cyclase were recognized in GCAP1 and GCAP2 (Krylov *et al.,* 1999; Otto-Bruc *et al.,* 1997; Olshevskaya *et al.,* 1999a; Schrem *et al.,* 1999). It is important to precisely establish the sites of interaction of both GCAPs within the intracellular domains of cyclase since it was shown that their stimulatory activity is not additive (Gorczyca *et al.,* 1995). This observation has suggested common or overlapping sites, in contrast to models proposed by others (Pugh *et al.,* 1997). GCAPs apparently form a complex with target enzymes independently of calcium concentration (Duda *et al.,* 1996; Gorczyca *et al.,* 1995). Thus, conformational changes induced by Ca^{2+} in GCAPs structure are transmitted to the adjacent cyclase and act as a switch between inhibition and activation. All GCAPs belong to the larger family of structurally related proteins, named neuronal calcium sensors (NCS) (reviewed by Braunewell & Gundelfinger, 1999). All members of this family have a similar molecular mass (22–24 kDa), 4 characteristic structural motifs known as EF-hands (which are potential sites of Ca^{2+} binding) and are acylated by small fatty acids at the N-terminal glycine. Recently, two other calcium-binding proteins have been shown to activate bovine RetGC at Ca^{2+} concentrations over 2 μM. The first of them was termed calcium-dependent GCAP (Pozdnyakov *et al.,* 1995), later identified as S100B protein (Margulis *et al.,* 1996), and the regulatory mechanism was shown to involve the catalytic domain of target cyclase. The second protein was neurocalcin, which also belongs to the NCS family (Kumar *et al.,* 1999). These findings extend the calcium-dependent regulation of the RetGCs to proteins that are expressed not only specifically in the retina and it is tempting to search if such regulatory mechanisms might be more general and present in cells outside the nervous system.

Phosphorylation

As it has been recently shown by Potters, the phosphorylated state is essential for the activity of cyclases GC-A and GC-B (Potter, 1998; Potter & Hunter, 1998). Six serine/threonine residues from the KLD region are phosphorylated and the mutation of any of them caused a reduction in the cyclase phosphorylation state and activity. These findings were preceded by earlier observations that particulate cyclases from the sea urchin sperm existed in a highly phosphorylated state and a rapid dephosphorylation took place after specific peptide ligand binding (reviewed by Garbers & Lowe, 1994). It is not yet clear if phosphorylation/dephosphorylation is a common regulatory mechanism for all particulate cyclases, however, the guanylyl cyclase purified from the bovine retina was recently reported to be an autophosphorylating kinase (Aparicio & Applebury, 1996). Phosphorylation is also one of the mechanisms regulating the activity of intestinal guanylyl cyclase (GC-C). It involves Ca^{2+} and protein kinase C (Khare *et al.,* 1994) and is probably different from the one observed in GC-A and GC-B. Phosphorylation of particulate cyclase present in olfactory receptor neurons by cAMP-dependent protein kinase (PKA) negatively influenced the enzyme activity (Moon *et al.,* 1998). Observations from our laboratory indicate that particulate cyclase(s) present in rat macrophages have to remain in the phosphorylated state to express their activity (Kobiałka & Gorczyca, unpublished). All the above data indicate that phosphorylation/dephosphorylation is a very important regulatory mechanism in the functioning of pGCs. It is still an open question, however, which kinases and phosphatases are involved in that mechanism.

CONCLUDING REMARKS

Perhaps the most important observation was that mutants of pGCs that contained only catalytic and dimerization domains were able to catalyze the conversion of GTP to cGMP (Wilson & Chinkers, 1995; Rudner *et al.*, 1995). Thus, the presence of the kinase-like domains, transmembrane domains, and extracellular domains from the chemical point of view is spare, however, they keep the enzyme activity under control in the specific cellular environment and trigger it only when needed. There is one common mechanism of catalysis in pGCs, regulated in different ways, which are specific for each isoenzyme and the site of its expression.

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