Identification of a *Xenopus* glutamine synthetase gene abundantly expressed in the embryonic nervous system but not in adult brain

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Abstract We used a PCR-based subtraction cloning procedure with concanavalin A-treated and -untreated animal caps from stage 9 *Xenopus* embryos to search for genes up-regulated during early neural development. One such gene was found to encode a protein homologous to several known glutamine synthetases, and we named it *xGS*. Molecular hybridization studies revealed that *xGS* mRNA is maternally transmitted and abundantly expressed in neuroectoderm-derived tissues during the gastrula and neurula stages. The expression of *xGS* mRNA in the nervous system continues until the larval stages, but declines thereafter and becomes undetectable in adult brain. Considering its metabolic activity and potential neuroprotective effect against the neurotoxic substances such as glutamate and ammonia, the glutamine synthetase may play an important role in the early stages of vertebrate neural development.

Key words: PCR; cDNA subtraction; Neural induction; Hybridization (in situ); Glutamine synthetase; Con A

1. Introduction

By cDNA subtraction cloning combined with in vitro neural induction system with *Xenopus* animal cap, we isolated several genes which are up-regulated during early neural development [1]. We previously reported that one such gene, *HMG-X*, was found to encode a protein similar to mammalian HMG1 and HMG2, known to be involved in transcriptional regulation [1]. Here we report that another gene encoding a protein (designated *xGS*) homologous to the glutamine synthetases found in various vertebrates is also up-regulated during the early development of the *Xenopus* nervous system.

Glutamine synthetase produces glutamine from glutamate and ammonia [2]. This reaction is important for neuroprotection, because it removes the two major neurotoxic substances, glutamate and ammonia [3]. Glutamate is abundant in the central nervous system: it is released from neurons as a neurotransmitter and taken up by glial cells and neurons through a glutamate transport system [4]. In addition, glutamine synthetase plays a role in glutamate recycling, i.e. glutamine is utilized as a source for glutamate synthesis [2].

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Abbreviations. xGS, *Xenopus* glutamine synthetase; HMG, high mobility group protein; PCR, polymerase chain reaction; Con A, concanavalin A; EF-1α, elongation factor-1α; bp, base pairs; kb, kilobase.

Glutamine synthetase activity, mainly in Müller glia, increases dramatically during the development of the chicken retina [5,6]. In mice, retinal glutamine synthetase activity increases about 200 fold between embryonic day 1 and day 21, with concomitant increases in its mRNA [7]. However, the expression patterns of this molecule in other parts of vertebrate embryos have not been studied. Here we report on the structure of the *xGS* gene and its expression patterns during early stages of *Xenopus* embryogenesis.

2. Materials and methods

2.1 Neural induction in animal caps

Eggs of *Xenopus laevis* were obtained as previously described [8]. Embryos were staged according to Nieuwkoop and Faber [9]. Eighty animal caps from stage 9 embryos were incubated for the initial 3 h with or without 500 μg/ml of Con A in Steinberg's solution (pH 7.0). After being washed twice with Steinberg's solution (pH 7.4), explants were cultured for 2 h at 20°C in the same solution before RNA extraction. Neural induction in some of the animal cap samples set aside was confirmed after 3 days by direct microscopic observation of brain-like structures.

2.2 RT-PCR assays

Total RNA was prepared using the acidic guanidine thiocyanate method [10] from 50 animal caps either treated or untreated with Con A. The RNA (1 μg) was subjected to the standard reverse transcription-polymerase chain reaction (RT-PCR) [11]. The following primers were used: N-CAM (nucleotide 2817–2835 and 3159–3142), 5′-CACAGTTCCACCAAATGC-3′ [12], s-actin (750–770 and 1199–1218), 5′-AACAGCAGCTTCTTCCTC-3′ and 5′-TACACAGAGCGACTTGAACA-3′ [13]; EF-1α (1104–1123 and 1372–1353), 5′-CAGATTGGTGCTGGATATGC-3′ and 5′-ACTGCCTTGATGACTCCTAG-3′ [14]. The conditions for PCR were as follows: (i) 94°C for 4 min, (ii) 55°C for 1 min, 72°C for 1 min and 94°C for 30 s (54 cycles), and (iii) 72°C for 4 min. After agarose gel electrophoresis, the PCR products were visualized with ethidium bromide.

2.3 Cloning and sequence analysis of xGS cDNA

Construction of a PCR-based directional cDNA library, subtractive hybridization and differential screening were performed as previously described [1]. We screened a unidirectional *Xenopus* embryonic cDNA library [8] with a cDNA probe, #14, obtained in the previous subtraction experiment [1]. The nucleotide sequence of the newly isolated clone (#14.1) was determined by a standard procedure [15] and submitted to the DDBJ, EMBL and GenBank under accession number D50062.

2.4. Northern blot hybridization

The probe was generated by a random priming method using the 1.6 kb *Eco*RI–*Xho*I fragment released from clone #14.1 as the template. PCR-generated cDNA fragments of *Xenopus* c-*src* [16] and *EF-1α* [14] were used as controls. Hybridization and densitometry were performed as previously described [8]

2.5. Whole-mount in situ hybridization

Digoxigenin (DIG)-labeled RNA probes in sense and antisense orientations were prepared from clone #14.1 and the gene using the DIG

SSDI 0014-5793(95)00913-2

RNA Labeling kit (Boehringer Mannheim, Germany). Whole mount in situ hybridization was performed essentially as described previously [17] with the following modifications: the embryos were washed with maleic acid buffer (MAB, 100 mM maleic acid, 150 mM NaCl, pH 7.5) instead of the PBS-based solutions that we previously used, and then incubated in MAB containing anti-DIG antibody and 20% bovine serum.

3. Results

3.1 *Con A-treated animal cap explants differentiate into neural tissues*

Our preliminary dose–response experiments indicated that neural tissues, including brain and eyes, were induced in vitro with high efficiency when animal cap explants were treated with 500 μg/ml Con A at pH 7.0. Immunohistochemical staining with a neural tissue-specific monoclonal antibody (NEU-1) detected brain-like structures surrounded by epidermis (data not shown). The expression of neural and mesodermal markers was examined using RT-PCR in the Con A-treated explants when the sibling controls reached the tailbud stage (Fig. 1). N-CAM and s-actin (sarcomeric actin) were used as neural and mesodermal markers, respectively. The EF-1α lanes demonstrate that a comparable amount of total RNA was used in each reaction. Expression of the neural marker, but not of mesodermal marker, was detected in the Con A-treated explants (lane 4), but neither was induced in the control explants (lane 3).

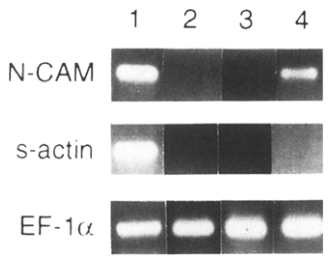

Fig. 1. RT-PCR analysis of Con A-treated explants. Lane 1: stage 28 whole embryos. Lane 2: explants immediately after resection. Lane 3: explants incubated in Steinberg's solution until the unoperated control embryos reached stage 28. Lane 4: explants treated with Con A (500 μg/ml) and then incubated in Steinberg's solution until the unoperated control embryos reached stage 28.

3.2. *Isolation of the cDNA clone corresponding to a gene up-regulated in animal caps during neural development*

cDNA clones derived from the genes expressed more abundantly in Con A-treated animal caps than in the control samples were isolated by a PCR-based subtraction cloning procedure as described elsewhere [1]. Since one of the cDNA fragments, designated clone #14, was found to contain novel nucleotide sequences, we screened a unidirectional cDNA library constructed from pooled poly(A)$^+$ RNA extracted from embryos at stages 10, 20 and 30 [8] with the #14 probe and obtained 4 positive clones out of 50,000 plaques. We then determined the total sequence of the longest cDNA, clone #14.1 (Fig. 2A). The cDNA consisted of 1620 bp and contained a single, long, open reading frame of 1179 bp preceded by three in-frame termination codons. The first methionine in the open reading frame is appropriate for translation initiation [18], and the predicted protein product consists of 392 amino acids with a calculated molecular mass of 43,936 daltons. A data-base search with this protein sequence revealed structural similarity with a group of glutamate-metabolizing enzymes, glutamine synthetases (GS), and we therefore named it *xGS*. Among the known glutamine synthetases, which are highly conserved across species, xGS is closest to a porcine homolog, with amino acid identity of 74% (Fig. 2B).

3.3. *Expression of xGS mRNA*

A 1.6 kb *xGS* transcript was detected in ovary and embryos at stages 6 through 35 by Northern blot hybridization (Fig. 3A and C). Since zygotic transcription is not active until the midblastula transition [19], the signals detected both in the ovary and in stage 6 embryos strongly suggest maternal transmission of *xGS* mRNA, although the transcript size is slightly larger in the latter case, probably because of elongation of the poly(A) tail, as has been observed for non-histone protein B4 [20]. In comparison with the relatively invariable expression of c-*src* [21], *xGS* was up-regulated after stage 8 and peaked around stage 12–14, followed by down-regulation at stage 16–20 and up-regulation again around stage 20–25 (Fig. 3B). Among the adult tissues examined, expression of *xGS* was detected in eye, kidney, muscle and ovary (Fig. 3C).

xGS expression was further analyzed by whole-mount in situ hybridization with embryos at various stages of development (Fig. 4A–J). In the blastula (stage 10), weak *xGS* signals were detected in the animal hemisphere (data not shown). In the late gastrula (stage 12), the transcript was detected in the anterior region (Fig. 4A). In the early neurula (stage 15), strong signals were detected throughout the neuroectoderm (Fig. 4B and C). In the tailbud embryo (stage 22), *xGS* is expressed along the neural tube and cement gland (Fig. 4D and E). In stage 25 larvae, the signals were detected in the brain, eye, spinal cord, cement gland, and branchial arch anlagen, which originates from neural crest, at least in part (Fig. 4F-H). The expression level in the spinal cord is reduced in stage 35 larvae (Fig. 4I and J) as compared with stage 25, although the general expression level is still high. Control samples hybridized with *xGS* sense probe showed no detectable signals at any stages (data not shown).

4. Discussion

Several endogenous and exogenous substances, including follistatin [22], noggin [23] and Con A [24,25], have been reported to possess neural inducing activity on presumptive neuroectoderm in the animal cap assay. However, the activity of follistatin seemed weak, if present at all, in our hands [26]. Noggin indeed induces neural tissue, but it sometimes induces mesoderm. Although Con A is structurally heterogeneous in nature and the mechanism of its action is yet to be determined

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Fig. 2. Structure of *xGS* cDNA and its encoded protein. (A) Nucleotide sequence of the #14.1 cDNA and predicted amino acid sequence. The polyadenylation signal is underlined. (B) Alignment of glutamine synthetase sequences. Amino acid residues identical in xGS and the homologs are indicated by dots

A

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AACAAACTTGCTGAGGAAACGAAGATCGGGGCTTCTTCCCTTGCTCATAAGATCAGCTGC     60
CGCCATGTCAGTCTCCCACAGTTCGAGACTCAACAAGGGGGTGAGGGAACAGTACATGAA    120
   M  S  V  S  H  S  S  R  L  N  K  G  V  R  E  Q  Y  M  K      (19)

ACTGCCCCAAGGAGAAAAGGTCCAGGTCACCTACGTGTGGATCGACGGCACCGGGGAAGG    180
 L  P  Q  G  E  K  V  Q  V  T  Y  V  W  I  D  G  T  G  E  G      (39)

AGTGAGGTGCAAAACCAGGACTCTGGATCAGGAACCCAAAACCATAGATGAAATCCCTGA    240
 V  R  C  K  T  R  T  L  D  Q  E  P  K  T  I  D  E  I  P  E      (59)

ATGGAACTTCGATGGATCCAGTACTCACCAAGCAGAAGGCTCAAACAGTGACATGTATCT    300
 W  N  F  D  G  S  S  T  H  Q  A  E  G  S  N  S  D  M  Y  L      (79)

CATCCCAGTCCAGATGTTCAGAGACCCATTCTGCCTGGACCCCAATAAACTGGTTATGTG    360
 I  P  V  Q  M  F  R  D  P  F  C  L  D  P  N  K  L  V  M  C      (99)

TGAAGTCTTGAAATACAACCGCAAGTCTGCAGAGACCAACCTGAGACACACATGCAAGAA    420
 E  V  L  K  Y  N  R  K  S  A  E  T  N  L  R  H  T  C  K  K      (119)

GATCATGGAGATGGTGAATGACCACCGCCCGTGGTTTGGAATGGAGCAGGAATACACCTT    480
 I  M  E  M  V  N  D  H  R  P  W  F  G  M  E  Q  E  Y  T  L      (139)

GCTGGGCATTAATGGGCACCCGTATGGCTGGCCAGAAAATGGTTTCCCAGGGCCACAAGG    540
 L  G  I  N  G  H  P  Y  G  W  P  E  N  G  F  P  G  P  Q  G      (159)

TCCCTATTACTGCGGCGTTGGAGCGGACAAGGTGTATGGCCGGGATGTGGTAGAGTCGCA    600
 P  Y  Y  C  G  V  G  A  D  K  V  Y  G  R  D  V  V  E  S  H      (179)

TTATAAGGCCTGTCTGTACGCTGGCATTAAAATCTGTGGCACCAACGCAGAAGTCATGCC    660
 Y  K  A  C  L  Y  A  G  I  K  I  C  G  T  N  A  E  V  M  P      (199)

CTCGCAGTGGGAGTTCCAAGTGGGTCCGTGCGAAGGTATCGACATGGGGGACCACCTGTG    720
 S  Q  W  E  F  Q  V  G  P  C  E  G  I  D  M  G  D  H  L  W      (219)

GATGGCCAGGTTCATCCTTCATCGGGTCTGTGAAGACTTTGGGGTGGTGGCGACTCTGGA    780
 M  A  R  F  I  L  H  R  V  C  E  D  F  G  V  V  A  T  L  D      (239)

CCCCAAACCCATGACCGGAAACTGGAACGGAGCCGGGTGCCACACCAACTACAGCACGGA    840
 P  K  P  M  T  G  N  W  N  G  A  G  C  H  T  N  Y  S  T  E      (259)

GAGCATGAGGGTGGAAGGAGGACTCAAACACATTGAAGATGCCATAGAGAAGCTGGGGAA    900
 S  M  R  V  E  G  G  L  K  H  I  E  D  A  I  E  K  L  G  K      (279)

GAGACACGACTACCACATCTGCGTCTACGACCCGCGGGGAGGGAAAGACAACTCCCGGAG    960
 R  H  D  Y  H  I  C  V  Y  D  P  R  G  G  K  D  N  S  R  R      (299)

ACTCACCGGCCAACACGAGACGTCGAGTATTCACGAGTTCTCGGCCGGCGTGGCCAACCG   1020
 L  T  G  Q  H  E  T  S  S  I  H  E  F  S  A  G  V  A  N  R      (319)

GGGCGCCAGTATCCGCATCCCGCGTCAGGTGGGCCAGGAAGGCTACGGCTACTTTGAAGA   1080
 G  A  S  I  R  I  P  R  Q  V  G  Q  E  G  Y  G  Y  F  E  D      (339)

CCGACGGCCGGCAGCCAACTGCGACCCCTACGCAGTAACCGAGGCGCTGGTCAGGACCAC   1140
 R  R  P  A  A  N  C  D  P  Y  A  V  T  E  A  L  V  R  T  T      (359)

CATCCTGAACGAAACCGGCAGCGAGACCAAAGACTATAAGAACGGAGCTGGATTCTCCCG   1200
 I  L  N  E  T  G  S  E  T  K  D  Y  K  N  G  A  G  F  S  R      (379)

GGCAATCGGTATGGCATCTCCCCGAGACGCCGCTGTGTTTTAACCCGTTAGTCTCCCGAC   1260
 A  I  G  M  A  S  P  R  D  A  A  V  F  .                      (392)

ACTACTTGAATTCTTGTGAAGTAAAAATTCCTTTGGAAAGGAGGGGCATTCCTAGAACCA   1320
GAGACCGTAACATGGTTTCTCCCGTGTTATCTGCCGAGATGGAGGGCCAATTTGGGCATG   1380
GGGGGGGGGGGGTTGCACAGATTTTATAGGAATAAGAAACAAAGCAAGTGACCTGCTGAA   1440
ATGTGGCCAGTGCCAAGTGATTCCTTCAGTTGATCCCTTTTGGGGAGGGCAGAATTGTTA   1500
ATTGGCTCCTCTTGGAGCTCGGGCTTTATATAAAACGACTGATAGGGTCTTGTACCCGCC   1560
ATATGACTGTATTCCATTGTATTGTTTTTACTGTCTAATAAAAATTTTAAACTTTAA      1617
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B

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Xenopus - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - M S V S H S S R L N K G V R E Q Y M K L P Q - G E K V Q V T Y V W I D G T G E G V R C K T -  45
mouse   - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - . A T . A . . H V . . . I K Q M . . S . . . - . . . I . A M . I . V . . . . . P L . . . . C  45
rat     - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - . A T . A . . H . . . . I K Q M . . N . . . - . . . I . L M . I . V . . . . . L . . . . -  45
porcine - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - . A T . A . . H . . . . I K Q V . . S . . . - . . . . . A M . I . . . . . . . L . . . . -  45
chicken - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - . A T . A . . H . S . A I K H M . . . . . - . . . . . A M . I . . . . . . . H L . . . . -  45
dogfish M R I C R S F L F L V K K C G N I T P T I W R N Q H T Y K . A T . A . A N . S . I . K K N . . E . . . - D G . . . A M . I . . . . . . . A . . . . . -  74
soybean - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - - . . L - - - - - . S D L I N - - - L N . S D I T D . . I A E . I . V G . S . M D M . S . A -  38

Xenopus R T L D Q E P K T I D E I P E W N F D G S S T H Q A E G S N S D M Y L I P V Q M F R D P F C L D P N K L V M C E V L K Y N R K S A E T N L R H T C K K 119
mouse   . . . . C . . . C V E . L . . . . . . . . . . F . S . . . . . N . . . H . . A . . . . . - - - R . . . . L . . . F . . . . . P . . . . . . . I . . R 117
rat     . . . . C D . . C V E . L . . . . . . . . . . F . S . . . . . . . . H . . A . . . . . . R R . . . . . F . . . F . . . . . P . . . . . . . S . . R 119
porcine . . . . S . . . C . E . L . . . . . . . . . F . S . . . . . . . . V . A A . . . . . R K . . . . . F . . . F . . . . . P . . . . . . . . . R 119
chicken . . . . H . . . S L E D L . . . . . . . . . . F . . . . . . . . . . R . A A . . . . . R K . . . . . L . . . F . . . . Q . . D . . . . . . . R R 119
dogfish . . . . N . . . S . A . L . . . . . . . . . . Y . S . . . . . . . . V . S A . . . . . R R . . . . . L . . . . . . . P . . S . . . . S . Q . 148
soybean . . . S G L V N D P S A L . K . . Y . . . . . G . . P . E D . E V I Y . H K . - . . . . . R R G N . I . . . . D A Y T P A G E P I P . . K . N K A A . 111

Xenopus I M - - E M V N D H R P W F G M E Q E Y T L L G I N G H - P Y G W P E N G F P G P Q G P Y Y C G V G A D K V Y G R D V V E S H Y K A C L Y A G I K I C 191
mouse   . . . . D . . S N Q H . . . . . . . . . . M . T D . . - . F . . . F . . . . . . . . . . . . . . . . . A . . . . I . . A . . R . . . . . . . V . . T 189
rat     . . . . D . . S S Q . . . . . . . . . . . M . T D . . - . F . . . S . . . . . . . . . . . . . . . . . A . . . . I . . A . . R . . . . . . . . T 191
porcine . . . . D . . S N Q H . . . . . . . . . M . T D . . - . F . . . S . . . . . . . . . . . . . . . . . A . . . . I . . A . . R . . . . . . . . G 191
chicken . . . . D . . S N Q H . . . . . . . . . . T D . . - . F . . . S . C . . . . . . . . . . . . . . . . . A . . . . I . . A . . R . . . . . . V . . G 191
dogfish . . . . S . I A N E Y . . . . . . . . . T D . . - . F . . . S . C . . . . . . . . . . . . . . . . . A . . . . I . . A . . R . . . . . . . . E L S 220
soybean . F I H P D . A A E E . . Y . L . . . . . . . Q K D V Q W . L . . . L G . . L . . . . . . . . . T . . N . A F . . . . I . D . . . . . . I . . . . F N . S 186

Xenopus G T N A E V M P S Q W E F Q V G P C E G I D M G D H L W M A R F I L H R V C E D F G V V A T L D P K P M T G N W N G A G C H T N Y S T E S M R V E G G 266
mouse   . . . . . . . . A . . . . . I . . . . . . Q . . . . . . I . C . . . . . . . . . . . . I . . F . . . . I P . . . D . . . . . . . F . . K A . . E . N . 264
rat     . . . . . . . . A . . . . . I . . . . . . R . . . . . . V . . . . . . . . . . . . . . I . . F . . . . I P . . . . . . . . . . . . F . . K A . . E . N . 266
porcine . . . . . . . . A . . . . . I . . . . . . . . . . . . V . . . . . . . . . . . . . . I . . F . . . . I P . . . . . . . . . . . . F . . K A . . E . N . 266
chicken . . . . . . . . A . . . . . . . . . . . . E . . . . . . I . . . . . . . . . . . . . . I V S F . . . . I P . . . . . . . . . . . . F . . K N . . E D . . 266
dogfish . . . . . . . . A A . . . Y . . . . . . . . Q . . . . . . I S . . . . . . . . . . . . I I . S F . . . . I P . . . . . . . . . . . . F . . K A . . D D . . 295
soybean . I . G . . . . G . . G . . . . . S V . . S A A . E . . V . . Y . . E . I T . I A . L . L S F . . . . I Q . D . . . P . A . . . . . K L . . D D . . 261

Xenopus L K H I E D A I E K L G K R H D Y H I C V Y D P R G G K D N S R R L T G Q H E T S S I H E F S A G V A N R G A S I R I P R Q V G Q E G Y G Y F E D R R 341
mouse   . . C . . E . . D . . S . . . Q . . . H T . . . K . . L . . . . . . . . F . . . . N . N D . . . S . . . . S . . . . . . W T . . . . K K . . . . . . . . 339
rat     . R C . . E . . D . . S . . . Q . . . R A . . . K . . L . . A . . . . . F . . . . N . N D . . . . . . . . S . . . . . . . . I . . . . K K . . . . . . . . 341
porcine . . Y . . E . . . . . S . . . Q . . . R A . . . K . . L . . T . . . . . F . . . . N . N D . . . . . . . . . . . . . . . . . . T G . . . K K . . . . . . . . 341
chicken . . . . . E . . . . . S . . . Q . . . R A . . . K . . L . . A . . . . . F . . . . . . . . . . . . . . . . . . . . . . . . N . . H . K K . . . . . . G 341
dogfish . . Y . . . S . . . . . . . Q . . . R A . . . K . . L . . A . A . . . H . . . . N . N . . . . . . . . . . . . . . . . . S . . . D K K . . . . . . . . 370
soybean Y E I . K K . . A . . E . . . K E . . A A . - - - - E G . E . . . . . P D . . A D M N T . L W . . . . . . . . . . . . V . V G . . T L K A . K . . . . E . . 332

Xenopus P A A N C D P Y A V T E A L V R T T I L N E T G S E T K D Y - K N G A G F S R A I G M A S P R D A A V F  100  392
mouse   . S . . . . . . . . . . . I . . . C L . . . . . D . P F Q . - . .  71  371
rat     . S . . . . . . . . . . . I . . . C L . . . . . D . P F Q . - . .  73  373
porcine . S . . . . . F . . . . . I . . C L . . . . . D . P F Q . - . .  74  373
chicken . S . . . . . . . . . . . . . . C L . . . . . D . P F E . - . .  74  373
dogfish . S . . . . . . . . . . . . . . C L . D . S . D K P I E . N . .  66  403
soybean . . S . M . . . V . . S M I A D . . . . W K P                    46  355
                                                                    Identity %
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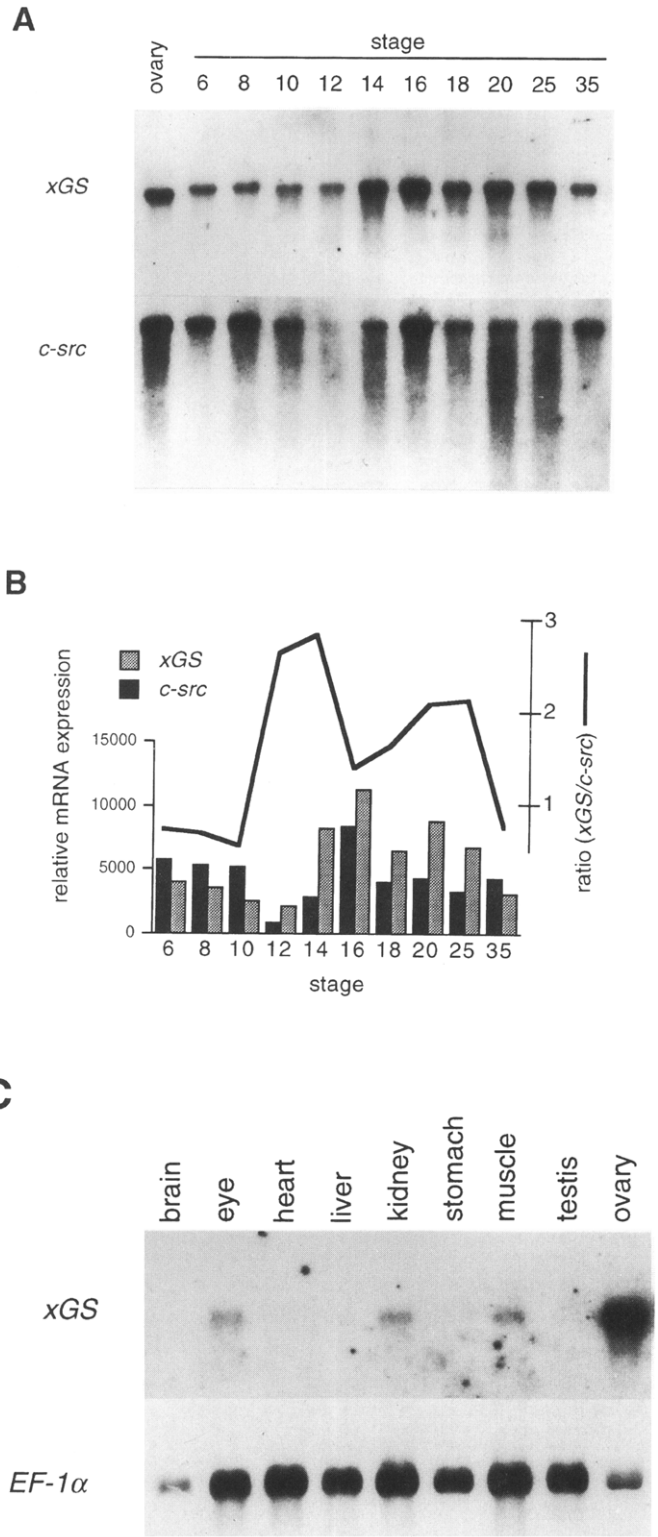

Fig. 3. Expression of *xGS* mRNA in *Xenopus* embryos and adult tissues. (A) Northern blot analysis. Each lane contained 2.5 μg of poly $(A)^+$ RNA isolated from whole embryos. (B) Densitometric analysis of the material shown in (A). The shaded and black bars represent the intensity of the *xGS* and c-*src* bands, respectively. The curve indicates the ratio between them. (C) Northern blot analysis. Each lane contained 2.5 μg of the poly $(A)^+$ RNA from adult *Xenopus* tissues.

[27], we used Con A in this series of experiments because it seems to mimic the unknown endogenous neural inducer(s) and organizes the animal cap into anterior neural tissues such as brain and eye without contamination by mesodermal components. Recently, Con A has been shown to raise intracellular calcium concentration by opening L-form calcium channels in the animal cap [28], and this is consistent with the idea that neural induction involves activation of protein kinase C [29].

Here we have shown that the mRNA for a *Xenopus* glutamine synthetase is expressed in the entire nervous system from early stages throughout development. Since the presumptive neuroectoderm in the gastrula and the neural precursor cells in the neurula express high levels of *xGS* mRNA, the encoded protein is probably present in undifferentiated neural precursor cells.

In contrast, the expression of *xGS* mRNA is down-regulated after stage 25 and almost undetectable in adult brain by Northern blotting. This suggests developmental switching between multiple forms of glutamine synthetases, because this enzymatic activity is known to be high in adult brain in higher vertebrates [7,30]. Thus, it is likely that the glutamine synthetase encoded by *xGS* gene is an embryonic isozyme and mainly expressed in immature neurons and/or glial cells. If this is true, the observed expression of *xGS* mRNA in adult frog eyes (Fig. 3A) may reflect the fact that amphibian retina keeps growing throughout life [31]. The finding that the known mouse glutamine synthetase is structurally closer to xGS rather than to the rat homolog also suggests a possible redundancy of glutamine synthetase genes in vertebrates.

The increase in glutamine synthetase activity in chick retina coincides with the appearance of circulating corticosteroid hormones [32]. Injected glucocorticoid induces glutamine synthetase activity in embryos [6, 33] by transactivating the gene(s) [30,34,35,36]. Thus, glucocorticoids may contribute to the induction of glutamine synthetase during chick development [6]. The spatio-temporal distribution of *xGS* gene expression significantly overlaps that of *HMG-X* mRNA, as we previously reported [1]. This suggest that the two genes are either under the control of a common transcriptional regulatory mechanism or that expression of the *xGS* gene is regulated, at least in part, by HMG-X. The latter possibility is consistent with the fact that one of its mammalian homologs, HMG1, enhances the DNA binding activity of progesterone receptors by ten fold through alteration of the higher structure of DNA [37]. Thus, genes positively regulated by progesterone receptors are likely to be further activated by HMG1. Since transcription of *xGS* gene is probably regulated by glucocorticoid receptors, it may also be enhanced by HMG-X.

We have been attempting to inhibit the function of *xGS* in vivo by injecting antisense RNA into 2-cell stage embryos and by treating embryos at various stages with methionine sulfoximine, a specific inhibitor of glutamine synthetase [38,39]. As yet we have not obtained consistent data indicating morphological changes caused by reduced glutamine synthetase activity after such treatment. These results, however, do not exclude the importance of this enzyme because the relatively high level of expression of endogenous *xGS* or potential complementation by other mechanisms may overcome such inhibition. One promising approach to test the importance of glutamine synthetase during vertebrate neural development would be targeted disruption of this gene in the mouse.

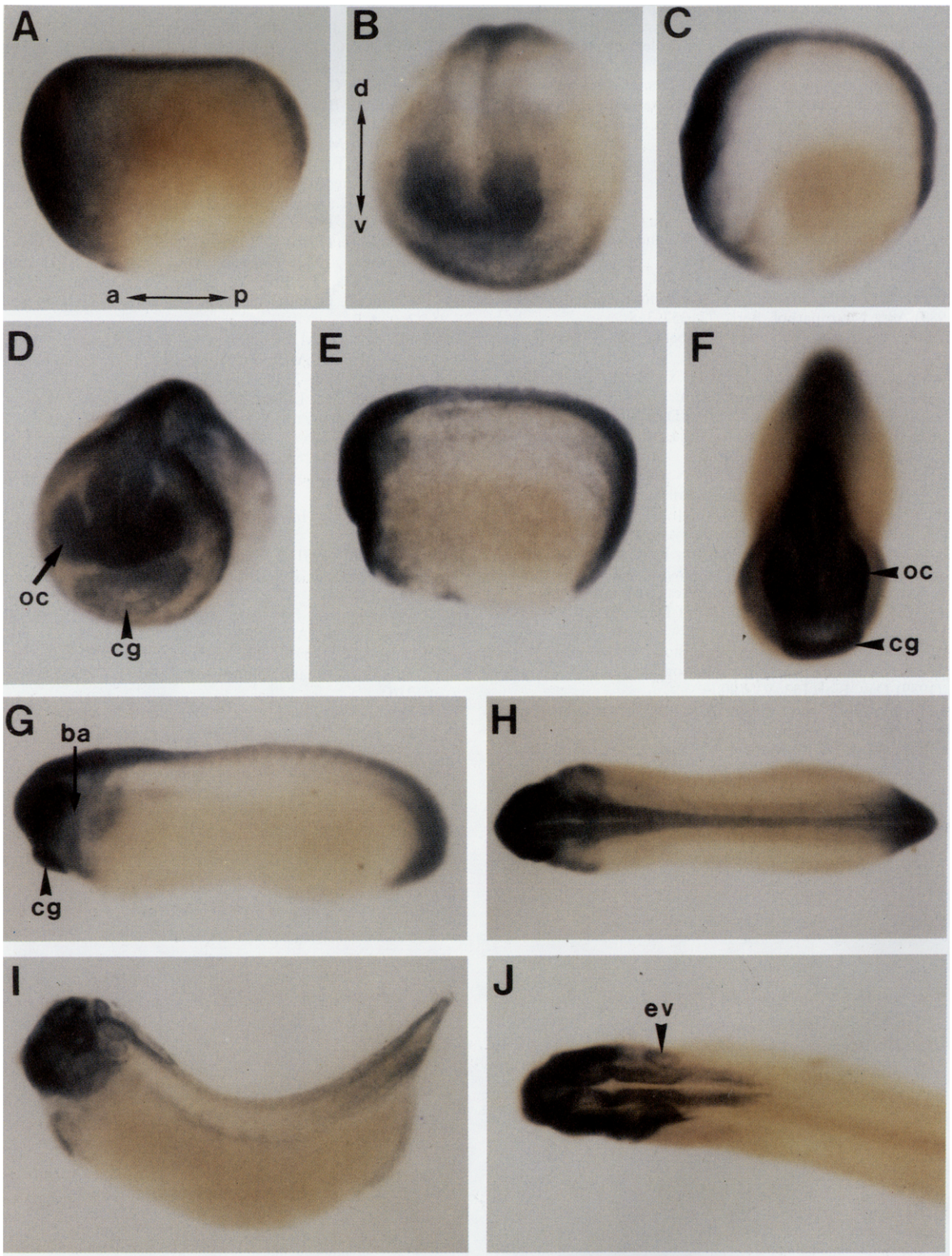

Fig. 4. Whole-mount in situ hybridization analysis of *xGS* mRNA in *Xenopus* embryos. (A) Lateral view of a stage 12 embryo (late gastrula). *xGS* is expressed in the anterior and dorsal regions. (B) Anterior and (C) lateral views of a stage 15 embryo (neurula), showing expression along the neural plate, especially in the head and the tail regions. (D) Anterior and (E) lateral views of a stage 22 embryo (tailbud), showing intense expression along the central nervous system. Also note the expression in cement gland and the tail region (F) Anterior, (G) lateral and (H) dorsal views of a stage 25 embryo Intense signals are localized in the CNS, eyes and cement gland. A relatively moderate signal is observed in the tail region and branchial arch anlagen. (I) Lateral view of a stage 35 embryo, showing intense expression in the head organs and tail. (J) Dorsal view of a stage 35 embryo with higher magnification. Note the reduced expression in the spinal cord. Abbreviations. anterior, a; posterior, p, dorsal, d; ventral, v; cement gland, cg; optic cup, oc, branchial arch anlagen, ba; ear vesicle, ev.

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