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# Molecular Phylogenetic Analysis of Diverse Forms of Echinoderm Guanylyl Cyclases

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**ABSTRACT**—We found many isoforms of membrane-bound guanylyl cyclase (membrane GC) in the gonads of several echinoid species including *Hemicentrotus pulcherrimus* (six isoforms), *Glyptocidaris crenularis* (four isoforms), *Clypeaster japonicus* (three isoforms), *Diadema setosum* (three isoforms), and *Brissus agassizii* (three isoforms). In addition to these membrane GC isoforms, several putative soluble GC isoforms were also found, one in *H. pulcherrimus*, two in *G. crenularis*, and three in *D. setosum*. Other echinoderm species also possess many GC isoforms in their gonads: the starfish, *Asterina pectinifera*, has five membrane GC isoforms; the brittle star, *Ophioplocus japonicus*, possesses six membrane and two putative soluble GC isoforms; and the sea cucumber, *Stichopus japonicus*, contains five membrane and two putative soluble GC isoforms. We also obtained the full-length cDNA sequence of some of these isoforms, one for sperm-activating peptide (SAP) receptor-type GC, and the other for a homolog of the vertebrate natriuretic peptide receptors/membrane GCs, and analyzed their phylogenetic relationship among various invertebrate and vertebrate GC isoforms.

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## INTRODUCTION

For over twenty years, it has been known that cGMP concentrations can be increased by a wide variety of agents (Goldberg and Haddox, 1977). The formation of cGMP from GTP is catalyzed by guanylyl cyclase [GTP pyrophosphatase (cycling), EC 4.6.1.2] (Mittal and Murad, 1982). Guanylyl cyclase is found in various cellular compartments of many organisms, in soluble and/or membrane-bound forms. The membrane-bound guanylyl cyclase (membrane GC) is a single polypeptide, the first established by cloning and sequencing of the cDNA encoding a sperm protein crosslinked to a sperm-activating peptide-IIA (SAP-IIA) and was initially obtained from a testis cDNA library of the sea urchin *Arbacia punctulata* (Singh *et al.*, 1988). The soluble guanylyl cyclase (soluble GC) consists of two different subunits ( $\alpha$  and  $\beta$ ) (Drewett and Garbers, 1994). In mammals, seven isoforms of membrane GC (GC-A, -B, -C, -D, -E, -F, and -G) have been found (Garbers, 1992; Garbers and Lowe, 1994; Schulz *et al.*, 1998), all of which are composed of an extracellular domain, a single membrane-spanning domain, and an intracellular domain that is further divided into two clearly defined domains; a protein kinase-like regulatory domain and a cyclase catalytic domain. The primary structure of the catalytic domain of both membrane and soluble GCs is highly conserved among vertebrates

and invertebrates (Garbers, 1992; Garbers and Lowe, 1994).

It has been reported that the binding of sperm-activating peptides (SAPs) to sperm surface receptors causes a marked and rapid increase, then subsequent rapid decrease, in cGMP concentrations in sperm cells (Kopf *et al.*, 1979; Suzuki, 1990, 1995). The transient increase in cGMP concentrations has been attributed to the transient activation and subsequent inactivation of the membrane GC, which is closely linked to the state of the enzyme (Garbers, 1989; Suzuki, 1999). A specific SAP such as SAP-IIA binds specifically to the sperm membrane GC, and causes an initial transient activation and subsequent inactivation of the enzyme (Ramarao and Garbers, 1985; Shimomura *et al.*, 1986; Suzuki *et al.*, 1984). SAP-I and SAP-II, isolated from the egg jelly of the sea urchin *Hemicentrotus pulcherrimus* or *Glyptocidaris crenularis* bind specifically to a 71 kDa and a 62 kDa sperm protein, respectively, and in either case the binding of the peptide to the protein results in activation of the respective sperm membrane GC (Harumi *et al.*, 1991; Shimizu *et al.*, 1994). Similarly, SAP-III isolated from the sand dollar *Clypeaster japonicus* causes increases of cGMP levels in the sperm cells after it binds to three sperm proteins, one of which appears to be a membrane GC (Yoshino and Suzuki, 1992). These facts indicate that the membrane GC is the receptor itself for SAP or for a protein associated with the receptor for SAP.

In the last decade, a number of SAP-I derivatives have been isolated from the egg jelly of several sea urchin species: two from *Strongylocentrotus purpuratus* and four from *H. pulcherrimus*. These derivatives all show the same biological

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activity on the respective spermatozoa (Suzuki, 1995, 1999), and their amino acid sequences are found in the respective precursor protein (Kinoh *et al.*, 1994; Ramarao *et al.*, 1990). Such structural diversity is also found in SAPs from other sea urchin species: five SAP-IIB derivatives in the egg jelly of *G. crenularis*; eight SAP-III derivatives in the *C. japonicus* egg jelly; and one SAP-IV derivative in the egg jelly of *Diadema setosum* (Suzuki, 1990, 1995). Therefore, it might be the case that the receptors for each group of SAP are heterogeneous with regard to the composition of their constituent proteins, and particularly in their primary structure, which allows for binding to the peptide.

The cDNA encoding the membrane GC has been isolated from three species of sea urchin testis cDNA library: *A. punctulata* (Singh *et al.*, 1989), *S. purpuratus* (Thorpe and Garbers, 1989), and *H. pulcherrimus* (Shimizu *et al.*, 1996). The primary structures of these membrane GCs are to some degree similar to those of the membrane GCs serving as the natriuretic peptide receptors. This is also the case for *Caenorhabditis elegans*, in which twenty-nine genes encoding putative GCs have been identified (Baude *et al.*, 1997; Wedel and Garbers, 1997). Vertebrates possess much smaller numbers of the membrane GC isoforms, but they can be divided into three major groups: (i) natriuretic peptide receptors, (ii) enterotoxin/guanylin receptors, and (iii) sensory organ-specific membrane GCs (Seimiya *et al.*, 1997). However, little is known about the origin of the membrane GC in each vertebrate group or about the molecular phylogenetic relationship among the membrane GC isoforms in vertebrates and invertebrates. In this study, to understand the diversity of the membrane GC isoforms in invertebrates, we began a characterization of the membrane GC isoforms in sea urchins, the spermatozoa of which contain a generous amount of the SAP receptor-type membrane GC, a condition conducive to biochemical analysis of the enzyme and mechanisms of interaction between peptide ligands such as SAPs and their receptors. The study extended to the characterization of the membrane GC isoforms in other echinoderms such as the starfish *Asterina pectinifera*, the brittle star *Ophioplocus japonicus*, and the sea cucumber *Stichopus japonicus*. In addition, we describe here in the isolation and characterization of cDNA clones for a SAP receptor-type membrane GC isoform obtained from a testis cDNA library of the sea urchin *Brissus agassizii* and from an echinoderm homolog of the vertebrate natriuretic peptide receptor/membrane GC isoform from an ovary cDNA library of the sea cucumber *S. japonicus*, and the phylogenetic relationship among these GC isoforms and known GC isoforms.

## MATERIALS AND METHODS

### Materials

Individual specimens of *H. pulcherrimus*, *C. japonicus*, *B. agassizii*, *A. pectinifera*, *O. japonicus*, and *S. japonicus* were collected off the coast near Noto Marine Laboratory, Kanazawa University. Individual specimens of *G. crenularis* and *D. setosum* were collected at Aomori Bay near the Asamushi Marine Biological Station of Tohoku

University and off the coast near Usa Marine Biological Institute of Kochi University, respectively. The gonads (testes and/or ovaries) were dissected out from the adult specimens and frozen with liquid nitrogen.

### Preparation of RNA and cDNA library construction

Total RNA was prepared from the growing gonads of various species of echinoderms and adult medaka fishes by the LiCl method of Cathala *et al.* (1983) or by the acid/guanidinium/thiocyanate/phenol/chloroform extraction method (Chomczynski and Sacchi, 1987). Poly(A)<sup>+</sup>RNA was isolated from the total RNA by chromatography on oligo(dT)-cellulose (Pharmacia Biotech.) (Davis *et al.*, 1986). Using the poly(A)<sup>+</sup>RNA, a cDNA library was constructed in a  $\lambda$ ZAP II vector with a ZAP-cDNA Synthesis Kit (Stratagene).

### Amplification of GC cDNA fragments by reverse transcription-polymerase chain reaction (RT-PCR)

Three degenerate oligonucleotide primers (P2: 5'-GAYATHGTN-GGNTTYAC-3'; P6: 5'-GTRTTNACNGTRTCNCC-3'; P7: 5'-ARR-CARTANCKNGGCAT-3') were synthesized based on the amino acid sequences of three conserved regions (DIVGFT, GDTVNT, and MPRYCL) in known GCs. These primers were used to amplify a cDNA fragment encoding GC in cDNA reverse-transcribed from the total RNA of the gonad as described previously (Seimiya *et al.*, 1997). The PCR product was purified and subcloned into the plasmid vector pBluescript II KS(-) (Stratagene), and used as a probe for the screening of a cDNA library as described previously (Mikami *et al.*, 1998).

### Screening of a cDNA clone for a membrane GC from a *B. agassizii* or a *C. japonicus* testis cDNA library

A cDNA fragment corresponding to the nucleotides (nt) 2943 to 3283 of a full-length sequence of *BaSTGC01* was labeled with <sup>32</sup>P-dCTP using the Random Primer DNA labeling Kit, Ver.2 (Takara Shuzo Co., Ltd.), then used as a probe for the screening of a *B. agassizii* testis cDNA library. About 1.5×10<sup>5</sup> recombinant phages were plated, and plaques were transferred to a nylon membrane (Hybond-N<sup>+</sup>, Amersham). The membrane was prehybridized for 2 hr at 37°C in 6×SSPE containing 0.5% SDS, 5×Denhardt's solution, 50 µg/ml denatured herring sperm DNA, and 50% formamide. The radioactive probe (36.7×10<sup>6</sup>cpm/ml) was added to the prehybridization solution and incubated overnight at 37°C. The membrane was washed three times for 15 min at 50°C in 2×SSC containing 0.1% SDS. After repeated screening under the same conditions as above, the cDNA insert of a positive clone (*BaSTGC01*) was excised *in vivo* into pBluescript SK(-) (Stratagene) in accordance with the manufacturer's protocol.

Similarly, using a 341-bp cDNA fragment (*CjPTGC03*) as a probe, two clones designated as *CjSTGC01* and *CjSTGC02* were obtained from a *C. japonicus* testis cDNA library.

### Screening of a cDNA clone for a membrane GC from a *S. japonicus* ovary cDNA library

A cDNA fragment corresponding to the nt 3215 to 3555 of a full-length sequence of *SjSOGC01* obtained by RT-PCR with total RNA of the *S. japonicus* ovary and degenerate oligonucleotide primers (P2, P6, and P7) was labeled with digoxigenin (DIG)-dUTP using the DIG High Prime (Boehringer Mannheim), then used as a probe for the screening of a *S. japonicus* ovary cDNA library. About 2.2×10<sup>5</sup> recombinant phages were plated, and plaques were transferred to a nylon membrane (Hybond-N<sup>+</sup>, Amersham). The membrane was prehybridized for 30 min at 42°C in 6×SSC containing 0.02% SDS, 0.1% N-laurorylsarcosine, 2% blocking reagent, and 50% formamide. Then, the membrane was immersed in the hybridization solution containing the labeled probe and incubated overnight at 42°C. The membrane was washed three times for 15 min at 50°C in 2×SSC containing 0.1% SDS, and one time for 10 min at 55°C in 0.1×SSC containing 0.1% SDS. After repeated screening under the same conditions

as above, the cDNA insert of a positive clone (*SjSOGC01*) was excised *in vivo* into pBluescript SK(-) (Stratagene) in accordance with the manufacturer's protocol.

### 5'-Rapid amplification of cDNA ends (5'-RACE)

To obtain a full length cDNA sequence of *SjSOGC01*, the 5'-portion of the cDNA was amplified by the 5'-RACE method (Frohman *et al.*, 1988) using the 5'-RACE System for Rapid Amplification of cDNA Ends, Ver 2.0 (Gibco BRL). Three µg of total RNA was reverse-transcribed with gene-specific antisense oligonucleotide primers (GSP4, GSP7). The cDNA was tailed with dCTP using terminal deoxynucleotidyl transferase, and amplified by PCR with the Abridged Anchor Primer (Gibco BRL) and other gene-specific antisense oligonucleotide primers (GSP5, GSP8). One-tenth of the volume of the PCR product was amplified by PCR with the Abridged Universal Amplification Primer (Gibco BRL) and other gene-specific antisense oligonucleotide primers (GSP6, GSP9). The following PCR conditions were used: for GSP5 and GSP6, denaturation at 94°C for 5 min followed by 30 amplification cycles (94°C for 1 min, 63°C for 1 min, and 72°C for 2 min) and a final extension at 72°C for 7 min; for GSP8 and GSP9, denaturation at 94°C for 5 min followed by 30 amplification cycles (94°C for 1 min, 57°C for 1 min, and 72°C for 2 min) and a final extension at 72°C for 7 min. The PCR products were subcloned into pBluescript II KS (-) and sequenced. The gene-specific primers used were complementary to nt 2390–2415 (GSP4), 2370–2392 (GSP5), 2342–2365 (GSP6), 847–864 (GSP7), 803–821 (GSP8), and 758–776 (GSP9), respectively. The 5'-RACE products overlapped in 63–136 bp with the 5'-end of *SjSOGC01*.

### DNA Sequencing

The DNA fragment in the pBluescript vector was used directly to determine the nucleotide sequence as a template by the dideoxy chain termination procedure (Sanger *et al.*, 1977) with an Applied Biosystems 373A or 377 DNA sequencer, and analyzed on DNASIS software (Hitachi Software Engineering Co.).

### Molecular phylogenetic analysis

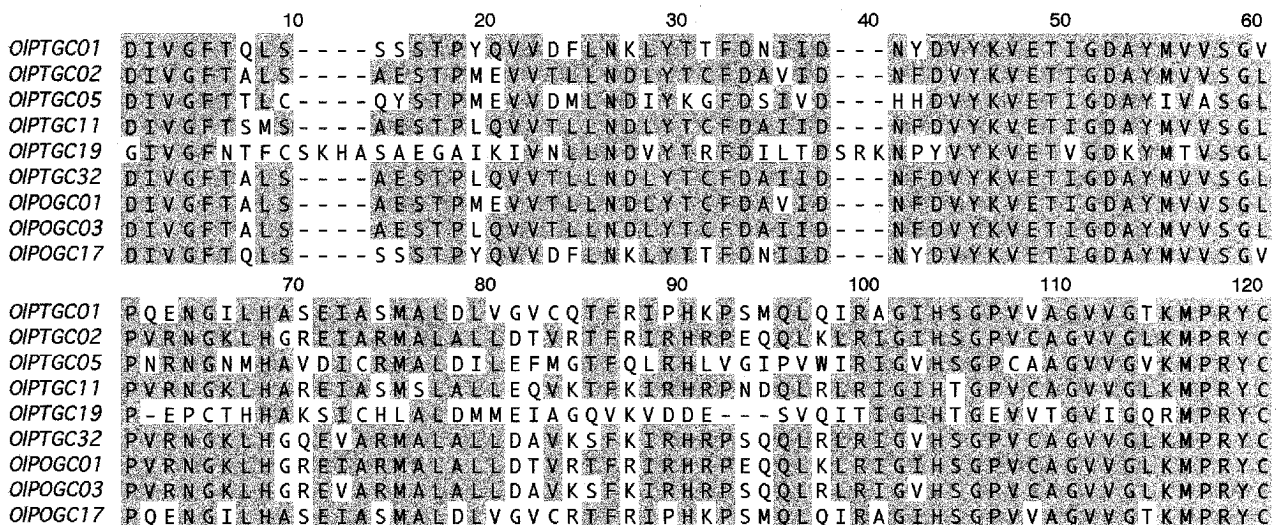
DNA sequences and the deduced amino acid sequences of various echinoderms and medaka fish GC isoforms were compared with those of known vertebrate GC isoforms using the ClustalW program (Thompson *et al.*, 1994) and the sequence editor SeqPup (Gilbert, Indiana University). A phylogenetic tree was constructed with the

aligned sequences by the neighbor-joining (Saitou and Nei, 1987) algorithms in the PROTRAS program of PHYLIP (version 3.572; Felsenstein, 1989) and the ClustalW program (Thompson *et al.*, 1994), respectively. For the neighbor-joining analysis, the evolutionary distance was estimated using Kimura's empirical method for protein distances (Kimura, 1983). GenBank/EMBL/DBJ accession numbers for the sequences used are: rat GC-A (X14773) (Chinkers *et al.*, 1989); rat GC-B (M26896) (Schulz *et al.*, 1989); rat GC-C (M55636) (Schulz *et al.*, 1990); rat GC-D (M26896) (Fülle *et al.*, 1995); rat GC-E (L36029) (Yang *et al.*, 1995); rat GC-F (L36030) (Yang *et al.*, 1995); rat GC-G (AF024622) (Schulz *et al.*, 1998); rat GCS-α<sub>1</sub> (M57405) (Nakane *et al.*, 1990); rat GCS-β<sub>1</sub> (M22562) (Nakane *et al.*, 1988); human GC-A (X15357) (Lowe *et al.*, 1989); human GC-B (113916) (Chang *et al.*, 1989); human GC-C (M73489) (de Sauvage *et al.*, 1991); human RetGC-1 (M92432) (Shyjan *et al.*, 1992); human RetGC-2 (L37378) (Lowe *et al.*, 1995); human GCS-α<sub>1</sub> (X66534) (Giulii *et al.*, 1992); human GCS-β<sub>1</sub> (X66533) (Giulii *et al.*, 1992); OIGC1 (AB004921) (Takeda K and Suzuki N, unpublished data); OIGC2 (AB016082) (Yamagami S, Muramatsu R, and Suzuki N, unpublished data); OIGC3 (AB000899) (Seimiya *et al.*, 1997); OIGC4 (AB000900) (Seimiya *et al.*, 1997); OIGC5 (AB000901) (Seimiya *et al.*, 1997); OIGC6 (AB007192) (Mantoku *et al.*, 1999); OIGCS-α<sub>1</sub> (AB000849) (Mikami *et al.*, 1998); OIGCS-β<sub>1</sub> (AB000850) (Mikami *et al.*, 1998); eel GC-A (AB012869) (Kashiwagi *et al.*, 1998); eel GC-B (D25417) (Katafuchi *et al.*, 1994); *D. melanogaster* DGC1 (L35598) (McNeil *et al.*, 1995); *D. melanogaster* GC (X72801) (Gigliotti *et al.*, 1993); *D. melanogaster* GCS-α<sub>1</sub> (U27117) (Shah *et al.*, 1995); *D. melanogaster* GCS-β<sub>1</sub> (U27123) (Shah *et al.*, 1995); *Manduca sexta* GC-1 (AF073342) (Simpson *et al.*, 1999); *M. sexta* GCS-α<sub>1</sub> (AF062750) (Nighorn *et al.*, 1998); *M. sexta* GCS-β<sub>1</sub> (AF062751) (Nighorn *et al.*, 1998); sea urchin (*H. pulcherrimus*) sperm GC (HpGC) (D21101) (Shimizu *et al.*, 1996); sea urchin (*S. purpuratus*) sperm GC (SpGC) (M22444) (Thorpe and Garbers, 1989); and *C. elegans* GCY-X1 (L80003) (Baude *et al.*, 1998).

## RESULTS

### Amplification of various GC cDNA fragments by RT-PCR

In order to confirm the applicability of RT-PCR for amplification of cDNA fragments for various GC isoforms, we first



**Fig. 1.** Alignment of the amino acid sequences of GC isoforms identified in the testis (OIPTGC\*\*) and ovary (OIPOGC\*\*) of medaka fish *O. latipes*. Amino acids identical among more than five isoforms are indicated by single-letter code on gray background. Gaps in the sequences are indicated by dash (-).

	10	20	30	40	50	60
<i>DsPTGC01</i>	DIVGFTTISADSNP	MQVYTL	LNDLYTCLD	GVIDNF	DVYKQVETIG	DAYMVVSGLP
<i>DsPTGC02</i>	DIVGFTTRISAHITPI	QVAMENDLYS	CFDSRI	DVYDVKVETIG	DAYMVASGCP	EKNKGD
<i>DsPTGC03</i>	DIVGFTNICSQITP	MAVVSMLNGMYTKYD	NLSETF	DVYKQVETIG	DAYMVVSGAPTAT	-KF
<i>DsPOGC01</i>	DIVGFTKLSSDSTPL	EVVDL	LNDLYT	CFDAI	IDNFVYKQVETIG	DAYMVVSGLP
<i>DsPOGC02</i>	DIVGFTNICSQITP	MSVVSMLNGMYTKYD	NLSETF	DVYKQVETIG	DAYMVVSGAPTAT	-KY
<i>DsPOGC04</i>	DIVGFTTAAVSSPL	QIVDMLNGLYS	MFDCI	DQYDVYKQVETIG	DAYMVVSGLPEAT	-DR
<i>GcPTGC01</i>	DIVGFTALSSASTPI	QVVDL	LNDLYT	MFDAV	IANYDVYKQVETIG	DAYMLVSGLP
<i>GcPTGC03</i>	DIVGFTTICSRISP	MAVVSMLNGMYTKYD	NLSETF	DVYKQVETIG	DAYMVVSGAPNTT	-KF
<i>GcPTGC21</i>	DIVGFTKLSSMSTPF	QVVDL	LNDLYT	LFDDI	ISYVYKQVETIG	DAYMLVSGLP
<i>GcPOGC01</i>	DIVGFTKLSSGSTPL	EVVDL	LNDLYT	CFDAI	IDNFVYKQVETIG	DAYMCSVSGLP
<i>GcPOGC02</i>	DIVGFTTAAVSSPL	QVVDL	LNDLYT	SFGVI	GGFDVYKQVETIG	DAYMVVSGLP
<i>GcPOGC03</i>	DIVGFTQLAAASTPL	QVVDMLNGLYS	MFDA	CLDKYDVYKQVETIG	DAYMVVSGMPEAS	-DR
<i>HpPTGC02</i>	DIVGFTTRISAHINPI	EVVSMENDLYS	CFDGR	IDVYDVYKQVETIG	DAYMVASGCP	ERNKGD
<i>HpPTGC04</i>	DIVGFTSLSSSTSTP	FEVVDL	LNDLYT	LFDEI	ISYDVYKQVETIG	DAYMLVSGLPL
<i>HpPTGC07</i>	DIVGFTKLSSASTPI	EVVDL	LNDLYT	CFDAV	IDNFVYKQVETIG	DAYMCSVSGLP
<i>HpPTGC08</i>	DIVGFTNICAECP	IDI	VNMLN	ALYTF	DFDKL	TTVHDVYKQVETIG
<i>HpPTGC12</i>	DIVGFTALSSASTPI	QVVDL	LNDLYT	LFDAI	ISNYDVYKQVETIG	DAYMLVSGLPL
<i>HpPOGC01</i>	DIVGFTKLSSMSTPF	QVVDL	LNDLYT	LFDDI	ISYVYKQVETIG	DAYMLVSGLP
<i>HpPOGC05</i>	DIVGFTSLSSSTSTP	FEVVDL	LNDLYT	LFDEI	ISYDVYKQVETIG	DAYMLVSGLPL
<i>HpPOGC11</i>	DIVGFTAISSESTPL	QVVA	LNDLYT	CFDGI	IANFDVYKQVETIG	DAYMVVSGLP
<i>CjPTGC03</i>	DIVGFTSLSSSTSTP	LQVVA	LNDLYT	CFDGI	IANFDVYKQVETIG	DAYMVVSGLP
<i>CjPTGC05</i>	DIVGFTKLSSDSTPL	EVVDL	LNDLYT	CFDAI	IDNFVYKQVETIG	DAYMVVSGLP
<i>CjSTGC01</i>	DIVGFTSLSSSTSTP	LQVVA	LNDLYT	CFDGI	IANFDVYKQVETIG	DAYMVVSGLP
<i>CjSTGC02</i>	DIVGFTSLSSASTSTP	LQVVDL	LNDLYT	CFDKI	IDGFDVYKQVETIG	DAYMVVSGLP
<i>BaPTGC03</i>	DIVGFTALSSASTPI	QVVDL	LNDLYT	LFDAI	IANYDVYKQVETIG	DAYMLVSGLP
<i>BaPTGC04</i>	DIVGFTKLSSMSTPL	EVVDL	LNDLYT	CFDAV	IDNFVYKQVETIG	DAYMVVSGLP
<i>BaPTGC37</i>	DIVGFTSLSSSTSTP	YEVVA	LNKLYT	FDSI	IDNYDVYKQVETIG	DAYMVVSGVP
<i>BaSTGC01</i>	DIVGFTALSSASTPI	QVVDL	LNDLYT	LFDAI	IANYDVYKQVETIG	DAYMLVSGLP

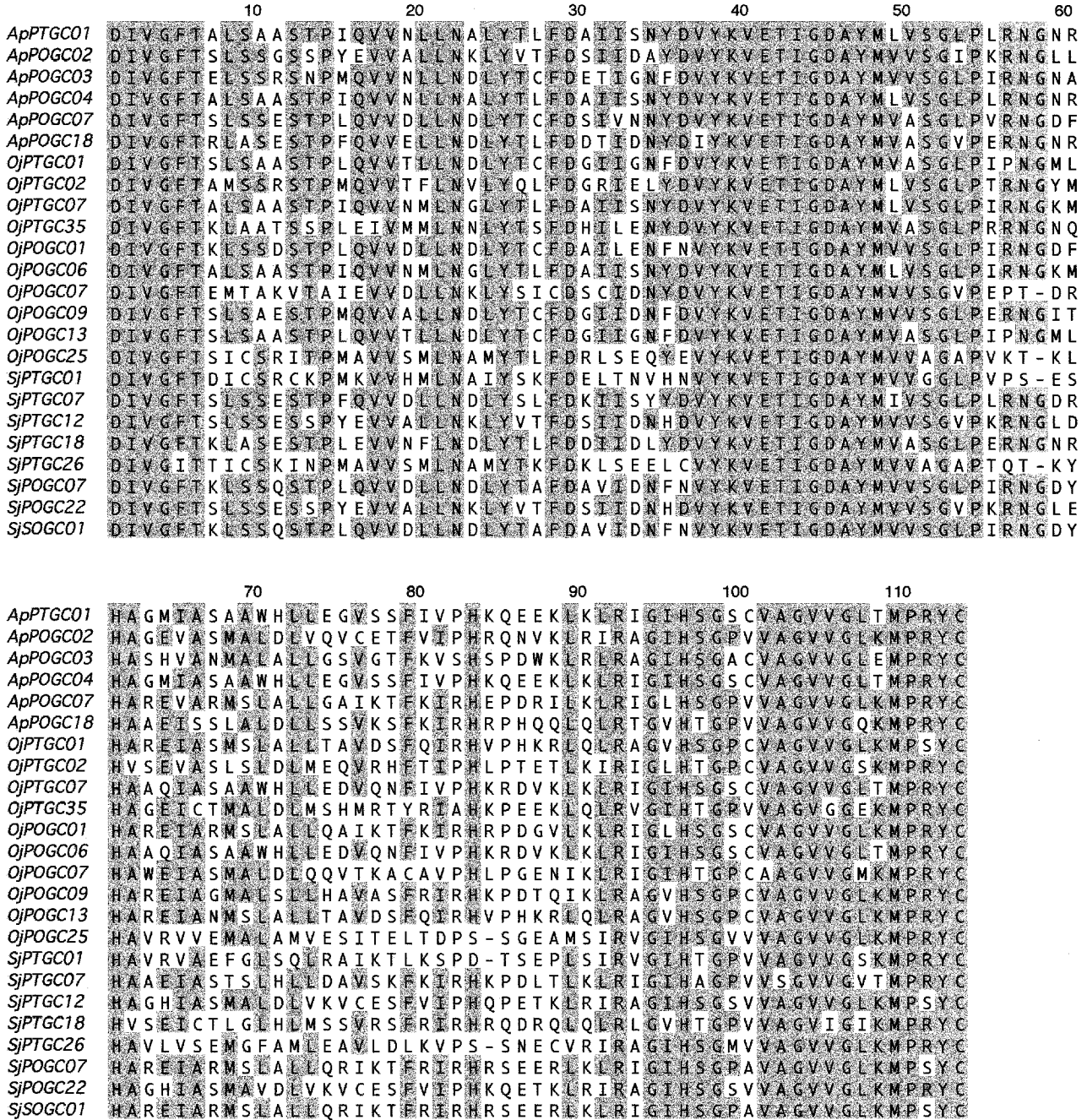
  

	70	80	90	100	110																								
<i>DsPTGC01</i>	HAKETISSMALAL	VEKVANKIKHKP	DEKLL	LRVGIHSGSV	VAGVVGLKMPRYC																								
<i>DsPTGC02</i>	HVTEIATMAIDL	RSAMKQVTVPH	ARTTKLQ	VRIQIHTG	PCVAGVVGIKMPRYC																								
<i>DsPTGC03</i>	HAVRIVEMGLAM	ISAIADV	KDPSSNDNM	KIRIGVHSGMV	VAGVVGLKMPRYC																								
<i>DsPOGC01</i>	HAREIARMSLAL	NEITTEIR	RHRPDERL	RLRIGVHTG	PPVAGVVGLKMPRYC																								
<i>DsPOGC02</i>	HAVRIVEMGLAM	ICAIADV	KDPSSNDNM	KIRIGVHSGMV	VAGVVGLKMPRYC																								
<i>DsPOGC04</i>	HAWEVCTMALE	LVRLVLSYKIP	HRTTERL	KLRVGIHSGS	CVAGVVGLKMPRYC																								
<i>GcPTGC01</i>	HAGQIASTAYH	LLESVKTEI	VPHRPEIF	LKRIGIHS	SCVAGVVGLTMPRYC																								
<i>GcPTGC03</i>	HAVRIAEMSLG	MLDSTKDL	KDPSSN-D	NV	KIRVGIHSGMV	VAGVVGLKMPRYC																							
<i>GcPTGC21</i>	HAAEIGSAAHL	IDATQKFK	RHRPDDT	LKLRVGIHSG	PVVTG	VVGLTMPRYC																							
<i>GcPOGC01</i>	HAREIARMSL	ALLNEITSF	RIRHRPDERL	RLRIGVHTG	PPVAGVVGLTMPRYC																								
<i>GcPOGC02</i>	HVKEIASMSL	ALLKEASTF	KIRHKPDEK	L	LRVGIHSGAC	VAGVVGLRMPRYC																							
<i>GcPOGC03</i>	HAWEISTLAL	ELRRLV	LGYPHLS	DKKCL	RIGIHS	NCVAGVVGVKMPRYC																							
<i>HpPTGC02</i>	HVKEIATMSID	LRSAMKQV	HVPRSP	STKLQ	VRIGLHTG	PCVAGVVGVKMPRYC																							
<i>HpPTGC04</i>	HAAEIASTSL	HLEDVKA	KVRHRPEVN	LKLRIGIHS	STVSGVVGLTMPRYC																								
<i>HpPTGC07</i>	HAREIARMSL	ALLHEITSF	RIRHRBEERL	RLRIGVHTG	PPVAGVVGLKMPRYC																								
<i>HpPTGC08</i>	HAARIANQAL	GMMVICKE	VMSPVTR	-EPI	GIRVGIHSG	PPVSGVVGEKMPRYC																							
<i>HpPTGC12</i>	HAGQIASTAH	LLESVKGEI	VPHKPEVFL	LKRIGIHS	SCVAGVVGLTMPRYC																								
<i>HpPOGC01</i>	HAAEIGSTAL	H	LIDEVQKFK	RHRPDDT	LKLRVGIHSG	PVVTG	VVGLTMPRYC																						
<i>HpPOGC05</i>	HAAEIASTSL	HLEDVKA	KVRHRPEVN	LKLRIGIHS	STVSGVVGLTMPRYC																								
<i>HpPOGC11</i>	HAREIAEMSL	SLL	LEAVKSF	KIRHRPDEQ	L	KLRA	GIHSGPCVAGVIGL	KMPRYC																					
<i>CjPTGC03</i>	HARHIAEMSL	ALLTAVQSF	KIRHRPDTQ	L	KLRA	GIHSGPCV	R	GI	GLKMPRYC																				
<i>CjPTGC05</i>	HAREIARMSL	ALLNQISTF	RIRHRPDVRL	RLRIGVHTG	PPVAGVVGLKMPRYC																								
<i>CjSTGC01</i>	HARHIAEMSL	ALLTAVQSF	KIRHRPDTQ	L	KLRA	GIHSGPCV	R	GI	GLKMPRYC																				
<i>CjSTGC02</i>	HAREISSMAL	ALIDGVSSFR	IRHKPGEK	L	LRVGIHSG	ACVAGVVGLRMPRYC																							
<i>BaPTGC03</i>	HAGQIASAAWH	LLESVKS	FVPHRDPVFL	LKRIGIHS	SCV	ARVVGLTMP	SYC																						
<i>BaPTGC04</i>	HAREIARMSL	ALLNEISSF	RIRHRPDERL	RLRIGVHTG	PPVAGVVGLKMPRYC																								
<i>BaPTGC37</i>	HAGEIASMAL	DLVSV	CETEV	I	PHRQDQK	L	R	I	R	A	G	I	H	S	G	S	V	A	G	V	V	G	L	K	M	P	R	Y	C
<i>BaSTGC01</i>	HAGQIASAAWH	LLESVKS	FVPHRDPVFL	LKRIGIHS	SCV	AGVVGLTMPRYC																							

**Fig. 2.** Comparison of the amino acid sequences of GC isoforms identified in the gonads of various sea urchin species. *DsPTGC\*\** and *DsPOGC\*\**, and *GcPTGC\*\** and *GcPOGC\*\** denote the isoforms in the testis and ovary of *D. setosum*, and in the testis and the ovary of *G. crenularis*, respectively. Similarly, *HpPTGC\*\** and *HpPOGC\*\** denote the isoforms in the testis and the ovary of *H. pulcherrimus*, respectively. The ovary of sea urchin species *C. japonicus* and *B. agassizii* was not used for RT-PCR experiments; all isoforms of these species used herein are in the testis. Amino acids identical among more than fifteen isoforms are indicated by single-letter code on gray background. Gaps in the sequences are indicated by dash (-).

applied the method to amplification of GC isoforms in the gonads of medaka fish, which have been known to possess six membrane GC isoforms and a soluble GC ( $\alpha$  and  $\beta$  subunits). By this method, the six GC isoforms (*OIPTGC01*, *OIPTGC02*, *OIPTGC05*, *OIPTGC11*, *OIPTGC19*, and *OIPTGC32*) were amplified from the testis sample and three GC isoforms (*OIPOGC01*, *OIPOGC03*, and *OIPOGC17*) were amplified from the ovary sample (Fig. 1). *OIPTGC02* and *OIPOGC01*

are identical, and each corresponded to *OIGC2* which could be a member of the natriuretic peptide receptor family (Yamagami S, Muramatsu R, and Suzuki N, unpublished data). *OIPTGC01* and *OIPOGC17*, and *OIPTGC32* and *OIPOGC03* were identical, respectively. *OIPTGC11* corresponded to *OIGC1*, which could be considered another member of the natriuretic peptide receptor family (Takeda K and Suzuki N, unpublished data). *OIPTGC05* corresponded to *OIGC6*, which



**Fig. 3.** GC isoforms identified in the gonads of several echinoderm species. The nomenclature for the isoforms is same as that in the legend for Fig. 2; ApPTGC\*\* and ApPOGC\*\* are the isoforms in the testis and ovary of the starfish *A. pectinifera*, respectively; OjPTGC\*\* and OjPOGC\*\* are the isoforms in the testis and ovary of the brittle star *O. japonicus*, respectively; and SjPTGC\*\* and SjPOGC\*\* are the isoforms in the testis and ovary of the sea cucumber *S. japonicus*, respectively. Amino acids identical among more than 13 isoforms are indicated by single-letter code on gray background. Gaps in the sequences are indicated by dash (-).

has been suggested to be a medaka fish homolog of the mammalian enterotoxin/guanylin receptor (Mantoku *et al.*, 1999). The amino acid sequence of *OIPTGC19* was identical to that of a part of *OIGCS- $\beta$* , which encodes the  $\beta$  subunit of a NO-sensitive soluble GC of medaka fish (Mikami *et al.*, 1998).

Using the same method as described above, three GC isoforms were identified in the testis sample of *D. setosum* and three GC isoforms were found in the ovary sample. Similarly, three GC isoforms were obtained from the testis and three from the ovary sample of *G. crenularis*; five isoforms were obtained from the testis of *H. pulcherrimus*, and three from the ovary. The sequence of HpPTGC04 was identical to that of HpPOGC05. HpPTGC12 corresponded to HpGC, which has been proved to be a protein associated with SAP-I receptors (Shimizu *et al.*, 1996). Thus, this isoform is referred to herein as a SAP receptor-type GC. The testis of *C. japonicus* and that of *B. agassizii* each contained three isoforms. Alignment of the deduced amino acid sequences of these GC isoforms are shown in Fig. 2. Some of these isoforms (DsPTGC03, DsPOGC02, DsPOGC04, GcPTGC03, GcPOGC03, and HpPTGC08) could be considered to be putative soluble GC isoforms. Other echinoderm species also possessed various GC isoforms in their testis and ovary (Fig. 3). ApPTGC01, the only isoform identified in the testis of *A. pectinifera*, was identical to ApPOGC04 in the ovary of the species. OjPTGC01 and OjPTGC07 in the testis of *O. japonicus* were identical to OjPOGC13 and OjPOGC06, respectively, in the ovary of the species. The ovary of the brittle star *O. japonicus* contained two putative soluble GC isoforms, and the testis of the sea cucumber *S. japonicus* possessed two putative soluble GC isoforms.

### Comparison and phylogenetic analysis of the amino acid sequences of various GCs

In order to understand structural and evolutionary relationship among various GC isoforms obtained in this study and already known various membrane and soluble GC isoforms, the amino acid sequences of the catalytic domain of these GC isoforms were compared and phylogenetic analysis was carried out. An unrooted phylogenetic tree indicated that these GC isoforms appear to fall into two groups: (i) the membrane GCs, and (ii) the soluble GCs, which can be further divided into  $\alpha$  and  $\beta$  subunits of soluble GCs and putative soluble GCs found in echinoderms and nematoda (Fig. 4). The membrane GCs can also be further divided, into vertebrate GCs and invertebrate GCs. The vertebrate membrane GCs can be divided into three major group GCs, as was previously reported (Seimiya *et al.*, 1997): (i) natriuretic peptide receptors, (ii) heat-stable enterotoxin/guanylin receptors, and (iii) sensory organ-specific membrane GCs. The SAP receptor-type GC was distantly related to all of the vertebrate membrane GCs (Fig. 5). Phylogenetic analysis with nucleotide sequences demonstrated similar results (data not shown).

Phylogenetic analysis with various echinoderm membrane GC isoforms and known membrane GC isoforms demonstrated that these membrane GC isoforms can be divided into

several groups: (i) a group comprised of five isoforms (HpPTGC12, BaPTGC03, GcPTGC01, ApPTGC01, and OjPTGC07), and (ii) several other groups, including Group A (Fig. 6). The phylogenetic tree with nucleotide sequences of the catalytic domains of these membrane GC isoforms demonstrated similar results (data not shown).

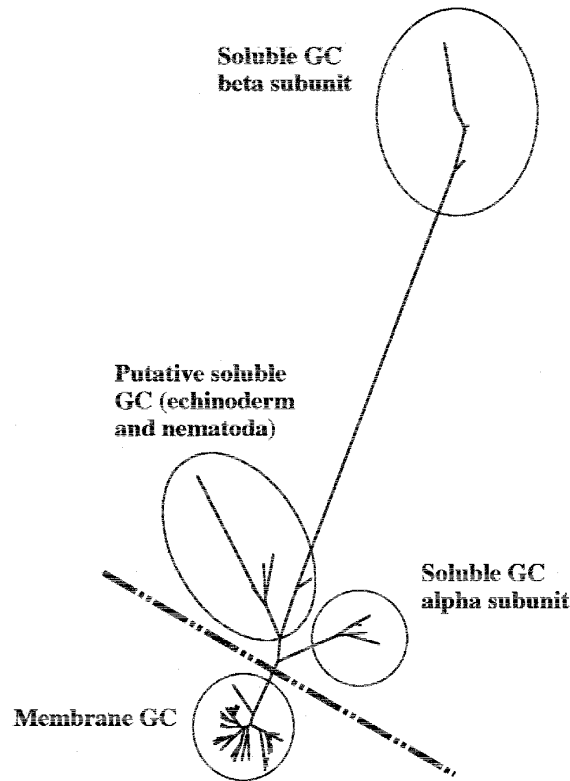
### Isolation and characterization of a cDNA clone encoding a membrane GC from a *B. agassizii* or *C. japonicus* testis cDNA library

Molecular phylogenetic analysis with the 341-bp cDNA fragment of *BaPTGC03* obtained by RT-PCR suggested that the cDNA fragment could be part of a SAP receptor-type GC of *B. agassizii* spermatozoa. Using *BaPTGC03* as a probe, a clone named *BaSTGC01*, 4110-bp in length, was obtained from a *B. agassizii* testis cDNA library. Comparison of the deduced amino acid sequence of *BaSTGC01* with those of known SAP receptor-type GCs indicated that *BaSTGC01* contains the entire coding region typical of these. *BaSTGC01* consists of a 149-bp 5'-untranslated region (5'-UTR), a 3414-bp open reading frame (ORF), and a 547-bp 3'-untranslated region (3'-UTR). Termination codons occur in all three frames upstream of the putative initiation codon of *BaSTGC01*. In addition, nucleotides around the putative initiation codon of *BaSTGC01* fit to the preferred sequence context for the initiation of protein synthesis in eukaryotic mRNAs (Kozak, 1983). The ORF of *BaSTGC01* encodes a polypeptide of 1138 amino acids. Hydropathic analysis (Kyte and Doolittle, 1982; data not shown) and comparison of the deduced amino acid sequence of *BaSTGC01* with those of known SAP receptor-type GCs suggest that the domain organization of *BaSTGC01* is similar to that of known SAP receptor-type GCs (Fig. 7). *BaSTGC01* contains an amino-terminal signal sequence of 22 amino acids (von Heijne, 1983). Cleavage of the signal sequence would result in a mature protein of 1116 amino acids. The mature protein is comprised of a large extracellular domain (residues 23–520), a single membrane-spanning domain (residues 521–542), a protein kinase-like domain (residues 576–859), and a cyclase catalytic domain (residues 879–1006). Comparison of the deduced amino acid sequence of *BaSTGC01* with that of HpGC, a SAP receptor-type GC, in *H. pulcherrimus* spermatozoa, indicates that thirteen serine residues at positions 574, 579, 665, 679, 684, 785, 907, 910, 927, 931, 940, 943, and 998 in the intracellular domain of *BaSTGC01* correspond respectively to those at positions 561, 565, 652, 666, 671, 772, 894, 897, 914, 918, 927, 930, and 985 in the intracellular domain of HpGC, all of which have been proved to be phosphorylated (Furuya *et al.*, 1998).

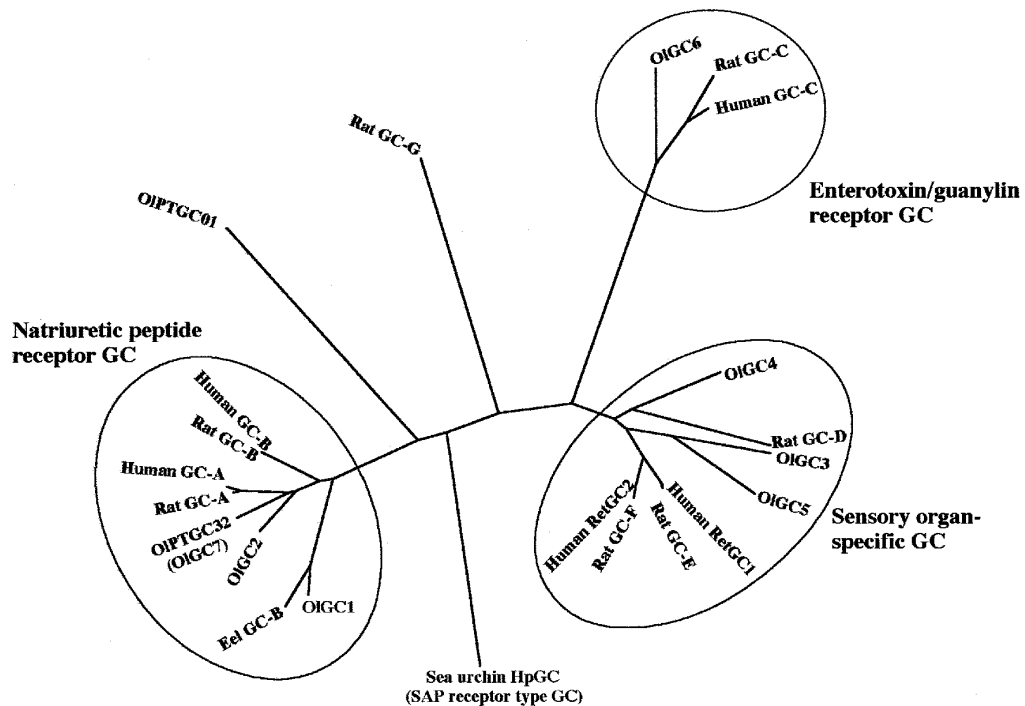
The clones *CjSTGC01* and *CjSTGC02* were 1922 bp and 2953 bp in length, respectively, and both lacking the 5'-portion of coding region. Portions of their deduced amino acid sequences are shown in Fig. 2.

### Isolation and characterization of a cDNA clone encoding a membrane GC from a *S. japonicus* ovary cDNA library

Molecular phylogenetic analysis with a 341-bp cDNA frag-

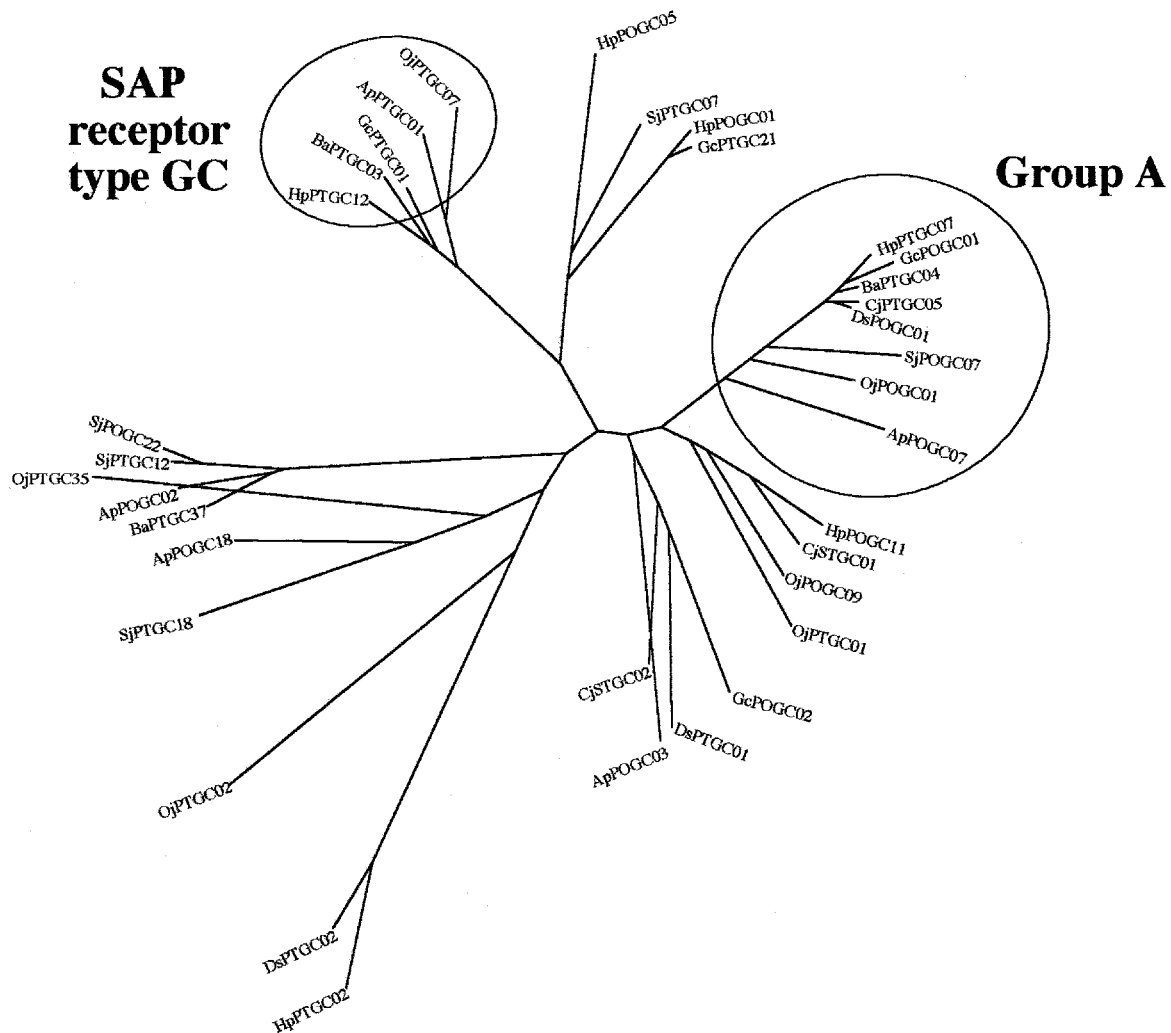


**Fig. 4.** Molecular phylogenetic relationship among various membrane and soluble GC isoforms. Amino acid sequences of the catalytic domain of various membrane GC isoforms and soluble GC isoforms were subjected to phylogenetic analysis. An unrooted phylogenetic tree was constructed by the neighbor-joining method. Branch lengths were proportional to evolutionary distances.



**Fig. 5.** Molecular phylogenetic analysis with the amino acid sequences conserved in the catalytic domain of various vertebrate membrane GC isoforms. An unrooted phylogenetic tree was constructed by the neighbor-joining method. Branch lengths were proportional to evolutionary distances.

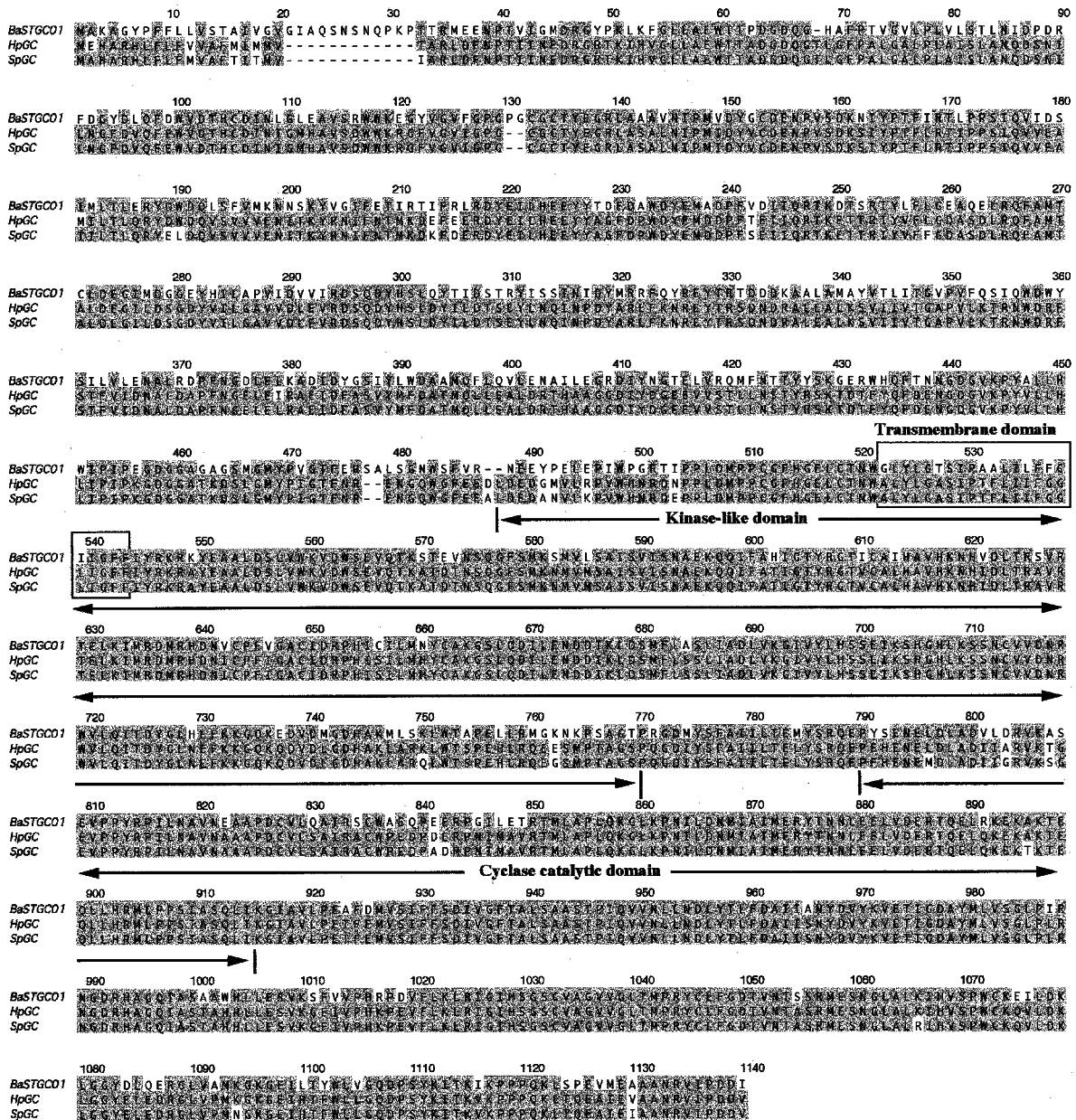




**Fig. 6.** Molecular phylogenetic relationship among various echinoderm membrane GC isoforms. Amino acid sequences of the catalytic domain of various echinoderm membrane GC isoforms were subjected to phylogenetic analysis. An unrooted phylogenetic tree was constructed by the neighbor-joining method. Branch lengths were proportional to evolutionary distances.

ment (*SjPOGC07*) obtained by RT-PCR indicated that *SjPOGC07* belongs to Group A and appears to be a sea cucumber homolog of the mammalian natriuretic peptide receptor/membrane GC. By screening a *S. japonicus* ovary cDNA library with the insert cDNA of *SjPOGC07* as a probe, a clone named *SjSOGC01* 1752-bp in length was obtained. Comparison of the deduced amino acid sequence of *SjSOGC01* with those of known natriuretic peptide receptors/membrane GCs suggested that *SjSOGC01* lacks the 5'-portion of the coding region. Therefore, the 5'-RACE method was adopted to obtain the 5'-portion of *SjSOGC01*. The primary 5'-RACE product for *SjSOGC01* was 1651-bp in length and did not contain an initiation codon. The secondary 5'-RACE product for *SjSOGC01* was 776-bp in length which contained a putative translation initiation codon. The full-length cDNA sequence for *SjSOGC01* consists of a 551-bp 5'-UTR, a 3225-bp ORF, and an 167-bp 3'-UTR. Termination codons occur in all three frames upstream of the putative initiation codon of *SjSOGC01*.

In addition, nucleotides around the putative initiation codon of *SjSOGC01* fit to the preferred sequence context for initiation of protein synthesis in eukaryotic mRNAs (Kozak, 1983). The ORF of *SjSOGC01* encodes a polypeptide of 1075 amino acids. Hydropathic analysis of the deduced amino acid of *SjSOGC01* (Kyte and Doolittle, 1982; data not shown) predicted that *SjSOGC01* contains an amino-terminal signal sequence of 28 amino acids (von Heijne, 1983). Cleavage of the signal sequence would result in a mature protein of 1047 amino acids. The mature protein is comprised of a large extracellular domain (residues 29–474), a single membrane-spanning domain (residues 475–493), a protein kinase-like domain (residues 525–811), and a cyclase catalytic domain (residues 832–1059) (Fig. 8). Five cysteine residues at positions 100, 125, 248, 455, and 463, and histidine and tryptophan at positions 138 and 139 in the extracellular domain of *SjSTGC01* were conserved in corresponding positions of natriuretic peptide receptors/membrane GCs including eel GC-B,



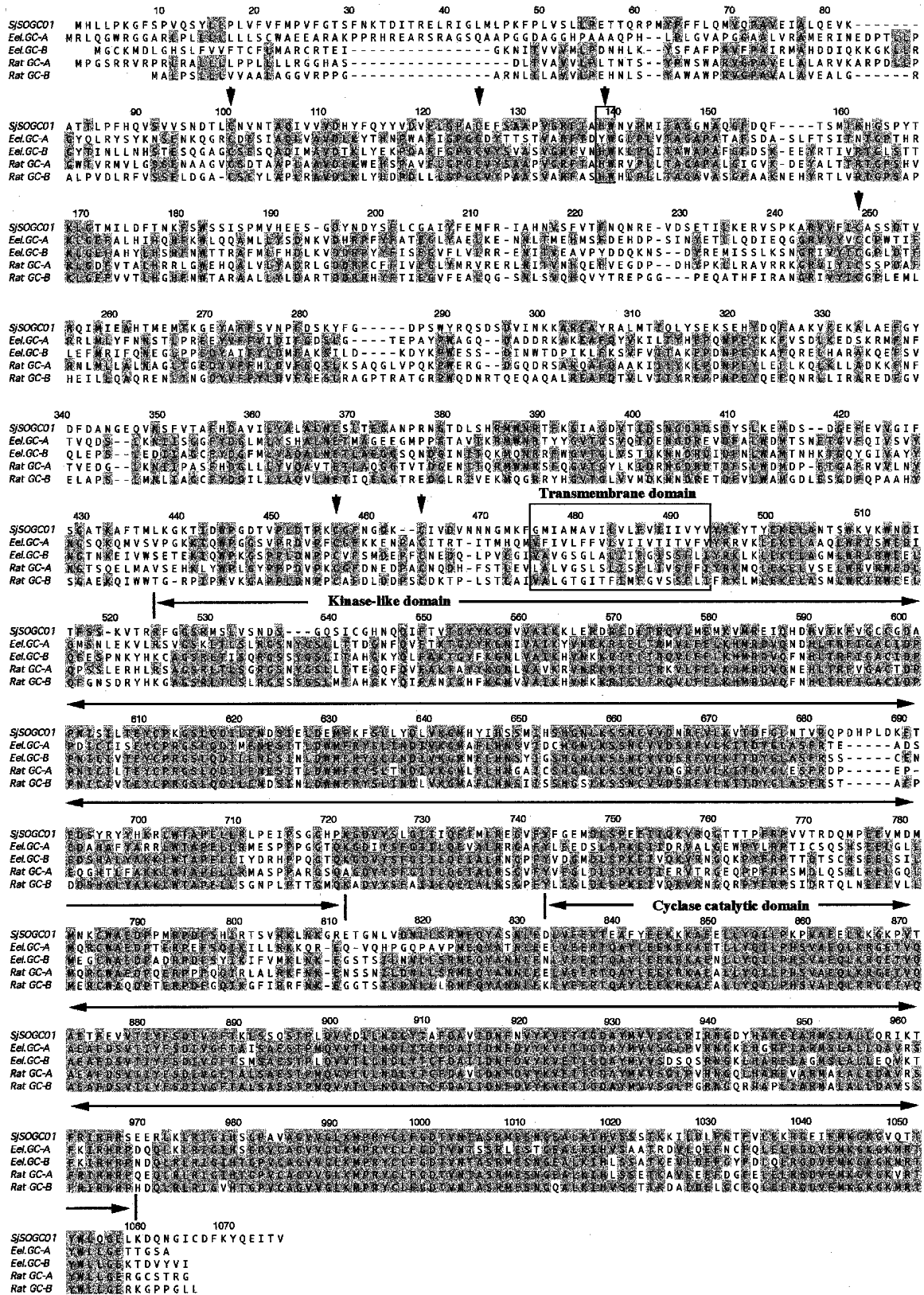
**Fig. 7.** Alignment of the amino acid sequences of three SAP receptor-type GCs. The deduced amino acid sequence of BaSTGC01 was compared with those of HpGC and SpGC. Transmembrane domain, kinase-like domain, and catalytic domain are indicated by an open box or lines with arrows. Amino acids identical in two isoforms are indicated by single-letter code on gray background. Gaps in the sequences are indicated by dash (-).

rat GC-A, and rat GC-B (Fig. 8).

### DISCUSSION

In this paper we demonstrated that the gonads of medaka fish contain many GC isoforms including natriuretic peptide receptors/membrane GCs and a soluble GC. This is consistent with the results obtained from RT-PCR analysis on *OIGC6* and *OIGCS-β*, transcripts in the ovary and/or testis of medaka fish (Mantoku et al., 1999; Mikami et al., 1998) (Fig. 1). This is also the case for the gonads of various echinoderms includ-

ing species of sea urchin (Figs. 2, 3). These facts suggest that the unfertilized eggs of these species possess messages for various GCs as stored mRNA, and the mature spermatozoa of these species may carry their translation products, although there is the possibility that somatic cells surrounding the immature eggs or spermatozoa possess such mRNAs. To our knowledge, no report has demonstrated that the gonads of echinoderms including species of sea urchin contain various GC isoforms such as the natriuretic peptide receptor/membrane GC and soluble GC excepting SAP receptor-type GC. In this regard, it should be mentioned that natriuretic pep-



**Fig. 8.** Comparison of the deduced amino acid sequence of SjSOGC01 with those of natriuretic peptide receptors. The conserved cysteine residues in the extracellular domain are indicated by arrows, and the conserved histidine-tryptophan (<sup>138</sup>H-<sup>139</sup>W) residues in the extracellular domain, which are important for ligand-binding, are indicated by an open box with an arrow. Transmembrane domain, kinase-like domain, and cyclase catalytic domain are indicated by open box or lines with arrows. Amino acids identical among three isoforms are indicated by single-letter code on gray background. Gaps in the sequence are indicated by dash (-).

tides and NO have been shown to induce a sperm acrosome reaction in mammals (Anderson *et al.*, 1994 ; Herrero *et al.*, 1998). These results suggest that a natriuretic peptide receptor/membrane GC and/or a NO-sensitive soluble GC are present in the spermatozoa and involved in these processes.

Phylogenetic analysis with the amino acid sequences of medaka fish and other vertebrate membrane GC isoforms demonstrates that vertebrate membrane GC isoforms could be divided into three groups: (i) natriuretic peptide receptors, (ii) heat-stable enterotoxin/guanylin receptors, and (iii) sensory organ-specific membrane GCs (Fig. 5). The analysis also shows that neither rat GC-G or OIPTGC01 from medaka fish testis is related to any member among the three groups. It is possible that the orthologues for rat GC-G and for OIPTGC01 are present in other vertebrates. Similarly, the phylogenetic analysis on echinoderm membrane GC isoforms indicates that echinoderm membrane GCs could be divided into several groups: (i) SAP receptor-type GC; (ii) Group A, and (iii) others. Further, the analysis indicates that every echinoderm species contains one membrane GC isoform belonging to Group A. However, the position of DsPOGC01 differs from the position expected in the phylogenetic tree from a morphological standpoint (Fig. 6; Shigei, 1974).

Though it remains to be proved that such a membrane GC exists in *B. agassizii* spermatozoa and can be activated by SAP-V, the molecular phylogenetic analysis carried out in this study suggests that BaSTGC01 (identical to BaPTGC03) is a SAP receptor-type GC isoform in *B. agassizii* spermatozoa. Similarly, our analysis demonstrated that BaPTGC03, GcPTGC01, and HpPTGC12 would be SAP receptor-type GCs in sea urchins *B. agassizii*, *G. crenularis*, and *H. pulcherrimus*, respectively; and that ApPTGC01 and OjPTGC07 would be this type of GC in the starfish and brittle star, respectively. However, despite uniform application of the RT-PCR method, we could not amplify a cDNA fragment for the SAP receptor-type GC in the sea urchin species of *C. japonicus* or *D. setosum* or the sea cucumber *S. japonicus*. This may be due to use of inadequate primers to amplify the cDNA fragment for the SAP receptor-type GC in those species. The SAP receptor-type GCs have several conserved regions other than those we used for the synthesis of primers in this study (Fig. 7). Therefore, we could perhaps amplify the cDNA fragment for the SAP receptor-type GC in *C. japonicus*, *D. setosum*, and *S. japonicus* if we used other conserved amino acid sequences such as QKGLKP, MIAIME, VSPWCK, and DKLGGY to design PCR-primers.

The extracellular domain of natriuretic peptide receptors/membrane GCs contains conserved ligand-binding sites such as cysteine residues, which form two disulfide-linked loops and histidine-tryptophan residues in the extracellular domain (Iwashina *et al.*, 1994). SjsOGC01, belonging to Group A, has such cysteine residues and histidine-tryptophan residues in the extracellular domain at positions corresponding to those in vertebrate natriuretic peptide receptors/membrane GCs (Fig. 8). Therefore, these conserved residues in SjsOGC01 might serve to bind unknown ligands that have a structure similar to

those ligands that bind to vertebrate natriuretic peptide receptors. In this regard, it may be valuable to note that CNP, a ligand for the natriuretic peptide receptors in mammals, has been reported to exist in a wide variety of tissues in various animals (Suga *et al.*, 1992; Vollmar *et al.*, 1993; Chrisman *et al.*, 1993; Hagiwara *et al.*, 1995) and to be involved in the regulation of cardiovascular homeostasis by means of potent natriuretic, diuretic, vasodilatory, and cell growth inhibitory activities. Thus, we presume that SjsOGC01 and the vertebrate natriuretic peptide receptors/membrane GC isoforms might be derived from an ancestral gene. The deduced amino acid sequence of *CjSTGC01* obtained from a *C. japonicus* testis cDNA library is similar to those of the vertebrate natriuretic peptide receptors/membrane GCs, although it is truncated at the 5'-portion of the coding region (data not shown).

It has been reported that a nematoda, *C. elegans*, contains a large family of GC isoforms, and some of these isoforms express at sensory neurons or interneurons (Yu *et al.*, 1997). In this study, we carried out phylogenetic analysis with the deduced amino acid sequences of the nematoda GC isoforms and other GC isoforms to determine whether orthologues for such nematoda GC isoforms are present in other animals, but the results were negative. Further, phylogenetic analysis performed on soluble GCs only suggests that genes for soluble GCs can be divided into three groups: (i) soluble GC  $\alpha$  subunit group, (ii) soluble GC  $\beta$  subunit group, and (iii) putative soluble GC group found in echinoderms and *C. elegans* (Fig. 4). At present, little data on soluble GCs is available for molecular phylogenetic analysis. When more data on soluble GCs has been accumulated, we can obtain a clearer understanding of the phylogenetic relationship among diverse GC isoforms.

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## REFERENCES

- Anderson RA, Feathergill KA, Drisdell RC, Rawlins RG, Mack SR, Zaneveld LJ (1994) Atrial natriuretic peptide (ANP) as a stimulus of the human acrosome reaction and a component of ovarian follicular fluid: Correlation of follicular ANP content with *in vitro* fertilization outcome. *J Androl* 15: 61-70
- Baude EJ, Arora VK, Yu S, Garbers DL, Wedel BJ (1997) The clon-

- ing of a *Caenorhabditis elegans* guanylyl cyclase and construction of a ligand-sensitive mammalian/nematode chimeric receptor. *J Biol Chem* 272: 16035–16039
- Cathala G, Savouret J-F, Mendez B, West BL, Karin M, Martial JA, Baxter JD (1983) A method for isolation of intact, translationally active ribonucleic acid. *DNA* 2: 329–335
- Chang M-S, Lowe DG, Lewis M, Hellmiss R, Chen E, Goeddel DV (1989) Differential activation by atrial and brain natriuretic peptides of two different receptor guanylate cyclases. *Nature* 341: 68–72
- Chinkers M, Garbers DL, Chang M-S, Lowe DG, Chin H, Goeddel DV, Schulz S (1989) A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* 338: 78–83
- Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem* 162: 156–159
- Chrisman TD, Schulz S, Potter LR, Garbers DL (1993) Seminal plasma factors that cause large elevations in cellular cyclic GMP are C-type natriuretic peptides. *J Biol Chem* 268: 3698–3703
- Davis LG, Dibner MD, Battey JF (1986) *Basic Methods in Molecular Biology*, Elsevier, New York
- de Sauvage FJ, Camerato TR, Goeddel DV (1991) Primary structure and functional expression of human receptor for *Escherichia coli* heat-stable enterotoxin. *J Biol Chem* 266: 17912–17918
- Drewett JG, Garbers DL (1994) The family of guanylyl cyclase receptors and their ligands. *Endocrine Rev* 15: 135–162
- Felsenstein J (1989) PHYLIP-phylogeny interference package (version 3.2). *Cladistics* 5: 164–166
- Frohman MA, Dush MK, Martin GR (1988) Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. *Proc Natl Acad Sci USA* 85: 8998–9002.
- Furuya H, Yoshino K, Shimizu T, Mantoku T, Takeda T, Nomura K, Suzuki N (1998) Mass spectrometric analysis of phosphoserine residues conserved in the catalytic domain of membrane-bound guanylyl cyclase from sea urchin spermatozoa. *Zool Sci* 15: 507–516
- Fülle H-J, Vassar R, Foster DC, Yang R-B, Axel R, Garbers DL (1995) A receptor guanylyl cyclase expressed specifically in olfactory sensory neurons. *Proc Natl Acad Sci USA* 92: 3571–3575
- Garbers DL (1989) Guanylate cyclase, a cell surface receptor. *J Biol Chem* 264: 9103–9106
- Garbers DL (1992) Guanylyl cyclase receptors and their endocrine, paracrine, and autocrine ligands. *Cell* 71: 1–4
- Garbers DL, Lowe DG (1994) Guanylyl cyclase receptors. *J Biol Chem* 269: 30741–30744
- Gigliotti S, Cavaliere V, Manzi A, Tino A, Graziani F, Malva C (1993) A membrane guanylate cyclase *Drosophila* homolog gene exhibits maternal and zygotic expression. *Dev Biol* 159: 450–461
- Giulli G, Scholl U, Bulle F, Guellaen G (1992) Molecular cloning of the cDNAs coding for the two subunits of soluble guanylyl cyclase from human brain. *FEBS Lett* 304: 83–88
- Goldberg ND, Haddox MK (1977) Cyclic GMP metabolism and involvement in biological regulation. *Annu Rev Biochem* 46: 823–896
- Hagiwara H, Hirose S, Takei Y (1995) Natriuretic peptides and their receptors. *Zool Sci* 12: 141–149
- Harumi T, Yamaguchi M, Suzuki N (1991) Receptors for sperm-activating peptides, SAP-I and SAP-IIb, on spermatozoa of sea urchins *Hemicentrotus pulcherrimus* and *Glyptocidaris crenularis*. *Develop Growth Differ* 33: 67–73
- Herrero MB, Cebral E, Franchi A, Motta A, Gimeno MF (1998) Progesterone enhances prostaglandin E2 production via interaction with nitric oxide in the mouse acrosome reaction. *Biochem Biophys Res Commun* 252: 324–328
- Iwashina M, Mizuno T, Hirose S, Ito T, Hagiwara H (1994) His145-Trp146 residues and the disulfide-linked loops in atrial natriuretic peptide receptors are critical for the ligand-binding activity. *J Biochem (Tokyo)* 115: 563–567
- Kashiwagi M, Miyamoto K, Takei Y, Hirose S (1999) Cloning, properties and tissue distribution of natriuretic peptide receptor-A of the euryhaline eel, *Anguilla japonica*. *Eur J Biochem* 259: 204–211
- Katafuchi T, Takashima A, Kashiwagi M, Hagiwara H, Takei Y, Hirose S (1994) Cloning and expression of eel natriuretic-peptide receptor B and comparison with its mammalian counterparts. *Eur J Biochem* 222: 835–842
- Kimura M (1983) *The neutral theory of molecular evolution*. Cambridge University Press, Cambridge, England, p 75
- Kinoh H, Shimizu T, Fujimoto H, Suzuki N (1994) Expression of a putative mRNA for sperm-activating peptide I in accessory cells of the ovary in the sea urchin *Hemicentrotus pulcherrimus*. *Roux's Arch Dev Biol* 203: 381–388
- Kopf GS, Tubb DJ, Garbers DL (1979) Activation of sperm respiration by a low molecular weight factor and 8-bromoguanosine 3',5'-monophosphate. *J Biol Chem* 254: 8554–8560
- Kozak M (1983) Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. *Microbiol Rev* 47: 1–45
- Kyte J, Doolittle RF (1982) A simple method for displaying the hydrophobic character of a protein. *J Mol Biol* 157: 105–132
- Lowe DG, Chang M-S, Hellmiss R, Chen E, Singh S, Garbers DL, Goeddel DV (1989) Human atrial natriuretic peptide receptor defines a new paradigm for second messenger signal transduction. *EMBO J* 8: 1377–1384
- Lowe DG, Dizhoor AM, Liu K, Gu Q, Spencer M, Laura R, Lu L, Hurley JB (1995) Cloning and expression of a second photoreceptor-specific membrane retina guanylyl cyclase (RetGC), RetGC-2. *Proc Natl Acad Sci USA* 92: 5535–5539
- Mantoku T, Kusakabe T, Suzuki N (1999) Sequence analysis of cDNA and genomic DNA, and mRNA expression of the medaka fish homolog of the mammalian guanylyl cyclase C. *J Biochem* 125: 476–486
- McNeil L, Chinkers M, Forte M (1995) Identification, characterization, and developmental regulation of a receptor guanylyl cyclase expressed during early stages of *Drosophila* development. *J Biol Chem* 270: 4668–4674
- Mikami T, Kusakabe T, Suzuki N (1998) Molecular cloning of cDNAs and expression of mRNAs encoding alpha and beta subunits of soluble guanylyl cyclase from medaka fish *Oryzias latipes*. *Eur J Biochem* 253: 42–48
- Mittal CK, Murad F (1982) In "Handbook of Experimental Pharmacology" Ed by Nathanson JA, Keabian JW, vol 58-1, pp 225–260, Springer-Verlag, Berlin
- Nakane M, Saheki S, Kuno T, Ishii K, Murad F (1988) Molecular cloning of a cDNA coding for 70 kilodalton subunit of soluble guanylate cyclase from rat lung. *Biochem Biophys Res Commun* 157: 1139–1147
- Nakane M, Arai K, Saheki S, Kuno T, Buechler W, Murad F (1990) Molecular cloning and expression of cDNAs coding for soluble guanylate cyclase from rat lung. *J Biol Chem* 265: 16841–16845
- Nighorn A, Gibson NJ, Rivers DM, Hildebrand JG, Morton DB (1998) The nitric oxide-cGMP pathway may mediate communication between sensory afferents and projection neurons in the antennal lobe of *Manduca sexta*. *J Neurosci* 18: 7244–7255
- Ramarao CS, Garbers DL (1985) Receptor-mediated regulation of guanylate cyclase activity in spermatozoa. *J Biol Chem* 260: 8390–8396
- Ramarao CS, Burks DJ, Garbers DL (1990) A single mRNA encodes multiple copies of the egg peptide speract. *Biochemistry* 29: 3383–3388
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4: 406–425
- Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463–5467
- Schulz S, Singh S, Bellet RA, Singh G, Tubb DJ, Chin H, Garbers DL

- (1989) The primary structure of a plasma membrane guanylate cyclase demonstrates diversity within this new receptor family. *Cell* 58: 1155–1162
- Schulz S, Green CK, Yuen PST, Garbers DL (1990) Guanylyl cyclase is a heat-stable enterotoxin receptor. *Cell* 63: 941–948
- Schulz S, Wedel BJ, Matthews A, Garbers DL (1998) The cloning and expression of a new guanylyl cyclase orphan receptor. *J Biol Chem* 273: 1032–1037
- Seimiya M, Kusakabe T, Suzuki N (1997) Primary structure and differential gene expression of three membrane forms of guanylyl cyclase found in the eye of the teleost *Oryzias latipes*. *J Biol Chem* 272: 23407–23417
- Shah S, Hyde DR (1995) Two Drosophila genes that encode the alpha and beta subunits of the brain soluble guanylyl cyclase. *J Biol Chem* 270: 15368–15376
- Shigei M (1974) Echinodermata. In "Animal Systematics (2) - Echinodermata", Ed by Uchida T, Vol 8, pp 208–332, Nakayama Shoten, Tokyo (in Japanese)
- Shimizu T, Yoshino K, Suzuki N (1994) Identification and characterization of putative receptors for sperm-activating peptide I. *Develop Growth Differ* 36: 209–221
- Shimizu T, Takeda K, Furuya H, Hoshino K, Nomura K, Suzuki N (1996) A mRNA for a membrane form of guanylyl cyclase is expressed exclusively in the testis of the sea urchin *Hemicentrotus pulcherrimus*. *Zool Sci* 13: 285–294
- Shimomura H, Dangott LJ, Garbers DL (1986) Covalent coupling of a resact analogue to guanylate cyclase. *J Biol Chem* 261: 15778–15782
- Shyjan AW, de Sauvage FJ, Gillett NA, Goeddel DV, Lowe DG (1992) Molecular cloning of a retina-specific membrane guanylyl cyclase. *Neuron* 9: 727–737
- Simpson PJ, Nighorn A, Morton DB (1999) Identification of a novel guanylyl cyclase that is related to receptor guanylyl cyclases, but lacks extracellular and transmembrane domains. *J Biol Chem* 274: 4440–4446
- Singh S, Lowe DG, Thorpe DS, Rodriguez H, Kuang W-J, Dangott LJ, Chinkers M, Goeddel DV, Garbers DL (1988) Membrane guanylyl cyclase is a cell surface receptor with homology to protein kinase. *Nature* 334: 708–712
- Suga S, Nakao K, Itoh H, Komatsu Y, Ogawa Y, Hama N, Imura H (1992) Endothelial production of C-type natriuretic peptide and its marked augmentation by transforming growth factor-beta. Possible existence of "vascular natriuretic peptide system". *J Clin Invest* 90: 1145–1149
- Suzuki N (1990) Structure and function of sea urchin egg jelly molecules. *Zool Sci* 12: 13–17
- Suzuki N (1995) Structure, function and biosynthesis of sperm-activating peptides and fucose sulfate glycoconjugate in the extracellular coat of sea urchin eggs. *Zool Sci* 12: 13–27
- Suzuki N (1999) Diversity of sperm-activating peptide receptors. In "The Spermatozoon: A Guide from Basic Knowledge to Clinical Applications" Ed by Gagnon C, Cache River Press, IL, USA (in press)
- Suzuki N, Shimomura H, Radany EW, Ramarao CS, Ward GE, Bentley JK, Garbers DL (1984) A peptide associated with eggs causes a mobility shift in a major plasma membrane protein of spermatozoa. *J Biol Chem* 259: 14874–14879
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22: 4673–4680
- Thorpe DS, Garbers DL (1989) The membrane form of guanylate cyclase. Homology with a subunit of the cytoplasmic form of the enzyme. *J Biol Chem* 264: 6545–6549
- Vollmar AM, Gerbes AL, Nemer M, Schulz R (1993) Detection of C-type natriuretic peptide (CNP) transcript in the rat heart and immune organs. *Endocrinology* 132: 1872–1874
- von Heijine G (1983) Patterns of amino acids near signal-sequence cleavage sites. *Eur J Biochem* 133: 17–21
- Wedel BJ, Garbers DL (1997) New insights on the function of the guanylyl cyclase receptors. *FEBS Lett* 410: 29–33
- Yang R-B, Foster DC, Garbers DL, Fülle H-J (1995) Two membrane forms of guanylyl cyclase found in the eye. *Proc Natl Acad Sci USA* 92: 602–606
- Yoshino K, Suzuki N (1992) Two classes of receptors specific for sperm-activating peptide III (SAP-III) in sand-dollar spermatozoa. *Eur J Biochem* 206: 887–893
- Yu S, Avery L, Baude E, Garbers DL (1997) Guanylyl cyclase expression in specific sensory neurons: A new family of chemosensory receptors. *Proc Natl Acad Sci USA* 94: 3384–3387

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