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Guanylyl Cyclases and Signaling by Cyclic GMP

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***Abstract*——Guanylyl cyclases are a family of enzymes that catalyze the conversion of GTP to cGMP. The family comprises both membrane-bound and soluble isoforms that are expressed in nearly all cell types. They are regulated by diverse extracellular agonists that include peptide hormones, bacterial toxins, and free radicals, as well as intracellular molecules, such as calcium and adenine nucleotides. Stimulation of guanylyl cyclases and the resultant accumulation of cGMP regulates complex signaling cascades through immediate downstream effectors, including cGMP-dependent protein kinases, cGMP-regulated phosphodiesterases, and cyclic nucleotide-gated ion channels. Guanylyl cyclases and cGMP-mediated signaling cascades play a central role in the regulation of diverse (patho)physiological processes, including vascular smooth muscle motility, intestinal fluid and electrolyte homeostasis, and retinal phototransduction. Topics addressed in this review include the structure and chromosomal localization of the genes for guanylyl cyclases, structure and function of the members of the guanylyl cyclase family, molecular mechanisms regulating enzymatic activity, and molecular sequences coupling ligand binding to catalytic activity. A brief overview is presented of the downstream events controlled by guanylyl cyclases, including the effectors that are regulated by cGMP and the role that guanylyl cyclases play in cell physiology and pathophysiology.**

I. Introduction

Guanylyl cyclases have evolved to synthesize cGMP in response to diverse signals, such as nitric oxide (NO),[2] peptide ligands, and fluxes in intracellular Ca^{2+} ($[Ca^{2+}]_i$). These signals use specific guanylyl cyclase-coupled receptors and cofactors to initiate the conversion of the cytosolic purine nucleotide GTP to cGMP. Intracellular cGMP ($[cGMP]_i$) regulates cellular physiology by activating protein kinases, directly gating specific ion channels, or altering intracellular cyclic nucleotide concentrations through regulation of phosphodiesterases (PDEs). The structure and function of the family of guanylyl cyclases, the molecular mechanisms regulating their activities, and the downstream effectors that underlie the physiology of cGMP-dependent processes are summarized in this review.

[2] Abbreviations: NO, nitric oxide; $[Ca^{2+}]_i$, intracellular calcium; AMPS, adenosine-5′-*O*-(3-thiomonophosphate); ANP, atrial natriuretic peptide; ANPCR, ANP clearance receptor; ATPγS, adenosine-5′-*O*-(3-thiotriphosphate); BNP, brain natriuretic peptide; CFTR, cystic fibrosis transmembrane conductance regulator; $[cGMP]_i$, intracellular cGMP; CNG channel, cyclic nucleotide-gated channel; CNP, C-type natriuretic peptide; CO, carbon monoxide; EGFR, epidermal growth factor receptor; EC_{50}, concentration of ligand yielding a half-maximum response; eNOS, endothelial nitric oxide synthase; GC, guanylyl cyclase; GCAP, guanylyl cyclase activating protein; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; G protein, heterotrimeric G protein; GST, glutathione *S*-transferase; K_d, concentration of ligand yielding half-maximum binding; KHD, kinase homology domain; K_i, concentration of ligand yielding half-maximum inhibition; K_m, concentration of substrate yielding half-maximum velocity; PCR, polymerase chain reaction; PDE, phosphodiesterase; pGC, particulate guanylyl cyclase; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PPIX, protoporphyrin IX; PAGE, polyacrylamide gel electrophoresis; sGC, soluble guanylyl cyclase; SNP, sodium nitroprusside; ST, heat-stable enterotoxin; V_{max}, maximum enzyme velocity; NOS, NO synthase; HCN, hyperpolarization-activated cy-

II. Guanylyl Cyclases

A. Molecular Biology

1. Identification of the Members of the Guanylyl Cyclase Family. It was established by the mid-1970s that guanylyl cyclase activity was found in both the soluble and particulate fractions of most cells (Hardman and Sutherland, 1969; Ishikawa et al., 1969; Schultz et al., 1969; White and Aurbach, 1969), and that these activities were due to different proteins (Garbers and Gray, 1974; Kimura and Murad, 1974; Chrisman et al., 1975). However, only with the development of molecular cloning techniques more than a decade later could the breadth of this enzyme family be fully explored (Tables 1 and 2). Purification of guanylyl cyclase from the cytosolic compartment revealed the soluble isoform was a heterodimer composed of α- and β-subunits. The β-subunit had a molecular mass of ~70 kDa, whereas the α-subunit was reported to be 73 to 82 kDa (Gerzer et al., 1981c; Kamisaki et al., 1986). Soluble guanylyl cyclase (sGC) was purified to apparent homogeneity from bovine or rat lungs (Koesling et al., 1988, 1990; Nakane et al., 1988, 1990). Degenerate oligonucleotide probes based on the structure of purified subunits were used to screen cDNA libraries and thereby clone α1- and β1-subunits. The C-terminal region of both subunits had a high degree of sequence identity with cloned adenylyl and particulate guanylyl cyclases (pGCs), suggesting this was the catalytic domain. Sodium nitroprusside (SNP)-sensitive guanylyl cyclase activity was expressed when the cloned cDNAs for α1 and β1 were cotransfected into a heterologous cell system, but not when transfected individually (Harteneck et al., 1990; Nakane et al., 1990). These data demonstrated both subunits of sGC are required for basal and nitrovasodilator-stimulated catalytic activity.

Studies of pGCs suggested a new paradigm for signal transduction. Sea urchin sperm is one of the richest sources of pGC. In echinoderms, peptides secreted by eggs activate pGC of sperm in a species-specific manner (Suzuki et al., 1984; Ramarao and Garbers, 1985). Moreover, radiolabeled egg peptides could be chemically cross-linked to a sperm cell surface protein of the same size as that recognized by antiserum against guanylyl cyclase (Shimomura et al., 1986). These observations suggested that pGC might also serve as a receptor for peptide ligands. While these studies were being conducted in the sea urchin, atrial natriuretic peptide (ANP) was demonstrated to activate guanylyl cyclase and to increase $[cGMP]_i$ in mammalian tissues (Hamet et al., 1984; Waldman et al., 1984; Winquist et al., 1984). Subsequently, ANP binding and guanylyl cyclase activity were copurified, strongly suggesting the two activities reside in a single molecule (Kuno et al., 1986; Paul et al., 1987; Shimonaka et al., 1987; Meloche et al., 1988). In 1988, pGC was first cloned from a sea urchin testis cDNA library using probes based on tryptic peptides obtained from the purified protein (Singh et al., 1988). This clone provided the necessary probe for isolating mammalian cDNAs encoding pGCs. The natriuretic peptide receptors, guanylyl cyclase A (GC-A) and B (GC-B), were the first pGCs cloned from mammalian tissues (Chang et al., 1989; Chinkers et al., 1989; Lowe et al., 1989; Schulz et al., 1989). The deduced primary sequences of the natriuretic peptide receptors predicted a protein with a single transmembrane domain that divides an extracellular ligand-binding domain from an intracellular domain. Deletion mutagenesis studies have demonstrated that the intracellular domain serves regulatory, dimerization, and catalytic functions (Chinkers and Garbers, 1989). This regulatory domain has sequence similarity with protein kinases, particularly the protein tyrosine kinases, which are also single transmembrane domain receptors (Singh et al., 1988). The sequences of the C-terminal catalytic domains are highly homologous to those of the α- and β-subunits of sGC and have limited identity with the two catalytic domains of adenylyl cyclases (Krupinski et al., 1989; Thorpe and Garbers, 1989).

Development of the polymerase chain reaction (PCR) facilitated the search for new members of the guanylyl cyclase family. Degenerate PCR primers based on conserved amino acid sequences in the catalytic domains of both sGCs and pGCs were used to preferentially amplify guanylyl, as opposed to adenylyl, cyclases and yielded sequences of a second α- and a second β-subunit of sGC and five unique pGC sequences (GC-C to GC-G) (Yuen et al., 1990; Harteneck et al., 1991). A third pair of sGC subunits, cloned by screening a human cDNA library with rat cDNA clones, is most likely the human ortholog of α1/β1 (Giuili et al., 1992). Guanylyl cyclase C (GC-C) is the receptor for the bacterial heat-stable enterotoxins (STs) (Schulz et al., 1990; de Sauvage et al., 1991), and for the endogenous peptides guanylin and uroguanylin (Currie et al., 1992; Hamra et al., 1993). The remaining cloned mammalian pGCs are orphan receptors without known extracellular ligands. Guanylyl cyclase D (GC-D)

TABLE 1
Soluble guanylyl cyclase isoform chromosomal localization and tissue distribution

Subunit	Chromosome Location[a]	Tissue Distribution
α1 (α3)	4q31.3–q33 (Giuili et al., 1993)[b]	Lung (Koesling et al., 1990); cerebellum, cerebrum, heart, kidney, liver, lung, skeletal muscle (Nakane et al., 1990); kidney (Ujiie et al., 1993)
α2	11q21–q22 (Yu et al., 1996)	Brain, retina (Harteneck et al., 1991); kidney (Ujiie et al., 1993); placenta (Russwurm et al., 1998)
β1 (β3)	4q31.3–q33 (Giuili et al., 1993)	Lung (Koesling et al., 1988); cerebellum, cerebrum, heart, kidney, liver, lung, skeletal muscle (Nakane et al., 1990); kidney (Ujiie et al., 1993); placenta (Russwurm et al., 1998)
β2	13q14.3 (Behrends et al., 1999)	Kidney, liver (Yuen et al., 1990); kidney (Ujiie et al., 1993)

[a] Human chromosome location.

TABLE 2
Particulate guanylyl cyclase isoforms, ligand and cofactor specificities, chromosomal localization, and tissue distribution

Receptor	Ligand(s)	Cofactors	Chromosome Location[a]	Tissue Distribution
GC-A (NPR-A)	ANP, BNP	ATP	1q21–q22 (Lowe et al., 1990b)[b]	Adipose tissue, adrenal gland, ileum, kidney, placenta (Lowe et al., 1989); adrenal gland, cerebellum, heart, kidney, pituitary (Wilcox et al., 1991); lamina propria (Li and Goy, 1993); cochlea (Furuta et al., 1995); thymus (Vollmar et al., 1996); ovary (Jankowski et al., 1997)
GC-B (NPR-B)	CNP	ATP	9p12–p21 (Lowe et al., 1990b)	Placenta (Chang et al., 1989); adrenal medulla, cerebellum, pituitary (Wilcox et al., 1991); adrenal gland, aorta, atrium, cerebellum, lung, intestine, pituitary, testis, ventricle (Ohyama et al., 1992); uterus/oviduct (Chrisman et al., 1993); thymus (Vollmar et al., 1996); ovary (Jankowski et al., 1997)
GC-C	ST, Guanylin, Uroguanylin		12p12 (Mann et al., 1996b)	Intestinal mucosa (Li and Goy, 1993); regenerating liver (Laney et al., 1994)
GC-D	?		11p15.4 or 11q13–q14.1[c] (Yang et al., 1996)	Olfactory epithelium (Fülle et al., 1995)
GC-E (Ret GC-1)	?	GCAP 1,2,3	17p13.1 (Oliveira et al., 1994)	Retina, pineal gland (Yang et al., 1995)
GC-F (Ret GC-2)	?	GCAP 2,3	Xq22 (Yang et al., 1996)	Retina (Yang et al., 1995)
GC-G	?		10q24–q26c (Schulz et al., 1998b)	Intestine, kidney, lung, skeletal muscle (Schulz et al., 1998b)

[a] Human chromosome location.
[b] References.
[c] Inferred from mouse chromosomal assignment.

is expressed in the olfactory neuroepithelium in a zonal pattern resembling that of the seven-transmembrane domain odorant receptors (Fülle et al., 1995). Two other members of the sensory tissue subfamily of guanylyl cyclases, guanylyl cyclase E (GC-E, retGC-1) and guanylyl cyclase F (GC-F, retGC-2), are expressed in retina (Shyjan et al., 1992; Lowe et al., 1995; Yang et al., 1995). GC-E also is expressed in the pineal gland (Yang et al., 1995). Although these enzymes are orphan receptors, their extracellular domains are homologous to that of GC-D and share a similar arrangement of cysteine residues in the extracellular domain with the other pGCs. This suggests they may have an extracellular ligand, although the catalytic activity of the retinal cyclases is regulated by $[Ca^{2+}]_i$ through guanylyl cyclase-activating proteins (GCAPs). The recently cloned GC-G most closely resembles the natriuretic peptide receptors, although it is not activated by natriuretic peptides (Schulz et al., 1998b). Apparently, the family of mammalian guanylyl cyclases is relatively small because low stringency library screening and degenerate PCR have not yielded an abundance of unique cDNAs. In contrast, *Caenorhabditis elegans* has approximately 30 genes encoding guanylyl cyclase-like sequences and is seemingly rich in cGMP-coupled pathways (Yu et al., 1997).

2. Structure and Location of Guanylyl Cyclase Genes.

The chromosomal loci of the genes encoding isoforms of guanylyl cyclase and their ligands have been mapped in the human and/or the mouse (Tables 1, 2) and are unlinked and scattered throughout the genome, with notable exceptions. Thus, the genes encoding the natriuretic peptide ligands for GC-A, ANP and brain natriuretic peptide (BNP) are organized in tandem in both

et al., 1996b). Similarly, guanylin and uroguanylin, the endogenous activators of GC-C, are encoded by closely linked genes (Whitaker et al., 1997). Retinal guanylyl cyclase activity is regulated by GCAPs, which are calcium-binding proteins. To date, three members of the GCAP family have been identified. GCAP1 and GCAP2 are found in a tail-to-tail arrangement on human chromosome 6, whereas GCAP3 is located on chromosome 3 (Subbaraya et al., 1994; Haeseleer et al., 1999).

The genes encoding human sGC subunits $\alpha 3$ (equivalent to $\alpha 1$) and $\beta 3$ (equivalent to $\beta 1$) have been mapped to chromosome 4q32 (Giuili et al., 1993). Because both subunits are required in a 1:1 stoichiometry for activity, their common chromosomal locus may imply a coordinated regulation of gene expression. The genes encoding $\alpha 1$ and $\beta 1$ sGC subunits in the medaka fish are organized in tandem within a 34-kb span (Mikami et al., 1999). The activity of the 5′-upstream region of each of the medaka fish genes was analyzed using green fluorescent protein reporter constructs expressed in medaka embryos (Mikami et al., 1999). Although the $\alpha 1$ upstream region promoted expression of green fluorescent protein, the $\beta 1$ 5′ region was insufficient, suggesting expression of the $\alpha 1$- and $\beta 1$-genes is coordinated. However, the $\alpha 2$-subunit, which also can form an active dimer in vitro with $\beta 1$, is encoded by a gene on chromosome 11 (Yu et al., 1996). That $\alpha 2$- and $\beta 1$-subunits dimerize under physiological conditions argues against the requirement for coordinated regulation of expression of α- and β-subunits (Russwurm et al., 1998).

The structure of several genes for pGC has been determined, and the organization of their domains is reflected in the conservation of the intron/exon arrange-

portion of the gene encoding the catalytic and kinase homology domains. The extracellular domains of the guanylyl cyclases are conserved among, but not between, subfamilies and the structure varies most in those parts of the genes. Genes for GC-A and -B are similar in size (16.5–17.5 kb) and structure, with 22 exons and virtually identical intron/exon boundaries (Yamaguchi et al., 1990; Rehemudula et al., 1999). However, the size of introns is not conserved between these genes. Similarly, the guanylyl cyclases in sensory tissue share a conserved gene structure and have only 20 exons (Yang et al., 1996). The gene for GC-C is much larger (>50 kb) than genes encoding the other guanylyl cyclases and has a unique intron/exon arrangement (S. Schulz, J. Park, and S. A. Waldman, unpublished data). The structures of the genes for sGC subunits have not yet been reported.

Little is known regarding the regulation of expression of the genes for guanylyl cyclase. The 5′ regulatory regions of genes that have been sequenced (GC-A, -C, -E) have no typical TATA box and an absent or inverted CAAT box. While consensus binding sites for many general transcription factors are present, the elements controlling tissue-specific expression are only now beginning to be explored. The GC-A gene promoter has at least three consensus binding sites for Sp1, a transcription factor that is implicated in the expression of a number of genes in the vasculature (Liang et al., 1999). Assays using electromobility shift and reporter gene techniques have demonstrated all three sites bind Sp1 and are essential for basal transcription of the GC-A gene (Liang et al., 1999). Expression of the gene for GC-A also is regulated by its ligand, ANP. Levels of GC-A mRNA were suppressed by ANP in a time- and concentration-dependent manner in cultured aortic smooth muscle cells (SMCs) and primary cultures of inner medullary collecting duct cells (Cao et al., 1995, 1998). A cell-permeable analog of cGMP also inhibited transcription of GC-A, suggesting the second messenger, rather than the natriuretic peptide, is responsible for modulating gene activity (Cao et al., 1995, 1998). The ANP/cGMP-responsive element in the promoter for GC-A has not been identified.

Whereas GC-A is expressed in a variety of cell types and in many tissues, expression of GC-C in the adult human appears to be confined to the intestinal epithelium and primary and metastatic colorectal cancers (Carrithers et al., 1996). In the marsupial North American opossum, a guanylyl cyclase-coupled ST receptor, possibly the opossum ortholog of GC-C, is expressed in epithelial cells of the kidney, liver, testis, trachea, and intestine (Forte et al., 1989; London et al., 1999). The mRNA for GC-C and binding of radiolabeled ST are detectable in neonatal and weanling mouse liver, and in fetal, neonatal, and regenerating rat liver (Laney et al., 1992, 1994; Scheving and Russell, 1996; Swenson et al., technique has been used to amplify the mRNA for GC-C in a number of tissues, production of cGMP in response to ST has only been observed outside the intestine in rodent stomach and inner ear (Krause et al., 1997; London et al., 1997).

An initial characterization of the 5′ flanking region of the gene for GC-C, using reporter gene constructs, suggested intestine-specific transcriptional activity lies within the proximal 128 bp (Mann et al., 1996a). An analysis of this region, which is conserved between the human and the mouse, revealed potential binding sites for several transcription factors. Hepatocyte nuclear factor-4 (HNF-4) binds to a specific element in the proximal promoter for GC-C and stimulates expression of GC-C when transfected into a cell line that normally expresses neither GC-C nor HNF-4 (Swenson et al., 1999). Mutation of the HNF-4 binding site abolished activity of the promoter for GC-C in intestinal cells, demonstrating that HNF-4 is necessary for basal gene expression (Swenson et al., 1999).

Recent observations suggest the transcription factor Cdx2 mediates the intestine-specific expression of GC-C. Cdx2 is a member of the homeodomain family of transcription factors related to caudal, a *Drosophila* protein, and is required for the selective expression of several other genes in intestinal tissues (Traber and Silberg, 1996). Deletion, or mutation, of a Cdx2 consensus binding site in the proximal GC-C gene promoter reduced the activity of a reporter gene construct expressed in intestinal cells to the level observed in extraintestinal cells (Park et al., 2000).

3. Genetic Disorders Associated with Guanylyl Cyclases. The only human diseases mapped to a gene for guanylyl cyclase involve retinal dystrophies. Leber's congenital amaurosis (LCA1), dominant cone-rod dystrophy (CORD6), cone dystrophy (CORD5), and central areolar choroidal dystrophy have been mapped to chromosome 17p12-p13, the interval containing the gene for GC-E (Balciuniene et al., 1995; Perrault et al., 1996; Hughes et al., 1998; Kelsell et al., 1998). In LCA1, the gene for GC-E contains mutations, including frameshifts, which result in truncated proteins that lack the kinase-like and catalytic domains due to premature termination of translation or a missense mutation in the kinase-like domain (Perrault et al., 1996). Expression of GC-E with this missense mutation in a heterologous cell line demonstrated that the mutant protein is stable but not activated by GCAP1 (Duda et al., 1999). In CORD6, GC-E contains mutations in the intracellular dimerization domain (Kelsell et al., 1998). It was postulated these mutations might cause a steric change in the protein that affects both mutant/mutant and mutant/wild-type dimers and thereby results in the dominant phenotype of CORD6. Indeed, one of the mutants has an increased affinity for GCAP-1, producing an enzyme that is stimulated at higher $[Ca^{2+}]_i$ than wild-type GC-E (Tucker et

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