REVIEW

Enterotoxin/Guanylin Receptor Type Guanylyl Cyclases in Non-mammalian Vertebrates

Mina Nakauchi and Norio Suzuki*

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan

Running title: Intestine-Specific membrane GC

∗Corresponding author. Tel.: +81-11-706-4908; Fax: +81-11-706-4461.

E-mail address: norio-s@sci.hokudai.ac.jp

ABSTRACT- Cyclic GMP is a ubiquitous intracellular second messenger produced by guanylyl cyclases (GCs). The enterotoxin/guanylin receptor type membrane GC (designated as GC-C in mammals) is activated by exogenous ligands such as heat-stable enterotoxins (STa), small peptides secreted by some pathogenic strains of *Escherichia coli* which cause severe secretory diarrhea and also activated by endogenous ligands such as guanylin and uroguanylin. The STa/guanylin receptor type membrane GC, as well as other type membrane GCs, is composed of an extracellular domain, a single transmembrane domain, and an intracellular region comprising a kinase-like domain and a catalytic domain. The STa/guanylin receptor type membrane GC is identified in various vertebrates including fishes, amphibians, reptiles, and birds, implying that it serves some important and undefined physiological roles in the intestine of non-mammalian vertebrates, e.g. the regulation of water and salt absorption. In mammals, only a single membrane GC (GC-C) is known to be the STa/guanylin receptor. On the contrary, two membrane GC cDNAs are cloned from the intestine of the European eel *Anguilla anguilla* (GC-C1 and GC-C2) and the medaka fish *Oryzias latipes* (OlGC6 and OlGC9). OlGC6 and OlGC9 are structurally distinct and show different ligand responsibility. Accumulated evidences indicate that the transcriptional regulatory mechanism of the human *GC-C* gene is different from that of the corresponding medaka fish *GC* gene; the human *GC-C* gene is regulated by Cdx2 and/or HNF-4, and the medaka fish *OlGC6* gene is regulated by OlPC4, which is a medaka fish homologue of the mammalian transcriptional positive co-factor 4 (PC4). Furthermore, the transcriptional regulatory mechanism of the *OlGC9* gene is different from those of both the *OlGC6* and human *GC-C* genes, indicating that the study on these two medaka fish GCs will be useful for further understanding of the STa/guanylin receptor type membrane GC in the vertebrates.

Key words: membrane guanylyl cyclase, cGMP, trimer, transcriptional positive co-factor 4, enterotoxin, guanylin, medaka fish, intestine

INTRODUCTION

Cyclic GMP is a ubiquitous intracellular second messenger produced by an ever-expanding family of guanylyl cyclases (GCs), which are classified into two major forms, those found in the plasma membrane (membrane GC) and those in the cytoplasm (soluble GC) (Kusakabe and Suzuki, 2000; Loretz and Pollina, 2000; Wedel and Garbers, 2001). The soluble GC is a heme-containing heterodimer and is activated by nitric oxide or carbon monoxide (Wedel and Garbers, 2001). The membrane GC is a protein having a single membrane-spanning region and is activated by various endogenous and exogenous peptides. The membrane GC is further divided into three subfamilies such as the natriuretic peptide (NP) receptors, the sensory organ-specific membrane GCs, and the enterotoxin/guanylin receptors (Fig. 1) (Kusakabe and Suzuki, 2000).

Heat-stable enterotoxins (STa), which are small peptides secreted by some pathogenic strains of *Escherichia coli*, are the first identified natural compound to activate a membrane GC in mammals (Field *et al*., 1978; Hughes *et al*., 1978). The receptor for STa has been shown to be located primarily on the apical or brush border membrane of mammalian intestinal epithelial cells which are known as a rich source of a membrane GC (De Jonge, 1975). Earlier works have demonstrated that STa actually serves as an extracellular activator of a membrane GC and it causes severe secretory diarrhea in mammals (Field *et al*., 1978; Hughes *et al*., 1978; Field *et al*., 1989). Subsequently, it has been shown that an intestinal membrane GC is different from the membrane GCs present in most other tissues which would be stimulated by NPs (Kuno *et al*., 1986; Waldman *et al*., 1986). In contrast, several initial studies on the membrane GC in mammalian intestine suggested that there are several proteins which

show both STa-binding and GC activity (Kuno *et al*., 1986; Waldman *et al*., 1986; Ivens *et al*., 1990; Thompson and Giannella, 1990). However, the cloning and expression experiments of an intestinal membrane GC (designated GC-C in mammals) revealed that the membrane GC itself is the STa receptor and GC-C showed a considerable homology to NP receptors such as GC-A and GC-B (Schulz, *et al*., 1990). Later, it was demonstrated that the endogenous ligands (guanylin and uroguanylin) also activate GC-C (Currie, *et al*., 1992; Hamra *et al*., 1993).

The STa/guanylin receptor type membrane GC has been identified from various vertebrates, including human (De Sauvage *et al*., 1991; Singh, *et al*., 1991), rat (Schulz *et al*., 1990), pig (Wada *et al*., 1994), guinea pig (Kruhoeffer *et al*., EMBL/Genbank/DDDJ accession number Z74734), cow (Goraczniak *et al*., EMBL/Genbank/DDDJ accession number AF081464), opossum (London *et al*., 1999), birds (Krause *et al*., 1995), reptilians (Krause *et al*., 1997), amphibians (MacFarland, EMBL/Genbank/DDDJ accession number D49837), European eel (Comrie *et al*., 2001), and medaka fish (Mantoku *et al*., 1999; Iio *et al*., 2005), as shown in Table 1. In mammals, only a single membrane GC (GC-C) is known to be the STa/guanylin receptor (Schulz, *et al*., 1990), although two membrane GCs are identified in the intestine of the European eel *Anguilla anguilla* (Comrie *et al*., 2001) and the medaka fish *Oryzias latipes* (Mantoku *et al*., 1999; Iio *et al*., 2005). Here, we describe recent progresses on the studies of the STa/guanylin receptor type membrane GC of the non-mammalian vertebrates, mainly on the medaka fish and discuss their potential contribution to understanding on the roles in vertebrates.

STRUCTURE OF THE ENTEROTOXIN/GUANYLIN RECEPTOR TYPE MEMBRANE GC

The STa/guanylin receptor type membrane GC, as well as other type of membrane GCs, is composed of an extracellular domain, a single transmembrane domain, and an intracellular region comprising a kinase-like domain and a catalytic domain.

The extracellular domain of the STa/guanylin receptor type membrane GC functions as the binding site for both (uro)guanylin and STa (Schulz *et al*., 1990; Lucas *et al*., 2000; Mantoku *et al*., 1999; Iio *et al*., 2005) (Fig. 2). The binding site for STa is thought to be located at the proximal region to the transmembrane domain (Hasegawa *et al*., 1999b; Wada *et al*., 1996a), which is also conserved in medaka fish STa/guanylin receptor type membrane GCs (OlGC6 and OlGC9) (Mantoku *et al*., 1999; Iio *et al*., 2005). The extracellular domain contains 8-10 N-glycosylation sites, depending on species, which are probably not absolutely required for binding to the ligand(s) but are important for proper folding of the domain for STa-binding (Hasegawa *et al*., 1999a; Nandi *et al*., 1996; Ghanekar *et al*., 2004).

The kinase-like domain in the intracellular region is located between the transmembrane domain and the catalytic domain and has homology to the catalytic site of receptor tyrosine kinases (Schulz, *et al*., 1990). However, the domain is thought to have no protein kinase activity since it lacks a conserved Gly-rich region and an Asp residue requiring for positioning of γ-phosphate of ATP and transferring the phosphate group to the substrate, respectively (Schulz, *et al*., 1990). It has been reported that the GC-C lacking the kinase-like domain is constitutivelly fully active and could not longer be activated by STa, suggesting that the kinase-like domain plays a critical role in the signal transduction from the ligand-binding domain to the catalytic domain (Rudner *et al*., 1995; Dashmane *et al*., 1997; Bhandari *et al*., 2001). The kinase-like domain is also thought to interact with adenine nucleotides based on the results of *in vitro* assays, although the activation by STa does not depend on the presence of ATP. Therefore,

occupation of the domain by ATP might stabilize the activated membrane GC and protect it against rapid desensitization (Katwa *et al*., 1992; Gazzano *et al*., 1991). The presence of Lys^{516} residue in the domain seems to be critical for the possible proper orientation of ATP (Bhandari *et al*., 2001). The Lys residue is conserved in OlGC6 and OlGC9 (Iio *et al*., 2005).

The catalytic domain of the STa/guanylin receptor type membrane GC is highly conserved with those of the NP receptor type and sensory-organ-specific type membrane GCs. The STa/guanylin receptor type membrane GC contains an approximately 60 amino acid long extension distal to the catalytic domain, similar to the sensory organ-specific type membrane GCs but not to the NP receptor type membrane GCs and deletion of this extended portion (tail) results in unresponsive to STa (Wada *et al*., 1996b). In relation to this, it is important to mention that Ser^{1029} residue in the tail of porcine GC-C is phosphorylated by PKC (Wada *et al*., 1996b; Crane and Shanks, 1996) (Fig. 2) and that phosphorylation of Ser^{1029} residue plays a critical role in activating the cyclase, especially in synergy with STa (Wada *et al*., 1996b; Crane and Shanks, 1996). Ser^{1029} is conserved in OlGC9, but not in OlGC6 (Iio *et al*., 2005). On the other hand, it is reported that a novel PDZ protein termed IKEPP interacts with the C-terminal 4 amino acid residues of the STa/guanylin receptor type membrane GC and is involved in the regulation of the cyclase activity (Scott *et al*., 2002) (Fig. 2). These C-terminal target residues in PDZ proteins are also conserved in OlGC6 and OlGC9 (Iio *et al*., 2005).

Based on the results of the studies by Vaandrager *et al.* (1994), it appears that the STa/guanylin receptor type membrane GC is a homomultimer without regard to the absence or presence of ligands. Recently, it was demonstrated that both the extracellular and intracellular domains exist as trimers and a ligand is required to

generate or stabilize the trimeric extracellular domain (Hasegawa *et al*., 1999a; Vijayachandra *et al*., 2000). A region located in the linker between the kinase-like and catalytic domains is implied as the intracellular multimerization sequence (Vijayachandra *et al*., 2000) (Fig. 2) and the amino acid sequences of the region are also conserved in OlGC6 and OlGC9 (Iio *et al*., 2005). From the point of view of the catalytic mechanism, a membrane GC requires two catalytic subunits to convert GTP into cGMP (Hurley, 1998). Therefore, further studies, probably three-dimensional structural studies, are needed for describing the detailed catalytic mechanisms of this type membrane GCs.

ACTIVATION OF THE ENTEROTOXIN/GUANYLIN RECEPTOR TYPE MEMBRANE GC

Mammalian STa/guanylin receptor type membrane GC has been shown to be activated upon endogenous or exogenous ligands binding to the receptor domain and the studies in the *in vivo* and *in vitro* effects of the ligands on the GC demonstrated the following functions of the GC in the mammalian intestine (Fig. 3): (1) Regultion of the fluidity of the intestinal contents by stimulation of secretion of Cl^- and inhibition of absorption of NaCl. As a result, it prevents dehydration and consequently obstruction of the intestine on one hand and the loss of water (diarrhea) on the other hand (Vaandrager and de Jonge, 1994). (2) Regulation of pH of the intestinal contents by stimulation of HCO_3^- secretion and inhibition of H^+ extrusion mediated by a Na^+/H^+ exchanger. As a result, it stimulates the digestion and absorption of food components (Fawcus *et al*., 1997; Joo *et al*., 1998; Guba *et al*., 1996). (3) Regulation of the Na^+ homeostasis by limiting intestinal Na^+ uptake and by stimulation of Na^+ excretion by the kidney especially in the case of high salt intake. In this regard, it should be

mentioned that uroguanylin secreted by the intestinal cells into the blood stream acts as an intestinal natriuretic hormone along a postulated endocrine intestine-kidney axis (Forte *et al*., 2000). (4) Protection against carcinogenesis caused by activation of cyclic nucleotide-gated (CNG) channels and/or inhibition of Na^+/Ca^{2+} exchange, which leads to alterations in the intracellular Ca^{2+} concentrations and thus inhibiting DNA synthesis in colon carcinoma cells (Pitari *et al*., 2001; Pitari *et al*., 2003). Despite the aforementioned potential physiological roles of mammalian STa/guanylin receptor type membrane GCs, targeted disruption of the *GC-C* gene induced no deleterious effects in mice and, in fact, confers resistance to STa-induced diarrhea (Schulz *et al*., 1997; Mann *et al*., 1997). However, all vertebrates have the STa/guanylin receptor type membrane *GC* gene, implying that it serves some more important and undefined physiological roles.

It has been reported that the receptors for STa are found throughout the intestinal tract of all birds examined (Krause *et al*., 1995). In certain kinds of birds, cellular cGMP-accumulating responses to both *E. coli* STa and rat guanylin have been observed in the proximal small intestine, suggesting that the functional receptors for endogenous peptides like guanylin and uroguanylin exist in the apical membranes of enterocytes throughout the avian intestine (Krause *et al*., 1995). The receptors for guanylin and uroguanylin have also been identified on the mucosal surface of the enterocytes lining the intestine of reptiles (Krause *et al*., 1997). Furthermore, guanylin-like peptides that stimulate cGMP accumulation in human T84 intestinal cells have also been isolated from the intestinal mucosa of reptiles (Krause *et al*., 1997). These suggest that the functional receptor/membrane GC, probably a STa/guanylin receptor type membrane GC and it's ligand(s) exist in the intestinal tract of birds and reptiles as in the intestine of mammals. However, cDNA of the STa/guanylin receptor type membrane GC have

yet been cloned neither in birds nor in reptiles.

Recently, two cDNAs for the STa/guanylin receptor type membrane GCs have been cloned from the European eel *Anguilla anguilla* (*GC-C1* and *GC-C2*) (Comrie *et al.*, 2001) and also from medaka fish (*OlGC6* and *OlGC9*) (Mantoku *et al*., 1999; Iio *et al*., 2005). Considering that the intestine is an essential organ for fish osmoregulation, the STa/guanylin receptor type membrane GCs may play the major roles in the teleost osmoregulation. In eels, it has been demonstrated that the expression of the *GC-C2* gene in the eel intestine was increased by 100% after transfer of fresh water-acclimated eels to sea water and developmental maturation of yellow eels into pre-migratory silver eels resulted in a significant increase in the intestinal expression of the *GC-C2* gene (Comrie *et al.*, 2001), although in the medaka fish such transcriptional change was found neither of the *OlGC6* gene nor the *OlGC9* gene upon changes in environmental salinity (Iio *et al*., 2005). In this regard, it should be mentioned that cDNAs for three distinct guanylin-like peptides are cloned from Japanese ell *A. japonica* and that the expression of all of guanylin-like peptides expression was increased after adaptation of the eel to seawater (Yuge *et al*., 2000). These strongly suggest that these peptides play important roles in seawater adaptation and act on regulation of water and salt absorption.

Although it is not known whether the European eel GC-C1 and GC-C2 are activated by STa and/or endogenous peptides (Comrie *et al.*, 2001), in medaka fish it is demonstrated that STa activates OlGC9 but not OlGC6 and on the contrary, the medaka fish intestine extract, in which endogenous ligands should be contained, activates OlGC6 but not OlGC9 (Fig. 4) (Iio *et al*., 2005). These results suggest that the structural differences between the extracellular domains of OlGC6 and OlGC9 are responsible for differential activation by endogenous ligand(s) and STa (Iio *et al*., 2005).

In this regard, the following facts should be useful for explanation of the differential activation by endogenous ligand(s) and STa. The structure of STa has been demonstrated to be similar to that of guanylin or uroguanylin (Nakazato, 2001) and the activation mechanisms of mammalian GC-C by STa have been explained by various standpoints of view (Fig. 2): (1) Importance of glycosylation sites (Asn^{195} and Asn^{402}) in the extracellular domain for proper folding of the domain for STa-binding (Nandi *et al*., 1996; Ghanekar *et al*., 2004), (2) Phospholylation of Ser^{1052} in the intracellular domain by PKC for conformation change suitable for an active form upon binding of STa to the extracellular domain (Wada *et al*., 1996b), (3) Oligomerlization (Vaandrager *et al*., 1994; Vijayachandra *et al*., 2000), (4) Interaction of the carboxyl termini with PDZ protein (Scott *et al*., 2002), and (5) ATP-binding to the kinase-like domain (Bhandari *et al*., 2001). Among them, a region for mediating oligomerization, PDZ domain, and a Lys residue in the kinase-like domain to stabilize the ATP-binding site are conserved in OlGC6 and OlGC9 (Fig. 2). In addition to these, the residues SPTFIWK which are suggested to be involved in STa-binding in porcine GC-C (Hasegawa *et al*., 1999b) is also conserved in both OlGC6 and OlGC9 (Fig.2). Considering these, the STa-binding site identified in porcine GC-C may not be related to the differential activation of OlGC6 and OlGC9 by STa. On the other hand, two glycosylation sites (Asn^{195} and Asn^{402}) which is essential for proper folding of the extracellular domain to allow ligand-binding in porcine GC-C (Hasegawa *et al.*, 1999a) are conserved in OlGC9, but not in OlGC6 and a RNNSFQK sequence (residues 1050-1056 in OlGC9) which corresponds to the consensus phosphorylation sequence ($RXXS^{1052}XK$) in porcine GC-C is found in OlGC9 but not in OlGC6. These structural differences between OlGC6 and OlGC9 may be responsible to differential binding and subsequent activation by possible endogenous ligand(s) and STa (Iio *et al*., 2005) and may be useful when

medaka fish STa/guanylin receptor type membrane GCs works in the regulation of salt and water transport in the medaka fish intestine. Further study on the differential activation mechanisms of two medaka fish STa/guanylin receptor type membrane GCs by endogenous ligand(s) and STa may be advantageous for further understanding of the ligand-stimulating mechanism of the STa/guanylin receptor type membrane GCs.

TRANSCRIPTION OF THE ENTEROTOXIN/GUANYLIN RECEPTOR TYPE MEMBRANE GC GENE

Using mammalian cultured cell lines, it has been demonstrated that the intestinal transcriptional regulator Cdx2 mediates the intestinal epithelial cell-specific expression of the humam *GC-C* gene through the 5'-flanking region between -83 and -75 (Park *et al*., 2000; Di Guglielmo *et al*., 2001) and that hepatocyte nuclear factor-4 (HNF-4) is a key regulator of the intestine-specific expression of the human *GC-C* gene through the 5'-flanking region between -46 and -29 (Mann *et al*., 1996; Swenson *et al*., 1999) (Fig. 5). However, the consensus binding sequences for HNF-4 are not found in the 5'-flanking region of the medaka fish *OlGC6* gene and those for Cdx2, which are found in the region, do not seem to be involved in the transcription of the *OlGC6* gene (Nakauchi and Suzuki, 2003), suggesting that the transcriptional regulatory mechanism of the *OlGC6* gene differs from that of the human *GC-C* gene. Using CACO-2 cells (human intestine-derived cell line) and COS1 cells (African green monkey kidney-derived cell line), it was demonstrated that the 5'-flanking region between -98 and -89 of the *OlGC6* gene is important for the transcriptional regulation only in the intestine-derived CACO-2 cells and that the sequence for this region are not similar to the regulatory element of the *GC-C* gene (Nakauchi and Susuki, 2003).

Foreign DNA transfer and expression experiments in medaka fish have been used

to investigate transcriptional regulation of tissue-specific genes, suggesting that medaka fish is adapted as a model vertebrate to study transcriptional regulation (Kusakabe and Suzuki, 2000). To understand the regulatory mechanisms of the *OlGC6* transcription *in vivo*, the transgenic medaka fish were generated by microinjection of the *OlGC6*-reporter fusion gene constructs into the medaka fish embryos (Fig. 6) (Nakauchi and Suzuki, 2003). Grown up transgenic founders were mated with non-transgenic littermates to establish a line, and the embryos of each F_1 were analyzed to examine the transmission of the transgene (Fig. 6). The experiments using these F_1 transgenic medaka fishes demonstrated that the AGACCTTTGC nucleotides in the 5'-flanking region of the *OlGC6* gene (region between -90 and –81) play a critical role in the transcription of the gene *in vivo* (Nakauchi and Suzuki, 2003). Furthermore, it was shown that a medaka fish homologue (designated as OlPC4) of mammalian positive co-factor 4 (PC4) activates the transcription of the *OlGC6* gene through the AGACCTTTGC element (Nakauchi *et al.*, 2005). The recombinant OlPC4 has been demonstrated to exhibit certain preferences with respect to binding sequences. In this regard, it should be mentioned that binding of human PC4 to a random sequence is much less efficient than binding to a promoter-containing sequence, although there is no direct evidence for the sequence-specific binding of human PC4 to any human gene (Kretzschmar *et al*., 1994; Ge and Roeder, 1994; Kaiser *et al*., 1995). Therefore, PC4 may exert still unknown functions in the recognition of the binding sequence (Nakauchi *et al*., 2005). PC4 is known as a transcriptional coactivator and it is demonstrated that human PC4 activates the transcription of some genes by interacting with several types of activators and general transcriptional factors, and also with TATA-binding protein (TBP)-associated factors (TAFs), as well as with a specific type of coactivator, thereby acting as an adaptor that links upstream activators with the basal transcriptional

machinery (Kretzschmar *et al*., 1994; Ge and Roeder, 1994) (Fig. 7). These results suggest that PC4 plays an important role in the regulation of the genes transcribed by RNA polymerase II, although its physiological roles are still largely unknown (Kretzschmar *et al*., 1994; Ge and Roeder, 1994). Considering that mammalian PC4 requires upstream activators and interacts with many factors (Kretzschmar *et al*., 1994; Ge and Roeder, 1994) and our recent results demonstrating that OlPC4 requires the additional factor(s) which would be expressed in medaka fish intestine, OlPC4 may also interact with some unknown factor(s) and/or activator(s) binding to the upstream region of the *OlGC6* gene yet to be identified (Nakauchi *et al*., 2005).

Since the size of the human genome is almost four times larger than that of medaka fish (Tanaka, 1995), more dynamic changes could be occurred in the human genome during evolutional processes and such changes may lead to different transcriptional regulatory mechanisms of human genome. In fact, the 5'-flanking regions involved in the intestinal cell-specific transcriptional regulation of the human *GC-C* gene are different from the corresponding region in the medaka fish *OlGC6* gene, and on the contrary, that of the *OlGC6* gene is also different from the corresponding region in *GC-C* gene (Fig. 5). These potential transcriptional regulatory sequences are not found in the 5'-flanking region of the *OlGC9* gene, suggesting that the transcriptional regulatory mechanism of the *OlGC9* gene is different from those of the *OlGC6* and human *GC-C* genes (Iio *et al*., 2005). Recently, it was demonstrated that the some parts in the 5'-flanking region of the *OlGC9* gene are involved in the transcriptional regulation when CACO-2 cells and COS1 cells were used (Iio *et al*., 2005). Further investigations on determination of the detailed *cis*-regulatory region in the *OlGC9* gene and on identification of the transcriptional factor(s) interacting with the region will reveal more details about the mechanism of the transcriptional regulation of the

STa/guanylin receptor type guanylyl cyclase genes.

It has been reported that the human *GC-C* gene locates on the chromosome 12 and the size of the *GC-C* gene is 85 kbp consisting of 27 exons (Lucas *et al*., 2000; Vaandrager, 2002). In medaka fish, the *OlGC6* gene is 16 kbp, much smaller than that of the human *GC-C* gene but the *OlGC6* gene consists of 27 exons, the number of which is the same as that of the human *GC-C* gene (Mantoku *et al*. 1999). The *OlGC6* gene was mapped to linkage group 19 (LG19) and the *OlGC9* gene was mapped to linkage group 8 (LG8). The genes on LG8 and LG19 share the same ancestral chromosome (proto-chromosome 2), suggesting that the *OlGC6* and *OlGC9* genes were duplicated from the same ancestral gene (Naruse *et al*., 2004; Iio *et al*., 2005). However, the differential activation of OlGC6 and OlGC9 by possible endogenous ligand(s) and STa and actually no similarity in the 5'-flanking region between the *OlGC6* and *OlGC9* genes suggest that after gene duplication the nucleotide sequences of both genes and subsequent biological functions of both genes' translation products were altered independently during evolutional processes. Further studies to solve the differential activation mechanisms would be useful for understanding of the ligand-stimulating mechanism of the mammalian STa/guanylin receptor type membrane GC.

ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (no. 11236202) and from the National Project on Protein Structural and Functional Analyses.

REFERENCES

Bhandari R, Srinivasan N, Mahaboobi M, Ghanekar Y, Suguna K, Visweswariah SS (2001) Functional inactivation of the human guanylyl cyclase C receptor: Modeling and mutation of the protein kinase-like domain. Biochemistry 40: 9196-9206

Comrie MM, Cutler CP, Cramb G (2001) Cloning and expression of two isoforms of guanylate cyclase C (GC-C) from the European eel (*Anguilla anguilla*). Comp Biochem Physiol B 129: 575-586

Crane JK, Shanks KL (1996) Phosphorylation and activation of the intestinal guanylyl cyclase receptor for *Escherichia coli* heat-stable toxin by protein kinase C. Mol Cell Biochem 165: 111-120

Currie MG., Fok KF, Kato J, Moore RJ, Hamra FK, Duffin KL, Smith CE (1992) Guanylin: an endogenous activator of intestinal guanylate cyclase. Proc Natl Acad Sci USA 89: 947-951

Dashmane SP, Parkinson SJ, Crupper SS, Robertson DC, Schulz S, Waldman SA (1997) Cytoplasmic domains mediate the ligand-induced affinity shift of guanylyl cyclase C. Biochemistry 36: 12921-12929

De Jonge HR (1975) The localization of guanylate cyclase in rat small intestinal epithelium. FEBS Lett 53: 237-242

De Sauvage FJ, Camerato TR, Goeddel DV (1991) Primary structure and functional expression of the human receptor for *Escherichia coli* heat-stable enterotoxin. J Biol Chem 266: 17912-17918

Di Guglielmo MD, Park J, Schulz S, Waldman SA (2001) Nucleotide requirements for CDX2 binding to the cis promoter element mediating intestine-specific expression of guanylyl cyclase C. FEBS Lett 507: 128-132

Fawcus W, Gorton VL, Lucas ML, McEwan GT (1997) Stimulation of three distinct guanylate cyclases induces mucosal surface alkalinisation in rat small intestine *in vitro*. Comp Biochem Physiol A Physiol 118: 291-295

Field M, Graf LHJr, Laird WJ, Smith PL (1978) Heat-stable enterotoxin of *Escherichia coli*: *In vitro* effects on guanylyl cyclase activity, cyclic GMP concentration and ion transport. Proc Natl Acad Sc. USA 75: 2800-2804

Field M, Rao MC, Chang EB (1989) Intestinal electrolyte transport and diarrheal disease. N Engl J Med 321: 800-806, 879-883

Forte LR, London RM, Freeman RH, Krause WJ (2000) Guanylin peptides: Renal actions mediated by cyclic GMP. Am J Physiol 278: F180-F191

Gazzano H, Wu HI, Waldman SA (1991) Activation of particulate guanylate cyclase by *Escherichia coli* heat-stable enterotoxin is regulated by adenine nucleotides. Infect Immun 59: 1552-1557

Ge H, Roeder RG (1994) Purification, cloning, and characterization of a human coactivator, PC4, that mediates transcriptional activation of class II genes. Cell 78: 513-523

Ghanekar Y, Chandrashaker A, Tatu U, Visweswariah SS (2004) Glycosylation of the receptor guanylyl cyclase C: role in ligand binding and catalytic activity. Biochem J 379: 653-663

Guba M, Kuhn M, Forssmann WG, Classen M, Gregor M, Seidler U (1996) Guanylin strongly stimulates rat duodenal HCO_3^- secretion: Proposed mechanism and comparision with other secretagogues. Gastroenterology 111: 1558-1568

Hamra FK, Forte LR, Eber SL, Pidhorodeckyj NV, Krause WJ, Freeman RH, Chin DT, Tompkins JA, Fok KF, Smith CE, Duffin KL, Seigel NR, Currie MG. (1993) Uroguanylin: structure and activity of a second endogenous peptide that stimulates

intestinal guanylate cyclase. Proc Natl Acad Sci USA 90: 10464-10468

Hasegawa H, Kawano Y, Matsumoto Y, Hidaka Y, Fujii J, Taniguchi N, Wada A, Hirayama T, Shimonishi Y (1999a) Expression and characterization of the extracellular domain of guanylyl cyclase C from a baculovirus and Sf21 insect cells. Prot Exp Pu. 15: 271-281

Hasegawa H, Hidaka Y, Matsumoto Y, Sanni T, Shimonishi Y (1999b) Determination of the binding site on the extracellular domain of guanylyl cyclase C to heat-stable enterotoxin. J Biol Chem 274: 31713-31718

Hughes JM, Murad F, Chang B, Guerrant RL (1978) Role of cyclic GMP in the action of heat-stable enterotoxin of *Escherichia coli*. Nature 271: 755-756

Hurley JH (1998) The adenylyl and guanylyl cyclase superfamily. Curr Opin Struct Biol 8: 770-777

Iio K, Nakauchi M, Yamagami S, Tsutsumi M, Hori H, Naruse K, Mitani H, Shima A, Suzuki N (2005) A novel membrane guanylyl cyclase expressed in medaka fish intestine. Com Biochem Physiol B Biochem Mol Biol 140: 569-578

Ivens K, Gazzano H, O'Hanley P, Waldman SA (1990) Heterogeneity of intestinal receptors for *Escherichia coli* heat-stable enterotoxin. Infect Immun 58: 1817-1820

Joo NS, London RM, Kim HD, Forte LR, Clarke LL (1998) Regulation of intestinal Cl^- and HCO_3^- secretion by uroguanylin. Am J Physiol 274: G633-G644

Kaiser K, Stelzer G, Meisterernst, M. (1995) The coactivator p15 (PC4) initiates transcriptional activation during TFIIA-TFIID-promoter complex formation. EMBO J 14: 3520-3527

Katwa LC, Parker CD, Dybing JK, White AA (1992) Nucleotide regulation of heat-stable enterotoxin receptor binding and of guanylate cyclase activation. Biochem J 283: 727-735

Krause WJ, Freeman RH, Eber SL, Hamra FK, Fok KF, Currie MG, Forte LR (1995) Distribution of Escherichia coli heat-stable enterotoxin/guanylin/uroguanylin receptors in the avian intestinal tract. Acta Anat (Basel) 153: 210-219

Krause WJ, Freeman RH, Eber SL, Hamra FK, Currie MG., Forte LR (1997) Guanylyl cyclase receptors and guanylin-like peptides in reptilian intestine. Gen.Comp Endocrinol 107: 229-239

Kretzschmar M, Kaiser K, Lottspeich F, Meisterernst, M (1994) A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators. Cell 78: 525-534

Kuno T, Kamisaki Y, Waldman SA, Gariepy J, Schoolnik G, Murad F (1986) Characterization of the receptor for heat-stable enterotoxin from *Escherichia coli* in rat intestine. J Biol Chem 261: 1470-1476

Kusakabe T, Suzuki N (2000) The guanylyl cyclase family in medaka fish *Oryzias latipes*. Zool Sci 17: 131-140

London RM, Eber SL, Visweswariah SS, Krause WJ, Forte LR (1999) Structure and activity of OK-GC: A kidney receptor guanylate cyclase activated by guanylin peptides. Am J Physiol 276: F882-F889

Loretz CA, Pollina CP (2000) Natriuretic peptides in fish physiology. Comp Biochem Physiol 125B: 169-187

Lucas KA, Pitari G.M, Kazerounian S, Ruiz-Stewart I, Park J, Schulz S, Chepenik KP, Waldman SA (2000) Guanylyl cyclases and signaling by cyclic GMP. Pharmacol Rev 52: 375-414

Mann EA, Jump ML, Giannella RA (1996) Cell line-specific transcriptional activation of the promoter of the human guanylyl cyclase C/heat-stable enterotoxin receptor gene. Biochi Biophys Act 1305: 7-10

Mann EA, Jump ML, Wu J, Yee E, Giannella RA (1997) Mice lacking the guanylyl cyclase C receptor are resistant to STa-induced intestinal secretion. Biochem Biophys Res Commun 239: 463-466

Mantoku T, Muramatsu R, Nakauchi M, Yamagami S, Kusakabe T, Suzuki N (1999) Sequence analysis of cDNA and genomic DNA, and mRNA expression of the medaka fish homolog of mammalian guanylyl cyclase C. J Biochem (Tokyo) 125: 476-486

Nakauchi M, Suzuki N (2003) Promoter analysis of a medaka fish intestinal guanylyl cyclase gene. FEBS Lett 536:12-18.

Nakauchi M, Yoshino K, Yonezawa, K, Suzuki N (2005) Involvement of General Transcriptional Coactivator PC4 in the Transcription of Medaka Fish Intestine-Specific Membrane Guanylyl Cyclase Gene (*OlGC6*). J Biochem (Tokyo) in press

Nakazato M (2001) Guanylin family: new intestinal peptides regulating electrocyte and water homeostasis. J Gastroenterol 36: 219-225

Nandi A, Mathew R, Visweswariah SS (1996) Expression of the extracellular domain of the human heat-stable enterotoxin receptor in *Escherichia coli* and generation of neutralization antibodies. Protein Expt Purif 8: 151-159

Naruse K, Tanaka M, Mita K, Shima A, Postlethwait J, Mitani H (2004) A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. Genome Res 14:820-828

Park J, Schulz S, Waldman SA (2000) Intestine-specific activity of the human guanylyl cyclase C promoter is regulated by Cdx2. Gastroenterol 119: 89-96

Pitari G.M, Di Guglielmo MD, Park J, Schulz S, Waldman SA (2001) Guanylyl cyclase C agonists regulate progression through the cell cycle of human colon carcinoma

cells. Proc Natl Acad Sci USA 98: 7846-7851

Pitari GM, Zingman LV, Hodgson DM, Alekseev AE, Kazerounian S, Bienengraeber M, Hajnoczky G, Terzic A, Waldman SA (2003) Bacterial enterotoxins are associated with resistance to colon cancer. Proc Natl Acad Sci USA 100: 2695-2699

Rudner XL, Mandal KK, de Sauvage FJ, Kindman LA, Almenoff JS (1995) Regulation of cell signaling by the cytoplasmic domains of the heat-stable enterotoxin receptor: Identification of autoinhibitory and activating motifs. Proc Natl Acad Sci USA 92: 5169-5173

Schultz S, Green CK, Yuen PST, Garbers DL (1990) Guanylyl cyclase is a heat-stable enterotoxin receptor. Cell 63: 941-948.

Schulz S, Lopez MJ, Kuhn M, Garbers DL (1997) Disruption of the guanylyl cyclase-C gene leads to a paradoxical phenotype of viable but heat-stable enterotoxin-resistant mice. J Clin Invest 100: 1590-1595

Scott RO, Thelin WR, Milgram SL (2002) A novel PDZ protein regulates the activity of guanylyl cyclase C, the heat-stable enterotoxin receptor. J Biol Chem 277: 22934-22941

Singh S, Singh G, Heim JM, Gerzer R (1991) Isolation and expression of a guanylate cyclase-coupled heat stable enterotoxin receptor cDNA from a human colonic cell line. Biochem Biophys Res Commun 179: 1455-1463

Swenson ES, Mann EA, Jump ML, Giannella RA (1999) Hepatocyte nuclear factor-4 regulates intestinal expression of the guanylin/heat-stable toxin receptor. Am J Physiol 276: G728-736

Tanaka M (1995) Characteristics of medaka genes and their promoter regions. Fish Biol J Medaka 7: 11-14

Thompson MR, Giannella RA (1990) Different crosslinking agents identify distinctly

different putative *Escherichia coli* heat-stable enterotoxin rat intestinal cell receptor proteins. J Recept Res 10: 97-117

Vaandrager AB (2002) Structure and function of the heat-stable enterotoxin receptor/guanylyl cyclase C. Mol Cell Biochem 230: 73-83

Vaandrager AB, de Jonge HR (1994) Effect of cGMP on intestinal transport. Adv Pharmacol 26: 253-283

Vaandrager AB, van der Wiel E, Hom ML, Luthjens LH, de Jonge HR (1994) Heat-stable enterotoxin receptor/guanylyl cyclase C is an oligomer consisting of functionally distinct subunits, which are non-covalently linked in the intestine. J Biol Chem 269: 16409-16415

Vijayachandra K, Guruprasad M, Bhandari R, Manjunath UH, Somesh BP, Srinivasan N, Suguna K, Visweswariah SS (2000) Biochemical characterization of the intracellular domain of the human guanylyl cyclase C receptor provides evidence for a catalytically active homotrimer. Biochemistry 39: 16075-16083

Wada A, Hirayama T, Kitao S, Fujusawa J, Hidaka Y, Shimonishi Y (1994) Pig intestinal membrane-bound receptor (guanylyl cyclase) for heat-stable enterotoxin: cDNA cloning, functional expression, and characterization. Microbiol Immunol 38: 535-541

Wada A, Hirayama T, Kitaura H, Fujusawa J, Hasegawa M, Hidaka Y, Shimonishi Y (1996a) Identification of ligand recognition sites in heat-stable enterotoxin receptor, membrane-associated guanylyl cyclase C by site-directed mutational analysis. Infect Immun 64: 5144-5150

Wada A, Hasegawa M, Matsumoto K, Niidome T, Kawano Y, Hidaka Y, Padilla PL, Kurazono H, Shimonishi Y, Hirayama T (1996b) The significance of Ser1029 of the heat-stable enterotoxin receptor (STaR): Relation of STa-mediated guanylyl cyclase

activation and signaling by phorbol myristate acetate. FEBS Lett 384: 75-77

Waldman SA, Kuno T, Kamisaki Y, Chang LY, Gariepy J, O'Hanley P, Schoolnik G, Murad F (1986) Intestinal receptor for heat-stable enterotoxin of *Escherichia coli* is tightly coupled to a novel form of particulate guanylate cyclase. Infect Immun 51: 320-326

Wedel B, Garbers D (2001) The guanylyl cyclase family at Y2K. Annu Rev Physiol 63: 215-33

Yamagami S, Xu SH, Tsutsumi M, Hori H, Suzuki N (2003) Expression and genomic organization of a medaka fish novel membrane form of guanylyl cyclase/orphan receptor. Zool Sci 20: 591-606

Yuge S, Inoue K, Hyodo S, Takei Y (2003) A novel guanylin family (guanylin, uroguanylin, and renoguanylin) in eels: possible osmoregulatory hormones in intestine and kidney. J Biol Chem 278: 22726-22733

FIGURE LEGENDS

Fig. 1. Molecular phylogenetic relationship of membrane GCs between medaka fish and rat. The amino acid sequences of the catalytic domains of GCs were subjected to phylogenetic analysis as described previously (Yamagami *et al*., 2003). The following GenBank/EMBL/DDBJ accession numbers for the sequences were used: X14773 (rat GC-A); M26896 (rat GC-B); M55636 (rat GC-C); L37203 (rat GC-D); L36029 (rat GC-E); L36030 (rat GC-F); AF024622 (rat GC-G); AB004921 (OlGC1); AB030274 (OlGC2); AB000899 (OlGC3); AB000900 (OlGC4); AB000901 (OlGC5); AB007192 (OlGC6); AB023489 (OlGC7); AS054814 (OlGC8); AB162944 (OlGC9); AB015874 (OlGC-R2); U21101 (sea urchin sperm membrane GC).

Fig. 2. Schematic drawing of the structure of the mammalian enterotoxin/guanylin receptor type membrane GC. Lines in each domain represent the location of the residue or region. Conserved residue or region between OlGC6 and OlGC9 are underlined . STa, heat stable enterotoxin; PKC, protein kinase; PDZ, Post-synaptic density-95/Dlg-1/ZO-1.

Fig. 3. Schematic drawing of the function of the mammalian enterotoxin/guanylin type membrane GC in the intestinal cells. STa: heat-stable enterotoxin, CFTR: , HNE-3: Na^+/H^+ exchanger-3, CNG channel: cyclic nucleotide-gated channels, PKG: cGMP dependant protein kinase II, PKA: cAMP dependant protein kinase, PDE III: phosphodiesterase III.

Fig. 4. GC activity in the COS-7 cells transfected with *OlGC6* or *OlGC9* in the

presence or absence of medaka fish intestine extract (A) or STa (B). Shaded boxes indicate the cGMP concentrations after treatment of the cells by the intestine extract (26.56 μg protein/μl) or 1x 10^{-6} M STa. Black boxes indicate cGMP concentrations without peptides. Transfection and cGMP assay were performed in four independent experiments and the values are expressed as mean ± S.D.

Fig. 5. Schematic drawing of several consensus sequences for transcription factors found in the 5'-flanking region of the *OlGC6*, *OlGC9*, and human *GC-C* genes. Open ellipses and closed boxes represent consensus sequence for hepatocyte nuclear factor-4 (HNF-4) and Cdx2, respectively. A TATA box is indicated as an open triangle. The numbers below the line indicate the length of the 5'-flanking sequence.

Fig. 6. The procedure used for the investigation of the transcriptional regulation *in vivo* using transgenic medaka fish. Using F1, the reporter gene expression can be analyzed by various methods, e. g. Reverse transcription-polymerase chain reaction (RT-PCR) analysis, *in situ* hybridization analysis using reporter gene RNA probe.

Fig. 7. Schematic drawing of the eukaryotic transcriptional machinery containing PC4. PC4 regulates transcription by interacting with general transcriptional factors, general co-factors, the TATA binding protein (TBP)-associated factors (TAFs), and the RNA polymerase II (pol II) holoenzyme-associated mediator complex that binds to the carboxy-terminal domain (CTD) of the largest subunit of RNA pol II, and activators. Various of these elements interact with one another.

Table 1. Enterotoxin/guanylin receptor type guanylyl cyclase in vertebrates

species	EMBL/Genbank/DDDJ accession number	references
Homo sapiens (human)	S57551 M73489	Singh, *et al*., 1991 De Sauvage *et al*., 1991
Rattus norvegius (rat)	NM013170	Schulz *et al*., 1990
Sus scrofa (pig)	D17513	Wada *et al*., 1994
Cavia porcellus (guinea pig)	Z74734	Kruhoeffer *et al*., unpublished data
Bos taurus (cow)	AF081464	Goraczniak *et al*., unpublished data
opossum		London *et al*., 1999
birds		Krause *et al*., 1995
reptiles		Krause *et al*., 1997
Xenopus laevis (amphibians)	D49837	MacFarland, unpublished data
Anguilla anguilla (european eel)	AJ291611 (forGC-C1) AJ291612 (for GC-C2)	Comrie *et al*., 2001
Oryzias latipes (medaka fish)	AB007192 (for*OlGC6*) AB162944 (for *OlGC9*)	Mantoku *et al*., 1999 Iio *et al*., 2005

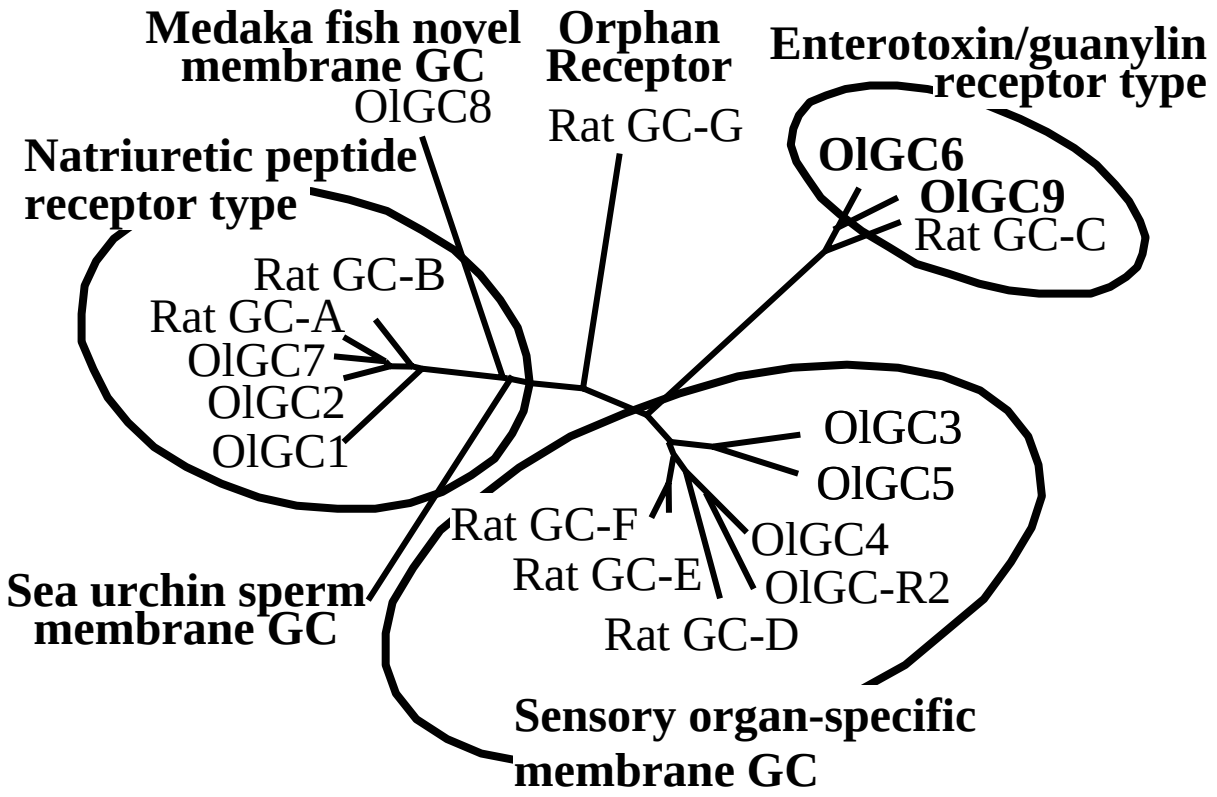
Medaka fish novel membrane GC
OlGC8
Orphan Receptor
Rat GC-G
Enterotoxin/guanylin receptor type
OlGC6
OlGC9
Rat GC-C
Natriuretic peptide receptor type
Rat GC-B
Rat GC-A
OlGC7
OlGC2
OlGC1
OlGC3
OlGC5
Rat GC-F
OlGC4
Rat GC-E
OlGC-R2
Rat GC-D
Sea urchin sperm membrane GC
Sensory organ-specific membrane GC

Fig. 1

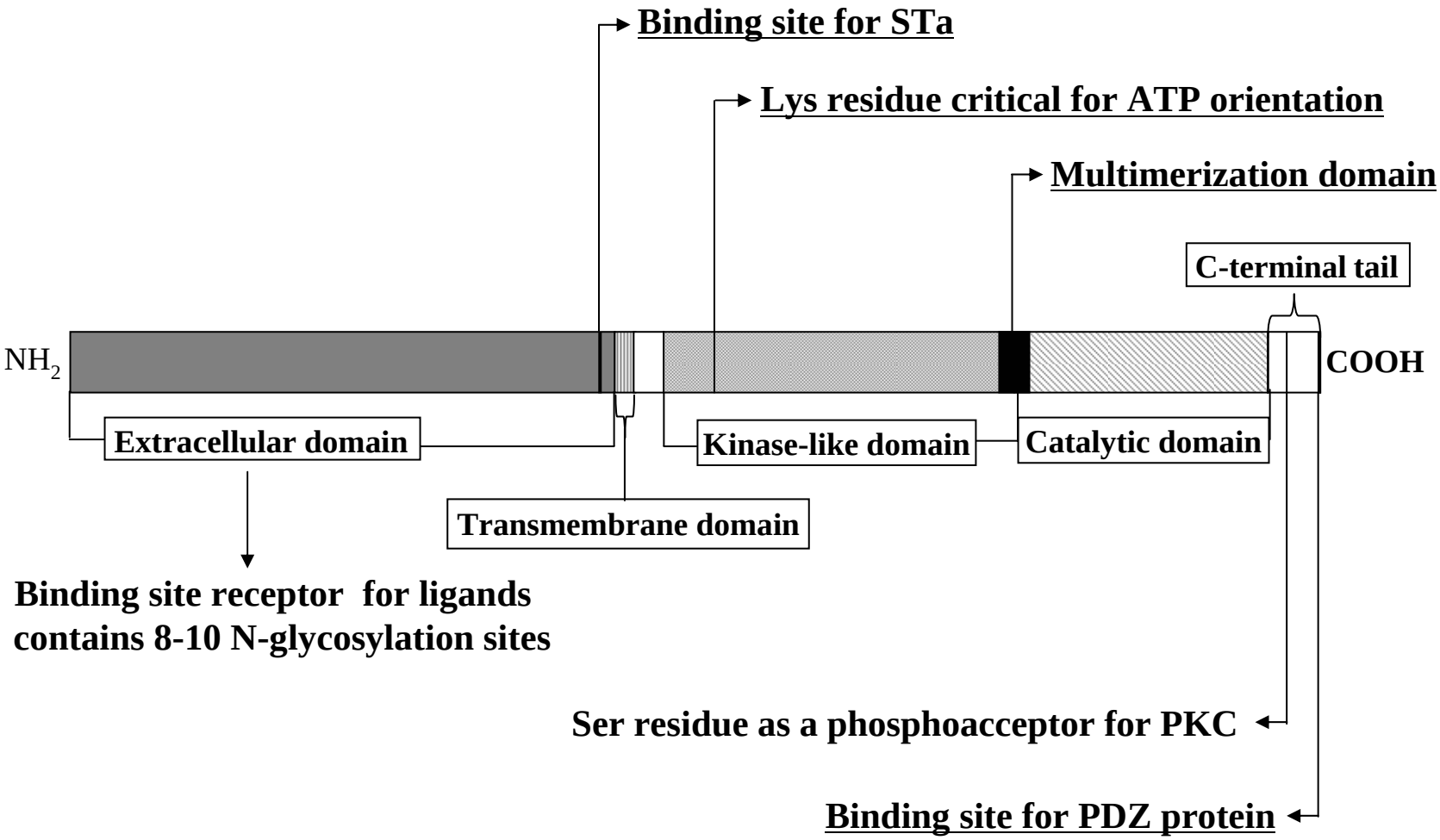
Binding site for STa
Lys residue critical for ATP orientation
Multimerization domain
C-terminal tail
NH2
COOH
Extracellular domain
Kinase-like domain
Catalytic domain
Transmembrane domain
Binding site receptor for ligands
contains 8-10 N-glycosylation sites
Ser residue as a phosphoacceptor for PKC
Binding site for PDZ protein

Fig. 2

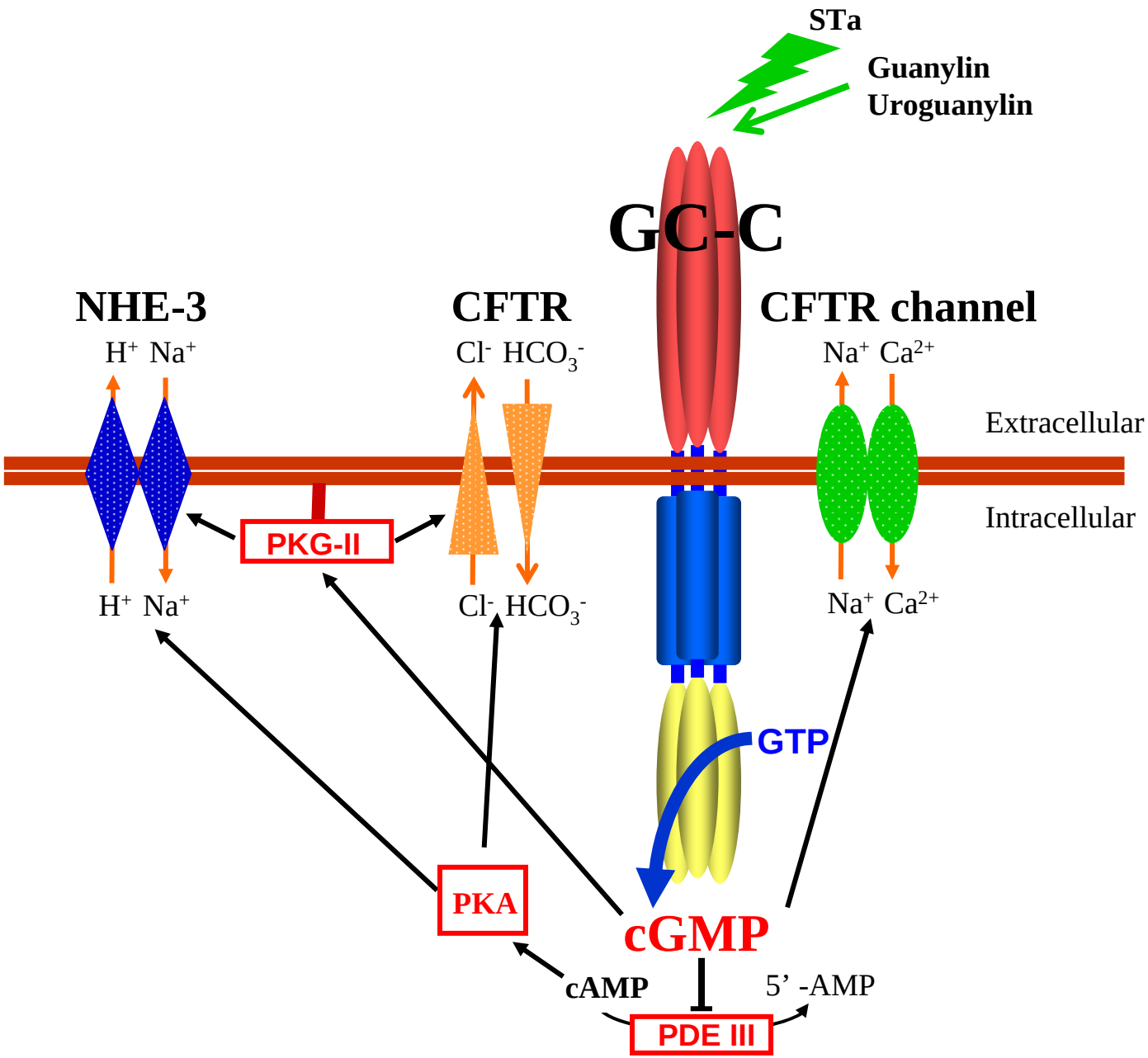
STa
Guanylin
Uroguanylin
GC-C
NHE-3
CFTR
CFTR channel
H+ Na+
Cl- HCO3-
Na+ Ca2+
Extracellular
Intracellular
PKG-II
H+ Na+
Cl- HCO3-
Na+ Ca2+
GTP
PKA
cGMP
cAMP
5' -AMP
PDE III

Fig. 3

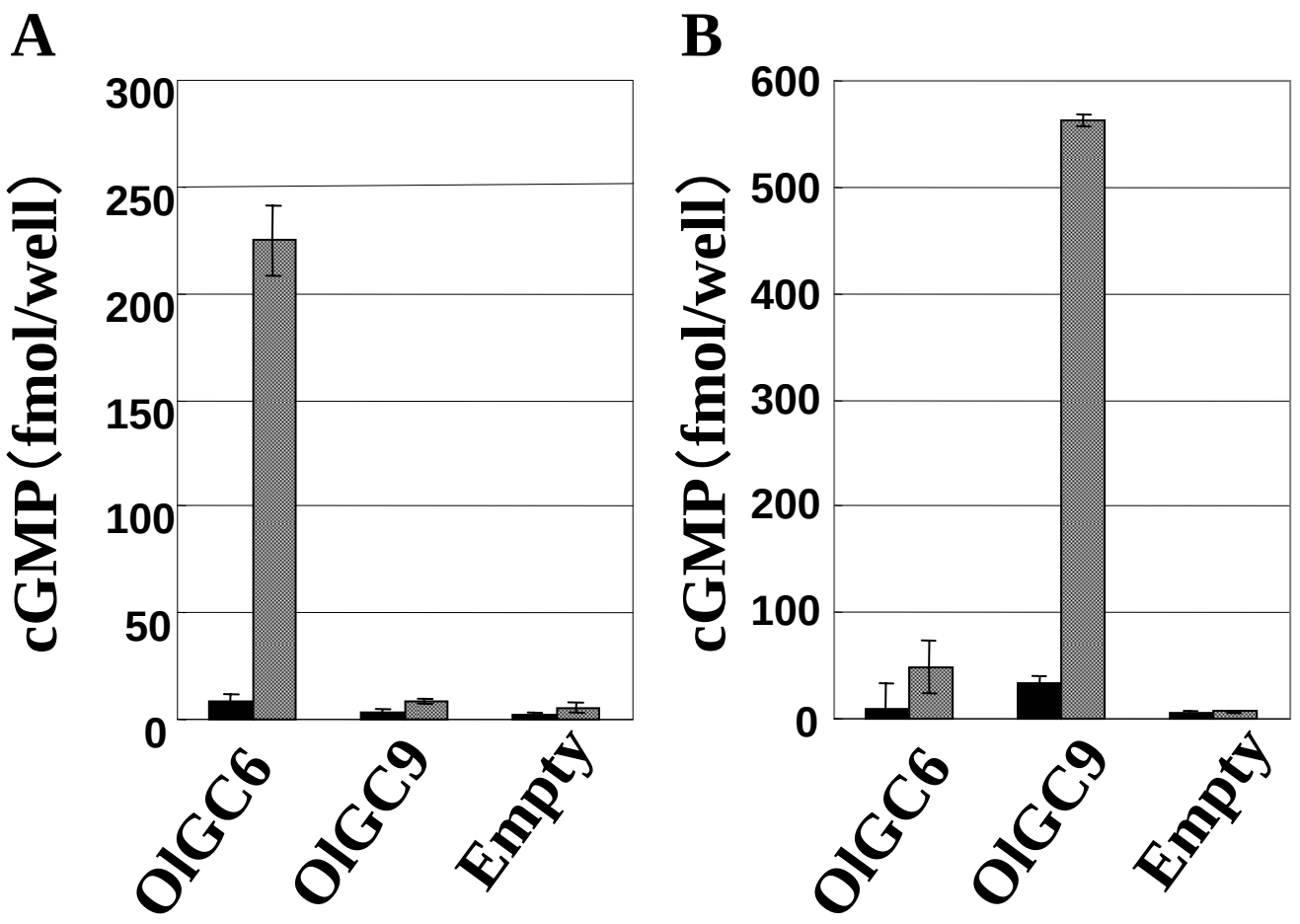
A
cGMP (fmol/well)
300
250
200
150
100
50
0
OlGC6
OlGC9
Empty
B
cGMP (fmol/well)
600
500
400
300
200
100
0
OlGC6
OlGC9
Empty

Fig. 4

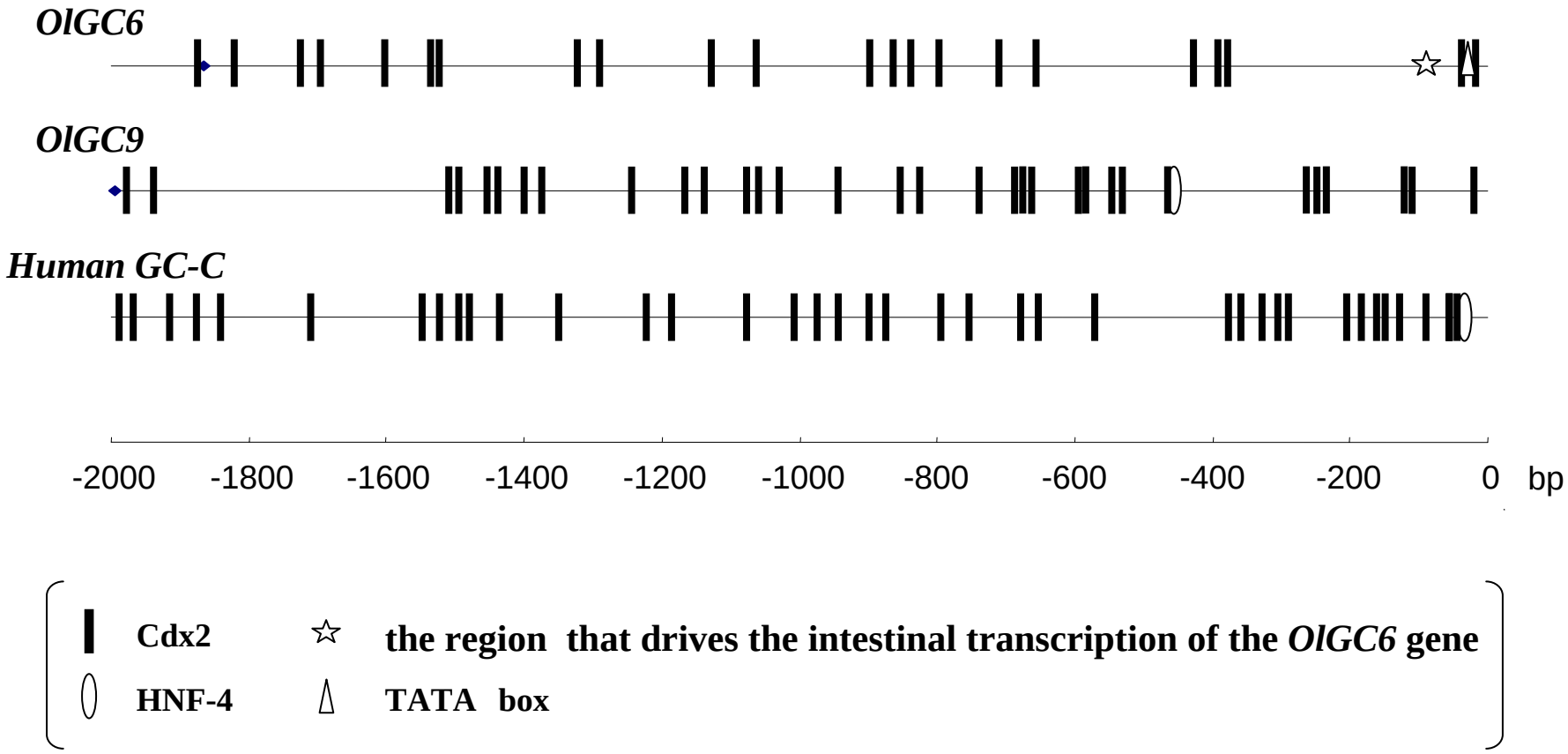
OlGC6
OlGC9
Human GC-C
-2000
-1800
-1600
-1400
-1200
-1000
-800
-600
-400
-200
0
bp
Cdx2
the region that drives the intestinal transcription of the OlGC6 gene
HNF-4
TATA box

Fig. 5

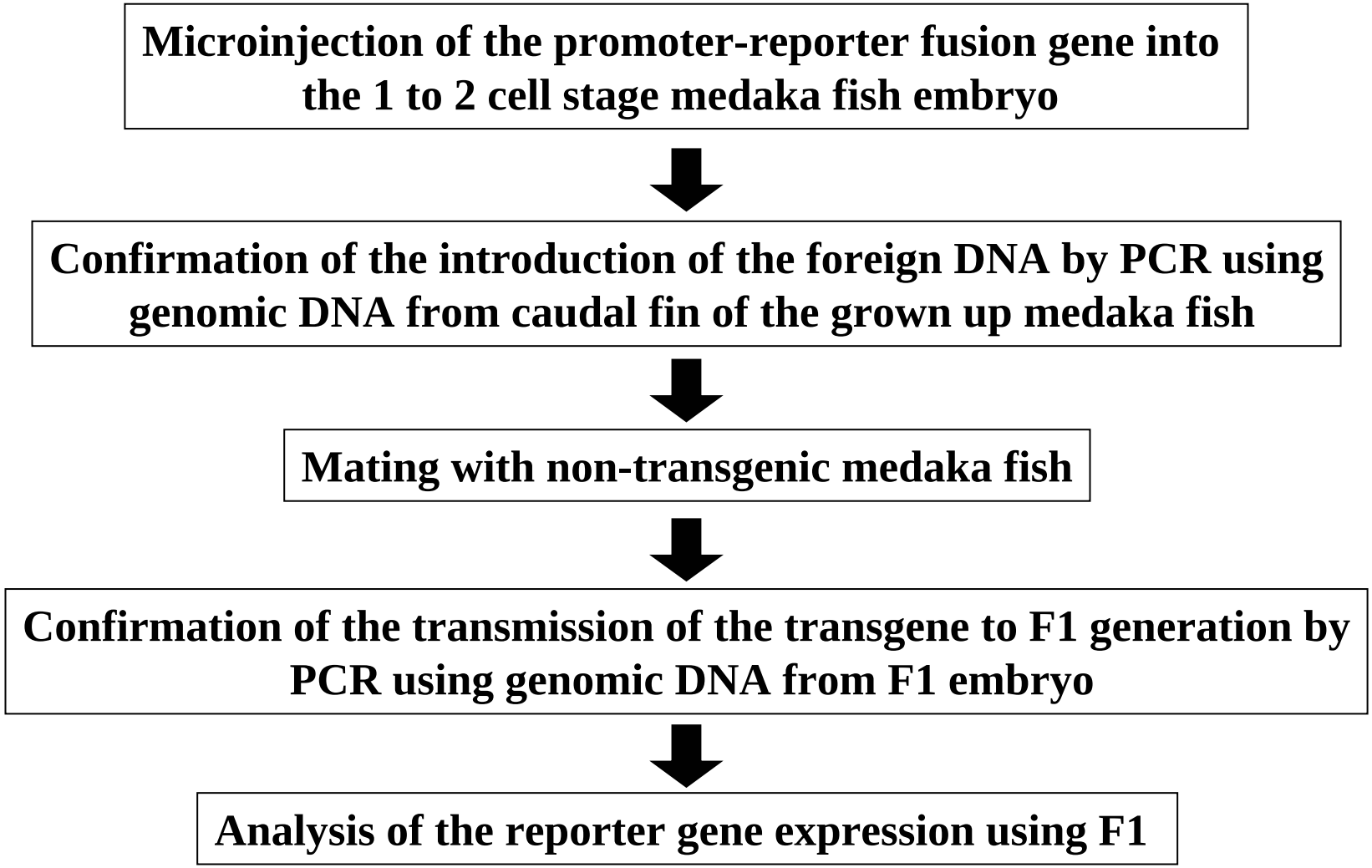
Microinjection of the promoter-reporter fusion gene into the 1 to 2 cell stage medaka fish embryo
Confirmation of the introduction of the foreign DNA by PCR using genomic DNA from caudal fin of the grown up medaka fish
Mating with non-transgenic medaka fish
Confirmation of the transmission of the transgene to F1 generation by PCR using genomic DNA from F1 embryo
Analysis of the reporter gene expression using F1

Fig. 6

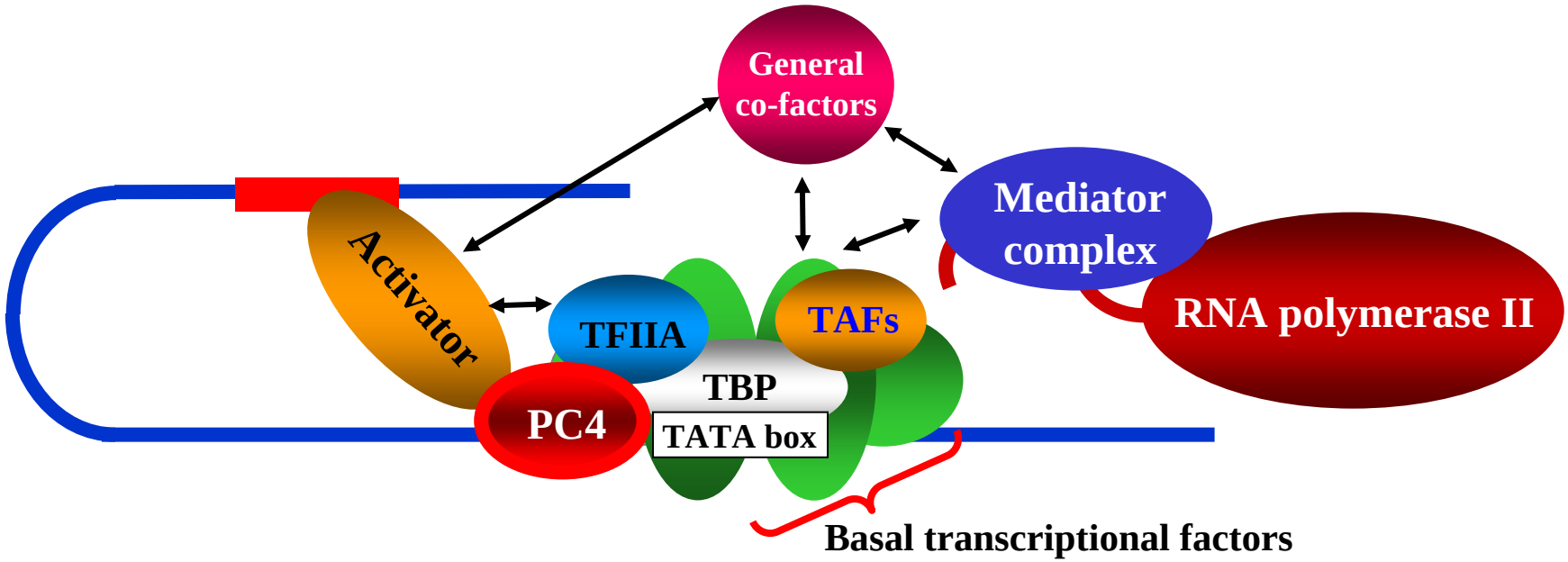
General
co-factors
Mediator
complex
RNA polymerase II
Activator
TFIIA
TAFs
TBP
PC4
TATA box
Basal transcriptional factors

Fig. 7