Biochemical and Biophysical Research Communications **270,** 34–39 (2000)
doi:10.1006/bbrc.2000.2379, available online at http://www.idealibrary.com on IDEAL®

Molecular Cloning and Characterization of *Xenopus* RGS5

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Received February 22, 2000

We identified six genes that encode putative RGS proteins (XRGSI-VI) in developing *Xenopus* embryos using PCR amplification with degenerate primers corresponding to the conserved region (RGS domain) of known RGS proteins. RT-PCR analysis revealed that mRNAs of these XRGSs are differentially expressed during embryogenesis. At stage 1, only XRGSII mRNA was detected. On the other hand, expression of XRGSVI mRNA increased apparently at stage 14 and expression of three of other XRGS (III, IV, V) elevated between stage 25 and 40. To further characterize XRGS proteins expressed in *Xenopus* embryos, we isolated a cDNA clone for XRGSIII. Based on determined nucleotide sequence, XRGSIII was considered as a *Xenopus* homologue of mammalian RGS5 (XRGS5). Genetic analysis using the pheromone response halo assay showed that expression of XRGS5 inhibits yeast response to α-factor, suggesting that XRGS5 negatively regulates the G-protein-mediated signaling pathway in developing *Xenopus* embryos.

Key Words: **RGS; G protein; *Xenopus;* embryogenesis; PCR; degenerate primers.**

Many extracellular signals including hormones and neurotransmitters stimulate cell surface receptors that activate heterotrimeric G proteins. These G proteins function as signal transducing molecules by regulating cellular effectors such as enzymes and ion channels (1, 2). Recently a new family of RGS (regulators of G protein signaling) was identified in organisms from yeast to mammals (3, 4). Genetic screenings for negative regulators for the pheromone response pathway in yeast identified a protein, Sst2 (5). By further analyses, Sst2 was revealed to interact directly with G protein α subunit (6).

Abbreviations used: G proteins, heterotrimeric guanine nucleotide-binding proteins; XRGS, *Xenopus* regulators of G protein signaling; ODC, ornithine decarboxylase; PCR, polymerase chain reaction; RT-PCR, reverse transcription PCR.

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In the last few years, full or partial sequences of 23 RGS proteins have been identified in mammals. All of them share a conserved "RGS domain" of approximately 120 amino acids with yeast Sst2 (7–17). Biochemical studies have demonstrated that RGS proteins have function as a GTPase-activating protein (GAP) for α subunits of G proteins (18).

Recently, some RGS members have been demonstrated to play some important roles in embryogenesis and cell differentiation. An Axin family, Axin and Axil were identified from mammals, and these proteins containing the RGS domain were shown to regulate an early step in embryonic axis formation by RNA injection into *Xenopus* embryos (12, 16). Moreover, *loco* gene was identified in *Drosophila* and its mutants revealed a severe glial cell differentiation defect. *loco* encodes two RGS domain proteins, and these proteins were found to show a significant similarity to rat RGS12. The interaction and the coexpression of LOCO and Gαi demonstrated a function of G-protein signaling for glial cell development (19). Thus it has been shown that RGS proteins are also involved in regulation of embryogenesis and cell differentiation. However, RGS proteins expressed during early embryonic development were not well studied yet.

To investigate which RGS proteins are actually expressed during early embryogenesis, we performed PCR amplification with degenerate primers corresponding to the conserved RGS domain. We identified six genes encoding putative RGS proteins (XRGSI–VI) in developing *Xenopus* embryos. In *Xenopus*, only axin homologue was identified from oocytes (20), and other *Xenopus* RGS proteins have not been reported. We examined expression patterns of mRNAs for these new XRGS proteins during embryogenesis. To further characterize XRGS proteins in *Xenopus* embryos, we isolated a cDNA for XRGSIII and investigated its biological activity using a yeast bioassay for response to mating pheromone.

MATERIALS AND METHODS

PCR identification of Xenopus RGS proteins. Total RNA was isolated from *Xenopus* embryos using SNAP total RNA isolation kit

0006-291X/00 $35.00

(Invitrogen). Embryonic stages were determined according to the table of Nieuwkoop and Faber (21). From total RNA of embryos at stage 15 and 40, the first strand cDNA was synthesized as a template of PCR. Degenerate oligonucleotide primers corresponding to the RGS domain of known RGS proteins of mammals were utilized as described (8). The PCR was allowed to proceed for 40 cycles of 94°C (45 s), 40°C (1 min), 72°C (2 min), and finally 72°C (5 min). Cloning of the PCR products into a pGEM-T vector (Promega) and subsequent sequence analysis were carried out.

RT-PCR assay. Total RNA was isolated from three *Xenopus* embryos at different developmental stages and subjected to reverse transcription with oligo(dT)15 as a primer. The reverse-transcribed cDNA was used as a template of PCR. The PCR conditions were the following: initial denaturation at 95°C for 5 min, followed by 27 or 30 cycles (27 for ODC and XRGSVI, 30 for XRGS–IV) of 55°C for 2 min, 72°C 3 min, 94°C 40 s, and a final incubation of 72°C for 15 min. The used primers were the following: for XRGSI, 5′-GAGGCCTGTGAAGACTACAAGAAG-3′, 5′-ACGTATCTGCTCTTGTGCTGCGTC-3′; XRGSII, 5′-TTAGCCTGCGAGAATTACAAAAAA-3′, 5′-GATAAATATCCTGTGCTGGGCCTC-3′; XRGSIII, 5′-GGCCTGTGAGGATTACAAAAAGGC-3′, 5′-GCAAAGATCTTCTTCTGGGCAAGC-3′; XRGSIV, 5′-AGGCCTGCGAGGACCTAAAGTA-3′, 5′-GCATGTAAATGTGGGTCTGTGC-3′; XRGSV, 5′-CCCGTTCACTCAATAAGCTTTCAGC-3′, 5′-AGGCTGTGTATTCTCAGCTGTGCA-3′; XRGSVI, 5′-AGACCAAATCCCCACACAAGCTCA-3′, 5′-AACTGTACACTCGTTTCTGGGCAG-3′; ODC, 5′-AATGGATTTCAGAGACCA-3′, 5′-CCAAGGCTAAAGTTGCAG-3′ (22). PCR products were analyzed on 3% MS-4 agarose (HISPANAGAR) gels.

Cloning of Xenopus RGS5 cDNA. The PCR-amplified DNA fragment of XRGSIII was used to screen *Xenopus* cDNA library prepared from stage 58 embryos (provided by Dr. Y. Yaoita, Tokyo Metropolitan Inst. for Neuroscience). The longest cDNA clone was sequenced on both strands.

Yeast pheromone response assay. A bioassay was used to measure the sensitivity of pheromone response in yeast that express RGS proteins. A DNA fragment containing the myc tag (MEQKLISEEDLSRGS) was introduced into pNV7 yeast expression vector under a galactose-inducible promoter. Protein coding regions of cDNAs of *Xenopus* RGS5 and rat RGS8 (15) were isolated by PCR-amplification. After confirmation by sequencing analysis, they were fused in frame immediately downstream of myc tag in pNV7. The sst2 deletion mutant yeast SNY86 (23) was transformed with each RGS cDNA in pNV7 and selected on ura$^-$ dropout plates. Independent colonies of each yeast tranformant were grown and a halo bioassay was performed as described (23).

Western blotting of epitope tagged RGS proteins. Single colonies of yeasts transformed with myc-tagged RGS constructs were inoculated into ura$^-$ dropout medium supplemented with 2% galactose or glucose and were grown to an identical density (A600 = 1). Yeast cells were precipitated and lysed in SDS sample buffer. Proteins were extracted by sonication and separated on SDS-polyacrylamide gels. After transferring to nitrocellulose membranes, expression levels of myc-tagged RGS proteins were examined by Western blotting using anti-myc tag monoclonal antibody (9E10, BabCO). Expression of actin was also examined using anti-actin monoclonal antibody (Chemicon). Signals were detected with ECL system (Amersham Pharmasia Biotech).

RESULTS

Six Xenopus Genes Encoding Putative RGS Proteins

All of known RGS proteins share a conserved RGS domain (45–80% homologous) of approximately 120 amino acids. To identify RGS proteins expressed in developing *Xenopus* embryos, we carried out PCR amplification with degenerate primers corresponding to the RGS domain (8). Although amplification was hardly detected at stage 15, apparent 200-bp DNA fragment was amplified at stage 40. The amplified 200-bp DNA was cloned into a pGEM-T vector, resulting in the isolation of 130 clones. These clones were classified into six groups based on sequencing analysis. All of them contained a DNA fragment of the expected length corresponding to 66–67 amino acids. Homology search in the nucleotide and protein data banks revealed that each of these six deduced sequences of amino acids has a significant homology (51–100%) to the RGS domain of a certain mammalian RGS protein. Therefore, these six *Xenopus* genes were considered to encode putative RGS proteins and were designated as XRGSI–VI. XRGSI was similar to rat RGS8 (51% identity, 65% similarity, 8, 15), XRGSII was close to mouse RGS4 (67% identity, 85% similarity, 23), XRGSIII was highly homologous to human RGS5 (77% identity, 91% similarity, 24), XRGSIV was, surprisingly, identical to human RGS9 within the PCR-amplified region (25), XRGSV was similar to rat RGS8 (59% identity, 82% similarity, 8, 15) and mouse RGS16 (53% identity, 76% similarity, 11), and XRGSVI was significantly similar to mouse and human RGS2 (73% identity, 83% similarity, 11, 26). Out of 135 clones, 40 clones for XRGSI, 72 for XRGSII, 4 for XRGSIII, 4 for XRGSIV, 7 for XRGSV, and 3 for XRGSVI were identified. The alignment of the deduced sequences of these six XRGSs is shown in Fig. 1.

Differential Expression of XRGS mRNAs during Xenopus Embryogenesis

The expression pattern of XRGS mRNAs in developing *Xenopus* embryos was investigated by RT-PCR (Fig. 2). At stage 1, only XRGSII mRNA was apparently detected. The expression of XRGSII mRNA started to decrease at stage 12 and increased after stage 30. In case of XRGSI, weak expression was detected at stage 10.5 and 12, then decreased, and increased again after stage 25. The expression of XRGSIII mRNA was detectable at stage 25 and increased up to stage 40. The expression of mRNAs of XRGSIV and XRGSV was weakly detected at stage 9 and then decreased. Their expression was again detectable at stage 25 (XRGSV) or 30 (XRGSIV) and increased up to stage 40. On the other hand, the expression of XRGSVI mRNA was apparently detected at stage 14 and further enhanced at stage 20. Its expression continued to stage 40. Expression of ornithine decarboxylase (ODC) was examined as a control. The expression levels of ODC mRNA stayed constant during the examined period. These results indicated that expressions of XRGS mRNAs are differentially regulated during early development of *Xenopus* embryos.

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                      10         20         30         40         50
XRGSI          1 EACEDYKKTR WVKKLPSKAQ DIFQEFLQFS SPREVNIDHR TRELIHKKMS      50
XRGSII         1 LACENYKKIK TPAKLPEQAQ KIYEDFISVE ATREVNLDST TREETSNNIL      50
XRGSIII        1 MACEDYKKAK SPSKMTTKAK KIYEEFIQTE APREVNIDHF TKAVTMKNLV      50
XRGSIV         1 EACEDLKYGD -QSKVKEKAE EIYKLFLAPG ARRWINIDGK TMDITVKGLK      50
XRGSV          1 MACEEYRKTR SLNKLSAKAH HIFQEFIDVQ APREVNLDYP SRELIKRNLL      50
XRGSVI         1 LACEDFKKTK SPHKLTAKSK KIYDEFIEKE APKEINIDFQ TRENIIQTIQ      50

                      60         70         80         90         100
XRGSI         51 VPCRNCFDAA QEQIRIL... .......... .......... ..........     100
XRGSII        51 QPTSSTFDEA QHRIFIL... .......... .......... ..........     100
XRGSIII       51 EPSSASFELA QKKIFAL... .......... .......... ..........     100
XRGSIV        51 HPHRYVLDAA QTHIYML... .......... .......... ..........     100
XRGSV         51 HPTLSCFDLA QLRIHSL... .......... .......... ..........     100
XRGSVI        51 EPSHSCFCAA QKRVYSL... .......... .......... ..........     100
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FIG. 1. Identification of six XRGS genes. Six *Xenopus* genes of putative RGS were identified from the products amplified by degenerate primer PCR. Their nucleotide sequences were determined (the accession number for them in the DDBJ/EMBL/GenBank nucleotide sequence databases is AB038434-9). Alignment of deduced amino acid sequences of six XRGSs is shown. Amino acids that appear in at least two XRGSs in the alignment sequences are shaded.

Cloning of Xenopus RGS5

To further characterize RGS proteins in *Xenopus* embryos, we first decided to isolate XRGSIII cDNA. XRGSIII was highly homologous to mammalian RGS5 and properties of RGS5 has not been understood well. Previous work has shown that several human RGS proteins can attenuate the mating pheromone response pathway in yeast and suppress supersensitivity of sst2 mutants (9). However, it was reported that mouse RGS5 have very little effect on pheromone response of yeast and could not suppress pheromone-induced halo formation (11). Using the PCR-amplified DNA fragment of XRGSIII, we screened a cDNA library prepared from *Xenopus* embryos at stage 58. The nucleotide sequence of the isolated longest clone was determined (Fig. 3A). The isolated cDNA was 1511 bp long and did not contain polyA tail. This cDNA sequence contained a single long open reading frame encoding a protein of 181 amino acids and its amino acid sequence was highly homologous to mammalian RGS5 [78% identical to mouse RGS5 (11) and 79% to human RGS5 (24), Fig. 3B]. This encoded protein was considered as a *Xenopus* homologue of RGS5 (XRGS5).

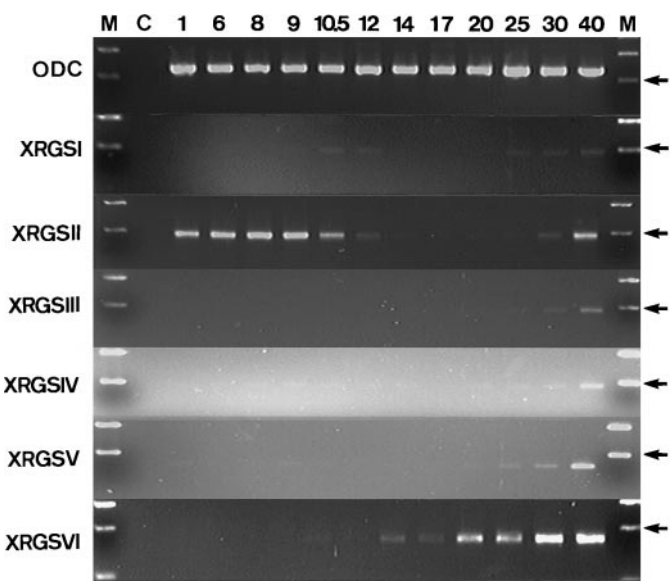

FIG. 2. Expression patterns of XRGS proteins during *Xenopus* embryogenesis. Total RNA was isolated from *Xenopus* embryos at various developmental stages (as indicated by numbers) and subjected to RT-PCR analysis. M, DNA molecular weight marker; C, control PCR without reverse-transcribed cDNA. Arrows indicate the position of 200 bp.

Biological Activity of Xenopus RGS5

We examined whether XRGS5 is able to inhibit the pheromone response in yeast. The protein-coding region of XRGS5 cDNA was PCR-amplified and was inserted into the yeast expression vector pNV7 under the control of a galactose-inducible promoter (XRGS5-pNV7). Rat RGS8 cDNA (15) was also cloned into pNV7 (RGS8-pNV7). The resultant constructs and the blank pNV7 vector were transformed into sst2 deletion mutant SNY86 as described (23). The sensitivity of these transformed cells to the mating pheromone was studied using a halo assay (Fig. 4A). SNY86 cells carrying the blank vector formed halos. On the other hand, those cells expressing rat RGS8 or XRGS5 did not form typical halos on galactose-containing plates and showed turbid zones surrounding the filter disks containing mating pheromone. When compared between the effect of rat RGS8 and that of XRGS5, the size of small clear area surrounding the disk seemed to be little larger with XRGS5 than with RGS8 at a highest dose of pheromone. The biological activity of XRGS5 might be little weaker than RGS8. The suppression of pheromone response was observed only in the galactose-containing cultures.

The expression levels of RGS8 and XRGS5 proteins in yeasts were investigated. Transformants of pNV7, RGS8-pNV7, and XRGS5-pNV7 were cultured in galactose containing medium and their SDS-extracts

A

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CTTTCTTCTC AAGAAACCTA AGAATCCATA AAAAGCCAAG ATGTGCAAAG GATTAGCTGC   60
                                            M  C  K  G  L  A  A
CCTGCCTCAC ACCTGCCTGG AAAGAGCCAA GGAGATAAAG ACCAAACTTG GGACTCTCCT  120
 L  P  H  T  C  L  E  R  A  K  E  I  K  T  K  L  G  T  L  L
CCAGAAGCCT GAATCAGCCA TCGATTTGAT TATCCCCTAC CCAGATAAGC CTGAAAAACC  180
 Q  K  P  E  S  A  I  D  L  I  I  P  Y  P  D  K  P  E  K  P
ACCGAAAGCT TCCAAGCCAT CAGCAGAAGA AGCAGGGCAA TGGCGTGATT CATTGGAAAA  240
 P  K  A  S  K  P  S  A  E  E  A  G  Q  W  R  D  S  L  E  K
GCTGCTAGAG AACAGCTATG GTCTCTCTGT CTTCCAGAGT TTCCTGAAGT CAGAGTTCAG  300
 L  L  E  N  S  Y  G  L  S  V  F  Q  S  F  L  K  S  E  F  S
CGAGGAGAAC ATTGAGTTCT GGATGGCCTG TGAGGATTAC AAAAAGGCCA AGTCTCCATC  360
 E  E  N  I  E  F  W  M  A  C  E  D  Y  K  K  A  K  S  P  S
CAAGATGACA ACCAAGGCTA AAAAAATCTA TGAAGAGTTC ATCCAAACAG AGGCTCCCAG  420
 K  M  T  T  K  A  K  K  I  Y  E  E  F  I  Q  T  E  A  P  R
AGAGGTCAAT ATCGACCATT TCACCAAAGC TGTTACCATG AAGAACTTGG TTGAACCATC  480
 E  V  N  I  D  H  F  T  K  A  V  T  M  K  N  L  V  E  P  S
GTCTGCAAGT TTTGAGCTTG CCCAGAAGAA GATCTTTGCT CTTATGGAGA AAGATTCACT  540
 S  A  S  F  E  L  A  Q  K  K  I  F  A  L  M  E  K  D  S  L
GCCCAGATTT GTGCGCTCTG AATTCTATCA AGAGCTAATA AAGTAACGGC ATGATGGAGC  600
 P  R  F  V  R  S  E  F  Y  Q  E  L  I  K  .
TGTGCAGTGG ATCCTCAGCA CTCCTTGAGT CCCAGACACT GGTTAGCTGG TATTTTCCTA  660
TTGCATCTGC TTTGGCCAAT GTGTGCTTTG TTTGGTTTTG TAGTTAAGGT ATCCTTCATA  720
ATATAAAAGT TTAAAAATAT ACGTCCCCAG GTACATCACA TCTAACCATT TCAGTGATGT  780
ATATATGATG GGGTCCATCA GTGCTGAGAT GTTATATCCC AAATTGTTCA CACAAACCTA  840
TCCCAGTGTG TGTTGGAGAG ACAGGTCTTA ACTAGGGCGT ATTGATATAT TCTAATGTTA  900
AATGCTTAGA ACATGAATAG GTTAGCACTC TAGAGTTAAT GTGCCATGAA GAGAAGCTGA  960
GAAAGTTAAG TGTTGCCCAC TATCTTGTAA CAGTAAAGAA TATACTTAAT CTTCAGGGAA 1020
GGAAACTGCT CACTATGTAT ACACAGGTGA AGGACATGGT AAAAGTAATC ACAAATGTAC 1080
ATAGTAAGTT AGGTTAAAAA AAAATGGACG CCCATCTAGT TCAACCTTTT AAGTCTATAT 1140
ATAACATGCC TAACTTCTAG TTGATCCAGA GGAAGGCAAT AAAAAAAGCT TTTGAAGCCT 1200
CTCTAAATGC CTTCCTCACT CCAAGATGTC AATGGGACCA ATCTCTGGAT CAATCGGACC 1260
AGTCCCTGGA TCATGTACTC ACACAAAGAG ACAAGTCCCA TGGGACCATC CATATATATT 1320
TAAACCTGTC ACTAACAAGG TATTCCATAA GACCCCGCCC TTCCATAATG ACCTACATGT 1380
AAGTTTTTTC CACGGTATGG GCCTTGTAAA ATGATTACAT CTGTTGGAAA AGGAAACTGA 1440
AAGAATGCAT GAATAGAACT TTGTAATAAG TCCAGCTAAA TGTGTACTTC CAAATTAAAT 1500
CTGTTTTAAA G                                                       1511
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B

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                      10         20         30         40         50
Xenopus RGS5        1 MCKGLAALPH TCLERAKEIK TKLGTLLQKP ESAIDLIIPY PDKPEKPPKA   50
human RGS5          1 MCKGLAALPH SCLERAKEIK IKLGILLQKP DSVGDLVIPY NEKPEKPAKT   50
mouse RGS5          1 MCKGLAALPH SCLERAKEIK IKLGILLQKP DSAVDLVIPY NEKPEKPANG   50

                      60         70         80         90        100
Xenopus RGS5       51 SKPSAEEAGQ WRDSLEKLLE NSYGLSVFQS FLKSEFSEEN IEFWMACEDY  100
human RGS5         51 QKTSLDEALQ WRDSLDKLLQ NNYGLASFKS FLKSEFSEEN LEFWIACEDY  100
mouse RGS5         51 HKPSLEEVLQ WRQSLDKLLQ NSYGFATFKS FLKSEFSEEN LEFWVACENY  100

                     110        120        130        140        150
Xenopus RGS5      101 KKAKSPSKMT TKAKKIYEEF IQTEAPREVN IDHFTKAVTM KNLVEPSSAS  150
human RGS5        101 KKIKSPAKMA EKAKQIYEEF IQTEAPKEVN IDHFTKDITM KNLVEPSLSS  150
mouse RGS5        101 KKIKSPIKMA EKAKQIYEEF IQTEAPKEVN IDHFTKDITM KNLVEPSPRS  150

                     160        170        180        190        200
Xenopus RGS5      151 FELAQKKIFA LMEKDSLPRF VRSEFYQELI K.......... ..........  200
human RGS5        151 FDMAQKRIHA LMEKDSLPRF VRSEFYQELI K.......... ..........  200
mouse RGS5        151 FDLAQKRIYA LMEKDSLPRF VRSEPYKELI K.......... ..........  200
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FIG. 3. Nucleotide and deduced amino acid sequence of *Xenopus* RGS5. (A) The deduced amino acid sequence of *Xenopus* RGS5 is shown in single-letter code under the respective codons. The line below the amino acid sequence indicates the region amplified by degenerate primer PCR and used for cDNA library screening. The shaded amino acid sequence corresponds to the putative RGS domain. (B) Amino acid sequences of RGS5 from *Xenopus*, human (24), and mouse (11) are aligned. Amino acids that appear at least two proteins in the alignment sequences are shaded.

were examined by Western blotting using anti-myc antibody (Fig. 4B). The expression of both RGS proteins was easily detected and levels of their expressions were not significantly different. No expression was observed in tranformants of pNV7. Also, the reactions with anti-myc antibody were not detected using yeasts cultured in glucose containing medium (data not shown). The expression of actin was examined as a control for loading. From these results, the cloned *Xenopus* RGS5 was found to be able to suppress super-

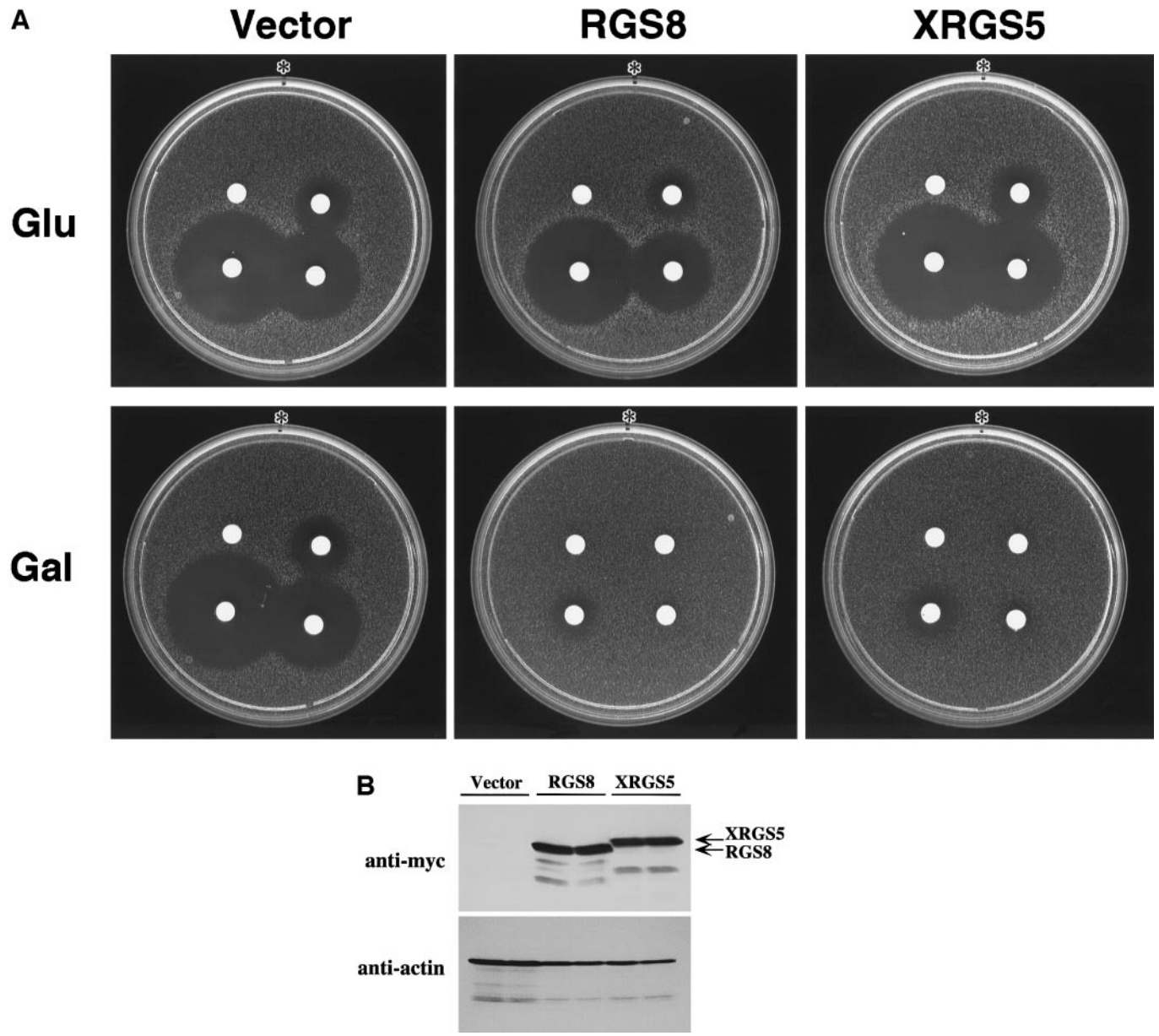

FIG. 4. Effect of *Xenopus* RGS5 gene on the response to mating pheromone. (A) Cells of the sst2 strain (SNY86) carrying pNV7, RGS8-pNV7, and *Xenopus* RGS5-pNV7 were plated on soft agar containing 2% glucose or 2% galactose. Sterile filter disks were placed on the nascent lawn, and synthesized α-pheromone was applied to the disks. Plates were incubated at 30°C for 36 h. The amount of α-pheromone added to each disk was: 0 ng (top left), 0.2 ng (top right), 2 ng (bottom right), 20 ng (bottom left). (B) Two colonies isolated from each yeast (SNY86) transformed with pNV7 vector (Vector), RGS8-pNV7 (RGS8), and *Xenopus* RGS5-pNV7 (XRGS5) were cultured in galactose-containing medium. Expression of RGS proteins was detected by Western blotting with anti-myc antibody. Expression levels of actin were also examined.

sensitivity of sst2 mutant and promote desensitization to mating pheromone.

DISCUSSION

By degenerate-primer PCR method, we described here that at least six genes for RGS proteins are expressed in developing *Xenopus* embryos. The sequencing analysis revealed that each gene product has a significantly high similarity to known RGS proteins of mammals. Not only mammals but also amphibians may express a number of specific RGS proteins and they may be capable to modify their responses to signaling molecules by using them.

We investigated expressions of XRGS transcripts during early development of *Xenopus* embryos. We found that XRGSII mRNA is already expressed at stage 1, and

that no other XRGS mRNA was apparently detected until stage 10.5. Thus, XRGSII is maternally expressed as a dominant RGS protein in unfertilized eggs. The expression of XRGSII mRNA continued during early period of development. This unique maternal XRGS may play important roles in quite early events of embryogenesis. On the other hand, after gastrulation, the expression of XRGSVI mRNA was induced at stage 14 (early neurula), further enhanced from stage 20 on until stage 40. The amino acid sequence of XRGSVI was similar to that of mammalian RGS2. Since RGS2 was demonstrated to interact selectively with Gαq (27), regulation of Gq-mediated signaling may be involved in neural induction and further cell differentiation. Thus, expression studies clearly demonstrated that the mRNA expression of XRGSs are differentially regulated during *Xenopus* embryogenesis. These results may demonstrate that each XRGS protein has some important roles in development and cell differentiation in *Xenopus* embryos.

We isolated a cDNA clone for XRGSIII and determined its nucleotide sequence. The isolated cDNA was found to encode a protein highly homologous to mammalian RGS5. Therefore, we referred to it as *Xenopus* RGS5 (XRGS5). Chen C. *et al.* reported that mouse RGS5 had very little effect on pheromone response in yeast (11). Despite of quite high similarity of amino acid sequence, we detected the significant activity of XRGS5 to attenuate the mating pheromone response in a halo bioassay. For the assay to measure activity of mouse RGS5, Chen C. *et al.* utilized yeast strain US356. This strain harbors a deletion mutation in the *bar1* gene, which encodes a secretory proteinase capable of cleaving mating pheromone. Here, we used *sst2* deletion mutant SNY86 cells and we could show the inhibitory function of XRGS5. *sst2* gene encodes a yeast RGS protein. It was reported that *sst2* mutant was more sensitive to α factor than *bar1* mutant (5). Therefore, when using more sensitive SNY86 cells, inhibition of pheromone response by mouse RGS5 may be observed. Alternately, mouse RGS5 might acquire different new functions.

We previously showed that rat RGS8 binds preferentially to the α-subunits Gαo and Gαi3 and that it functions as a GTPase-activating protein (15). In this report, we showed that RGS8 and XRGS5 could suppress the mating pheromone response in yeasts. Therefore, it is considered that XRGS5 might also interact with α subunits of G proteins and regulate G protein-coupled signalings. Expression studies indicated that expression of XRGS5 mRNA was detected from stage 25. The XRGS5-mediated regulations of G protein-signalings may occur during late embryogenesis or in certain differentiated cells.

ACKNOWLEDGMENTS

We are grateful to Dr. Y. Yaoita for *Xenopus* cDNA library. We thank Dr. H. Nakata for helpful discussion. This work is supported by research grants from the Ministry of Education, Science, Sports and Culture of Japan (to N.K. and O.S.), from the Naito Foundation (to O.S.), and from the Brain Science Foundation (to O.S.).

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