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Central nervous system-specific expression of G protein α subunits in the ascidian Ciona intestinalis

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ABSTRACT—Heterotrimeric G proteins play crucial roles as mediators of signaling by many extracellular stimuli. The receptors that activate G proteins constitute the largest and most diverse family of cell surface molecules involved in signal transmission of metazoan cells. To investigate G protein signaling in the central nervous system (CNS) of chordates, we isolated cDNA fragments encoding five different G protein α subunits (CiG\textalpha_{x}, CiG\textalpha_{q}, CiG\textalpha_{i1a}, CiG\textalpha_{i1b}, and CiG\textalpha_{i2}) from larvae of the ascidian, a simple chordate, Ciona intestinalis. In situ hybridization analysis revealed that each isoform had distinct patterns of spatial distribution in embryos. Among them, CiG\textalpha_{i1a} and CiG\textalpha_{i1b} mRNAs were specifically expressed in the CNS of the larva, whereas CiG\textalpha_{q} transcripts were expressed in small parts of the trunk epidermis and the tip of the tail, but not in the CNS. The CiG\textalpha_{x} expression was widely observed throughout the trunk and tail of the embryos, and the signals were stronger in the epidermis, mesenchyme, and tail muscle cells. Comparison of cDNA sequences and the exon-intron organization indicate that CiG\textalpha_{i1a} and CiG\textalpha_{i1b} are produced by alternative splicing of transcripts from a single gene, CiG\textalpha_{i1}. In the cleavage and gastrula stages, transcripts of CiG\textalpha_{i1} were widely distributed in embryos, and the expression then became restricted to the CNS of tailbud embryos and larvae. An exhaustive search has failed to find transducin-type α subunits in C. intestinalis. Since CiG\textalpha_{i1} is expressed in the ocellus, CiG\textalpha_{i1} may mediate signals from Ci-opsin1, a visual pigment of the ocellus photoreceptor cells.

Key words: ascidians, heterotrimeric G protein, phototransduction, central nervous system, alternative splicing

INTRODUCTION

Ascidians, or sea squirts, are lower chordates, and their simple, tadpole-like larvae share a basic body plan with vertebrates (Corbo et al., 2001). Ascidian embryos have been favored for developmental research because they have low cell numbers, contain only a few different tissue types, develop rapidly, and have a well-known cell lineage (Corbo et al., 2001; Satoh, 2001). The larva of Ciona intestinalis, the cosmopolitan ascidian species, has 2,600 cells, including only 40 notochord cells and 36 muscle cells. The larva has a remarkably simple central nervous system (CNS) with about 330 cells, of which less than one-third are neurons, the remainder being glial cells (Meinertzhagen and Okamura, 2001). An anterior brain vesicle contains two sensory organs, an eyespots (ocellus) and a gravity sense organ (otolith). These two sensory organs are responsible for the swimming behavior of the larva.

It was shown that the larvae were induced to swim upon a step-down of light, and the action spectrum of photic behavior of ascidian larvae was similar to the absorption spectrum of human rhodopsin (Nakagawa et al., 1999; Tsuda et al., 2001). Localization of rhodopsin in the ocellus was shown by the retinal protein imaging method (Ohkuma and Tsuda, 2000). Ci-opsin1, the opsin of Ciona intestinalis, has been identified and shown to be closely related to vertebrate retinal and pineal opsins (Kusakabe et al., 2001).

Opsins are apoproteins of visual pigments and a model of G protein-coupled receptors (GPCRs). Among signaling receptors, GPCRs are especially important because they constitute the largest and most diverse families of receptor proteins. More than 1000 GPCRs identified in the human genome are involved in the regulation of virtually all physio-
logical processes (Marinissen and Gutkind, 2001). In a project analyzing the expressed sequence tags (EST) of C. intestinalis larvae (Kusakabe et al., 2002), we identified several cDNA clones encoding GPCRs. It is expected that tremendous numbers of GPCRs will be discovered in the whole genome sequencing projects of Ciona intestinalis, which are currently in progress at the National Institute of Genetics, Japan and Joint Genome Institute, USA.

Most cells contain GPCRs that choose between multiple G proteins to regulate a host of intracellular signaling processes (Gilman, 1987; Tsuda, 1987; Neer, 1995; Hamm and Gilchrist, 1996; Offermanns, 2001). Heterotrimeric G proteins are composed of α, β, and γ subunits, and there are multiple isoforms of each subunit (Nürnberg et al., 1995; Hildebrandt, 1997). In response to extracellular signals, the GPCRs exert guanine nucleotide exchange factor activity that substitutes GDP for GTP on the α subunit (Gα), resulting in dissociation of the αβγ trimer into an active Gα-GTP monomer and a Gβγ dimer. The activated G proteins, in turn, regulate the activity of a variety of effector proteins, including intracellular enzymes and ion channels. A given GPCR can activate more than one G protein subtype, leading to divergent signaling pathways. The importance of G proteins has been suggested in the regulation of developmental events, such as those regulated by Wnt and Hedgehog signaling (Hammerschmidt and McMahon, 1998; Offermanns, 2001). Heterotrimeric G proteins are composed of α, βγ, and G proteins to regulate a host of intracellular signaling processes (Gilman, 1987; Tsuda, 1987; Neer, 1995; Hamm and Gilchrist, 1996; Offermanns, 2001). Heterotrimeric G proteins to regulate a host of intracellular signaling processes (Gilman, 1987; Tsuda, 1987; Neer, 1995; Hamm and Gilchrist, 1996; Offermanns, 2001). Heterotrimeric G proteins to regulate a host of intracellular signaling processes (Gilman, 1987; Tsuda, 1987; Neer, 1995; Hamm and Gilchrist, 1996; Offermanns, 2001). Heterotrimeric G proteins.

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In this study, as an initial step to understand specific coupling between GPCRs and G proteins in ascidian embryos, we isolated and characterized cDNA clones encoding five different Gα subunits from the ascidian Ciona intestinalis. Each Gα isoform showed distinct expression patterns during embryogenesis, suggesting the involvement of G protein signaling in a variety of physiological and developmental processes in ascidian embryos and larvae. We also report that two CNS-specific Gα isoforms are produced from a single gene by alternative splicing. The CNS-specific Gα isoforms may couple with C-i-opsin1 in the photoreceptor cells of the ocellus.

MATERIALS AND METHODS

Animals and embryos

Mature adults of C. intestinalis were collected from harbors in Murotsu and Aoi, Hyogo, Japan. The adults were maintained in indoor tanks of artificial seawater (Marine Art BR, Senju Seiyaku, Osaka, Japan) at 18°C. The embryos were prepared using gametes obtained from the gonoducts, as described previously (Nakagawa et al., 1999).

Isolation and sequencing of cDNA clones encoding G protein α subunits

The total RNA was prepared from the C. intestinalis larvae, and a cDNA library was constructed with a ZAP vector (Stratagene, La Jolla, USA), as described previously (Iwasa et al., 2000). The cDNA library was directly used as a template to amplify the cDNA fragments of about 500 bp encoding the G protein α subunits by polymerase chain reaction (PCR), using a pair of degenerate oligonucleotide primers corresponding to two conserved amino acid sequences, KQMK(R/K)IHH and KVW(I/G)CF, respectively. The 5'- and 3'- portions of cDNAs were amplified from the cDNA library by PCR using a gene-specific primer and a vector primer, as described previously (Iwasa et al., 2000). Full-length cDNA clones were amplified by PCR using a thermostable DNA polymerase bearing proofreading activity (Takara LA Taq; Takara Shuzo, Japan), with primers corresponding to the 5'- and 3'-untranslated region (UTR) sequences of cDNAs (5'-ATACGAGCAAGCACAGCGGGAA-3' for 5'-UTR, and 5'-TATGCATGCGATGACGTCAC-3' for 3'-UTR). The PCR products were subcloned into plasmid vectors and sequenced on both strands with an automatic DNA sequencer (Shinadzu DQS 1000L, Shimadzu, Kyoto, Japan).

Molecular phylogenetic analysis

The deduced amino acid sequences of Gα encoded by the 500-bp cDNA fragments amplified by PCR from C. intestinalis larvae were aligned with the amino acid sequences of Gα from other animals. A neighbor-joining tree was constructed with the alignment using the Clustal W program (Thompson et al., 1994). The evolutionary distances were estimated using Kimura’s empirical method. The sequences used were: eleven Homo sapiens Gα isoforms (Gαi, XM_011603, Gαo, NM_002072, Gαo, X04408, Gαr, L10665, Gαo, XM_0098967, Gα11, AF011497, Gα, L01694, Gαr, L20725, Gα15, XM_0099220, cone transducin D13077, rod transducin X63749), two Ratmus norvegicus isoforms (Gαo, M17526, gustducin X65741), four Octopus vulgaris Gα isoforms (Gαo, AB025780, Gαr, AB025782, Gαr, AB025781, Gαo, AB025783), and Drosophila melanogaster Gαo, L09700.

In situ hybridization

Digoxigenin-labeled RNA probes were synthesized using a DIG RNA labeling kit (Roche, Japan), according to the manufacturer’s protocol. For CiGα12, CiGα12, and CiGα12, the 500-bp cDNA fragments obtained by PCR were used as templates to synthesize the probes. For CiGα12 and CiGα12, the RNA probe was synthesized from the 136-bp coding region (nt 607–742 for CiGα12 and nt 497–632 for CiGα12) corresponding to the alternatively-spliced exon of each transcript. To detect all transcripts from the CiGα12 gene, an RNA probe was also synthesized from a cDNA fragment containing 3'-coding and untranslated regions (spanning from the nt 603 to the 1925 of CiGα12).

Whole-mount in situ hybridization was carried out basically according to the protocol by Wada et al. (1995). After the coloring reaction, the embryos and larvae were dehydrated in an ethanol series and incubated in ethanol for 5–10 min. Following rehydration with PBST, the embryos were incubated in 25% glycerol in PBST for 5 min, and then transferred to 50% glycerol in PBST. The embryos were photographed and stored in 50% glycerol/PBST.

Analysis of partial genomic structure of the CiGα12 gene

The genomic DNA of C. intestinalis was extracted from the sperm of one individual, according to a standard method (Sambrook et al., 1989). A genomic DNA fragment containing exons for both CiGα12 and CiGα12 was amplified by PCR using LA Taq with a pair of gene-specific primers 5'-TGGGAGACTGCTAGAAAGATGATAGCAG-3' (corresponding to nt 520–541 of the CiGα12 cDNA), and 5'-GCTACACAGAAGATGATAGCAG-3' (corresponding to nt 808–829 of the CiGα12 cDNA). The PCR products were cloned into pBluescript II SK (+) (Stratagene), the 500-bp cDNA fragments amplified by PCR using LA Taq with a pair of gene-specific primers 5'-TGGGAGACTGCTAGAAAGATGATAGCAG-3' (corresponding to nt 520–541 of the CiGα12 cDNA), and 5'-GCTACACAGAAGATGATAGCAG-3' (corresponding to nt 808–829 of the CiGα12 cDNA). The PCR products were cloned into pBluescript II SK (+) (Stratagene). The nucleotide sequence was determined on both strands with an automatic DNA sequencer (Shinadzu DQS 1000L, Shimadzu).
RESULTS
Isolation and characterization of C. intestinalis cDNAs encoding Gα subunits

Five DNA fragments, each encoding a central part (164–166 aa) of Gα with a distinct amino acid sequence, were amplified from a C. intestinalis larval cDNA library by PCR with the degenerate primers. The Gα isoforms encoded by the cDNA fragments were designated as CiGαx, CiGαq, CiGαx1a, CiGαx1b, and CiGαq2. The deduced amino acid sequences of the five isoforms were shown in Fig. 1.

To investigate the structural and evolutionary relationships among CiGαx, CiGαq, CiGαx1a, CiGαx1b, CiGαq, and known metazoan Gα isoforms, phylogenetic analysis was performed by the neighbor-joining method (Saitou and Nei, 1987; Fig. 2). CiGαq is most closely related to vertebrate Gαq/Gα11 isoforms. CiGαx is also a member of the Gq class. Within the Gq class, however, CiGαx is fairly diverged from both the Gαq/Gα11 subfamily and the Gα15/Gα16 subfamily. Therefore, CiGαx may represent a novel subfamily, the members of which have not been identified in other animals. CiGαx1a, CiGαx1b, and CiGαq2 were closely related to vertebrate and invertebrate Gαq isoforms. Among these, the C. intestinalis Gα isoforms CiGαx1a and CiGαx1b are most closely related to each other.

Fig. 1. Comparison of amino acid sequences among C. intestinalis Gα isoforms. Amino acid sequences encoded by the 500-bp cDNA fragments of five C. intestinalis Gα are aligned. Dashes indicate gaps introduced in the sequence to optimize the alignment. Asterisks indicate the positions where all isoforms exhibit the same amino acid. Dots indicate the positions where all of the residues are similar to each other. Arrows above the CiGαx sequence indicate the positions of the two primers used to amplify the cDNA fragments by PCR.

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otide substitutions between their cDNA sequences. These features of CiGαi1a and CiGαi1b indicate the possibility that transcripts for these isoforms are splicing variants of a single gene. To assess this possibility, we first determined the entire cDNA sequences of CiGαi1a and CiGαi1b. The full-length cDNAs encode a 354-amino acid polypeptide, and the predicted amino acid sequences are identical between CiGαi1a and CiGαi1b, except for the 12 positions found by the analysis of the PCR fragments (Fig. 3A). The 5' and 3' UTR sequences are almost identical between the CiGαi1a and CiGαi1b transcripts (identity >98%, data not shown).

To further confirm that the CiGαi1a and CiGαi1b transcripts originate from a single gene, we then examined the structure of the gene. Genomic DNA containing the coding regions that had different sequences between CiGαi1a and CiGαi1b were amplified from the C. intestinalis sperm DNA.

Fig. 2. Molecular phylogenetic tree of G protein α subunits. A phylogenetic tree was inferred from the amino acid sequences by the neighboring method. The scale bar indicates 0.1 amino acid replacements per site. The numbers at the nodes are bootstrap values based on 1,000 replicates. The five C. intestinalis Gα isoforms are boxed. The four major classes (Gi, Gq, Gs, and G12) of Gα (Simon et al., 1991) are indicated at the right of the corresponding branches.
Fig. 3. Two splicing variants of the CiGαi1 gene: CiGαi1a and CiGαi1b. (A) The deduced amino acid sequences of CiGαi1a and CiGαi1b. The entire sequence of CiGαi1a is shown. Dots represent the amino acid residues of CiGαi1a identical to those of CiGαi1b, and the letters represent the variable positions in CiGαi1b. The variable region encoded by the alternatively spliced exons is boxed. (B) Genomic structure of a part of the CiGαi1 gene containing alternatively spliced exons for CiGαi1a and CiGαi1b. The exons are indicated by uppercase letters, whereas the introns are indicated by lowercase letters. The predicted amino acid sequences are indicated beneath the corresponding codons by the single-letter codes.

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by PCR. A 2.2-kb genomic DNA fragment was amplified and cloned. The entire nucleotide sequence of the genomic DNA fragment revealed that two homologous exons were tandemly aligned on the same DNA strand (Fig. 3B). One of the exons that locates upstream to the other encodes the variable region of CiGαi1a, and the downstream exon

Fig. 4. Spatial expression patterns of mRNAs for five Gα isoforms in C. intestinalis tailbud embryos. Transcripts of each Gα isoform were detected at the mid tailbud stage by whole-mount in situ hybridization. (A, B) Lateral (A) or dorsal view (B) of a tailbud embryo hybridized with a CiGαx probe. (C, D) Lateral (C) or dorsal (D) view of a tailbud embryo hybridized with a CiGαs probe. (E–G) Lateral view of tailbud embryos hybridized with a CiGαi1a (E), CiGαi1b (F), or CiGαi2 (G) probe. See text for details of the expression patterns. Scale bar, 100 µm.
encodes that of CiGα1b. The positions of the introns adjacent to the isoform-specific exons of CiGα1a/b are conserved with respect to the Gα sequences in mammals and insects (Fig. 3A,B). The genomic organization of the CiGα1i gene strongly suggests that the CiGα1a and CiGα1b mRNAs are produced by alternative splicing.

**Expression patterns of Gα mRNAs in C. intestinalis embryos**

The expression patterns of mRNAs for *C. intestinalis* Gα isoforms were examined in tailbud embryos by whole-mount *in situ* hybridization (Fig. 4). The CiGαx expression was widely observed throughout the trunk and tail of the embryos, and the signals were stronger in the epidermis, mesenchyme, and tail muscle cells (Fig. 4A,B). The CiGαq transcripts were expressed in the anterior and dorsal trunk epidermis as well as the dorsal side of the tip of the tail (Fig. 4C). The anterior CiGαq-expressing regions seem to contain the developing adhesive organ. On the dorsal trunk epidermis, the CiGαq-expressing regions were bilaterally located as two pairs of patches (Fig. 4D). Both of the two splicing variants of CiGα1, CiGα1a, and CiGα1b, were specifically expressed in the brain and adhesive organ (Fig. 4E, F). A difference in expression patterns was not clear between CiGα1a and CiGα1b, although the hybridization signals were much weaker for CiGα1b. We failed to detect clear hybrid-

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**Fig. 5.** Whole-mount *in situ* hybridization using a CiGα1 sense (A) or antisense (B–N) probe. (A, C) Four-cell embryo. (B) Fertilized egg. (D) Eight-cell embryo. (E) 16-cell embryo. (F) 64-cell embryo. (G) Gastrula. (H) Neurula. (I–K) Ventral (I), lateral (J), or dorsal (K) view of an early tailbud embryo. (L, M) Lateral (L) or dorsal (M) view of mid tailbud embryo. (N) Tadpole larva. Scale bar, 100 µm.
ization signals for CiGα2, even after observing the staining reaction for three days; only faint signals were observed in the brain (Fig. 4G).

The distinct expression of the CiGαi1 gene in the nervous system of tailbud embryos prompted us to further examine the expression patterns of this gene throughout embryonic development. Probably due to the small size (136 bp) of the splice variant-specific probes, the hybridization signals were very weak, especially for CiGαi1b (Fig. 4E,F). Therefore, we used a longer probe that can be hybridized with both CiGαi1a and CiGαi1b mRNAs (see Materials and Methods). The CiGαi1 transcripts were present in the eggs and cleavage stage embryos as maternal messages (Fig. 5B–F). The transcripts were ubiquitously distributed in the eggs and early embryos (Fig. 5B–G). The ubiquitous distribution of the CiGαi1 mRNA was observed until the gastrula stage (Fig. 5G). At the neurula stage, the CiGαi1 expression was restricted to the anterior ectoderm, especially the presumptive brain vesicle, although the hybridization signal was ambiguous (Fig. 5H). The CiGαi1 expression became restricted to the palps (adhesive organ), the central nervous system (CNS), including the brain vesicle and the visceral ganglion, and the dorso-distal part of the tail (Fig. 5I–K). At the mid tailbud stage, the hybridization signals were very strong and were restricted to the palps, the entire brain vesicle, and the visceral ganglion (Fig. 5L,M). The entire gene expression persisted to the CNS until the tadpole larva stage (Fig. 5N).

**DISCUSSION**

**Diversity of Gα isoforms in ascidians**

In the present study, we showed that mRNAs encoding at least five different Gα isoforms are present in ascidian embryos. Mammals have at least 17 functional Gα genes, several of which are spliced alternatively, that encode 23 distinct protein products (Nürnberg et al., 1995). The presence of multiple Gα isoforms has also been demonstrated in various invertebrates, including insects, nematodes, octopus, hydra, and sponges (Wilkie and Yokoyama, 1994; Suga et al., 1999; Iwasa et al., 2000). Metazoan Gα subunits can be classified into Gαs, Gαi, Gαo, and Gα12 classes (Simon et al., 1991; Suga et al., 1999). The present analysis identified two members of the Gαq class and three of the Gαi class in *C. intestinalis* embryos. Although we have not found Gαz isoforms in *Ciona*, we recently identified a cDNA clone encoding Gα closely related to vertebrate Gαz isoforms in another ascidian species, *Halocynthia roretzi* (Iwasa et al., 2001). To date, however, no Gα isoforms have been assigned to the Gα12 class in ascidians.

Among the Gαq class members, CiGαq is closely related to the vertebrate Gαq and Gαt1. However, the vertebrate Gαq and Gαt1 are more closely related to each other than to CiGαq, suggesting that the vertebrate Gαq and Gαt1 isoforms originated by gene duplication during vertebrate evolution after the divergence between the vertebrate and the urochordate. The metazoan Gαq-class isoforms are further classified into Gαq and Gα15/16 subfamilies. Another *Ciona* Gαq-class isoform, CiGαz, is fairly diverged both from the Gαq and Gα15/16 subfamilies. Therefore, CiGαq may be a member of a novel Gαx subfamily. It will be interesting to see whether Gαx closely related to CiGαx, and is present in other animals, including vertebrates.

Based on their primary structure, Gαi-class isoforms are further classified into four distinct subfamilies: Gαi1, Gαi2, Gαi3, and Gαi4 (Suga et al., 1999). All three Gαi class members of *Ciona* belong to the Gαi subfamily, and so far no ascidian Gα isoforms have been assigned to the Gαt, Gαz, and Gα12 subfamilies. Since Gαt isoforms have been reported in diverse invertebrate phyla, including sponges (Suga et al., 1999), arthropods (Thambi et al., 1989; Horgan et al., 1995), and molluscs (Kojima et al., 1997; Iwasa et al., 2000), it is likely that ascidians also have this isoform. Gαt is abundant in the CNS both in vertebrates (Strathmann et al., 1990) and in insects (Thambi et al., 1989; Horgan et al., 1995), and play important roles in the function and development of the nervous systems (Offermanns 2001). Therefore, future studies are needed to clarify whether Gαt isoforms are present in ascidians.

**Alternative splicing of CiGαi1 transcripts**

A comparison of the CiGαi1a and CiGαi1b cDNA sequences and the genomic structure of the CiGαi1 gene strongly suggest that CiGαi1a and CiGαi1b are products of alternative splicing. In vertebrate, splice variants of Gαo, Gαi, and Gαt are known. Alternative splicing of the mammalian Gαo gene generates two different isoforms of Gαo (Tsukamoto et al., 1991). These isoforms, Gαo1 and Gαo2, are identical to each other in the N-terminal 248 amino acids; the sequences thereafter diverge. Gαo1 and Gαo2 result from alternative splicing of exons 7 and 8. The diverged carboxyl-terminus contains an effector-interacting domain. Therefore, Gαo1 and Gαo2 exhibit different properties in signal transduction (Kleuss et al., 1991). There are four splice variants of mammalian Gαt (Kozasa et al., 1988). These Gαt variants directly activate adenylyl cyclases and calcium channels (Mattera et al., 1989). The relative proportion and tissue distribution of the two variants of Gαt change during cellular differentiation, development, aging, and adaptive processes (Ihnatovych et al., 2001). Mammalian Gαo proteins are encoded by three different genes, Gαo1, Gαo2, and Gαo3, which are closely-related to each other (Itoh et al., 1988). Transcripts of the mammalian Gαo2 gene undergo alternative splicing, which in turn give rise to two distinct proteins with different carboxyl-terminal amino acid sequences (Montmayeur and Borrelli 1994). The Gαo2 variants exhibit differential cellular localization and function. Multiple Gαq isoforms are also produced by alternative splicing of a single Gαq gene in *Drosophila* (de Sousa et al., 1989). Therefore, alternative splicing seems to be a common mechanism to produce diversity of Gα isoforms in a wide variety of animals. Interestingly, the way alternative
splicing occurs differs between CiGαi1 and vertebrate Gα genes, suggesting that these alternative splicing events evolved independently in ascidians and in vertebrates. In future studies, it will be very important to investigate and learn the functional differences between CiGαi1a and CiGαi1b in the regulation of signal transduction during ascidian development.

Spatially restricted expression of Ciona Gα genes and roles of G-protein signaling in ascidian embryos and larvae

The present study demonstrated that multiple Gα genes are expressed with distinct expression patterns in Ciona embryos. Although autonomous cell-fate specification is a dominant mechanism in ascidian embryos, the importance of cell-cell communications has increasingly become evident in the determination and differentiation of embryonic cells in ascidians, especially in their nervous systems (Meinerzahgen and Okamura 2001; Wada and Satoh 2001; Darras and Nishida 2001). It is quite probable that G proteins mediate signaling in these cell-cell interactions during ascidian development. It is also known that G proteins are located on intracellular membranes, and are involved in membrane trafficking and vesicular transport mechanisms of the cell (Nürnberg et al., 1995). Therefore, some of the Ciona Gα isoforms may participate in these cellular activities.

Among the four Gα genes identified in this study, CiGαq4 and CiGαq1 showed distinct and spatially restricted expression patterns. In the tailbud stage, CiGαq4 is expressed in the anterior and dorsal trunk epidermis and the tail tip. Trunk regions expressing CiGαq4 seem to include the future adhesive organ and siphon rudiments (Nakayama et al., 2001). The CiGαq4 protein may be involved in cell signaling during development of these organs.

From fertilization to the gastrula stage, CiGαi1 mRNA is present ubiquitously as maternal messages. Therefore, CiGαi1 may mediate signaling between blastomeres during the cleavage stages. Later in embryogenesis, the CiGαi1 expression is restricted to the CNS and the adhesive organ. This expression pattern suggests that CiGαi1 isoforms play important roles in the development and function of the nervous systems which require intracellular signaling in various aspects.

Recently, we have reported that Ciona larvae express a vertebrate-type opsin gene Ci-opsin1 in the photoreceptor cells of the ocellus (Kusakabe et al., 2001). In vertebrates, Gt (transducin) are responsible for signal transduction in the photoreceptor cells of the retina by coupling with rhodopsin, while invertebrate opsins activate Gt and Gt (Tsuda and Tsuda, 1990). However, our extensive search has failed to find Gt in the Ciona EST and genome sequence databases. Interestingly, both Gt and Gt belong to the Gt class and the vertebrate rhodopsin can activate Gt in vitro (Terakita et al., 2002). We have shown that CiGαi1 is expressed in the brain vesicle including photoreceptor cells. Therefore, CiGαi1 may interact with Ci-opsin1 in phototransduction of the ascidian larval ocellus. Since no Gt has been reported in invertebrates to date, Gt may have appeared during early vertebrate evolution after the separation between vertebrates and urochordates.

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