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Original Article

Xoom: a novel oocyte membrane protein maternally expressed and involved in the gastrulation movement of *Xenopus* embryos

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ABSTRACT During a process of differential display screening for lithium-responsive mRNA in *Xenopus* embryos, we found a maternal transcript which is remarkably reduced by lithium. The isolated cDNA, designated *Xoom*, encoded a novel oocyte membrane protein with a signal sequence in the N-terminus and a single transmembrane domain. The extracellular domain contained a cysteine-rich region and a serine/threonine-rich region, which suggests an extracellular association with some proteins. Expression of *Xoom* maternally occurred in the whole oocyte at the early stage of oogenesis and the transcript was gradually localized in the animal hemisphere of full-grown oocytes. The zygotic expression was detected at first in the dorsoanterior region of the neural fold stage embryo. Thereafter, localized expression of *Xoom* was observed in neural crest cells of the neural tube stage embryo and in optic and otic vesicles of tadpole. *Xoom* has been expressed ubiquitously in adult organs, especially with a high level in the eye, heart, liver and kidney. In examining a relation between *Xoom* and the dorsoventral patterning, lithium-treatment at 32-cell stage embryo decreased *Xoom* mRNA level within an hour, but coinjection of lithium with *myo*-inositol reversed the decreasing *Xoom* mRNA to normal level. UV-irradiation had no effect on the maternal mRNA level of *Xoom*. Overexpression of *Xoom* showed no effect on development, but antisense *Xoom* RNA causes interference with normal gastrulation movement. These results suggest that maternally expressed and membrane-associated *Xoom* is closely involved in the gastrulation movement through a lithium-inducible signal pathway.

KEY WORDS: *Xenopus laevis, lithium, Xoom, gastrulation*

Introduction

In *Xenopus* development, lithium-treatment at cleavage stage causes an expansion of dorsoanterior mesoderm, leading to the dorsalizing pattern of embryo (Kao and Elinson, 1988). A widely accepted explanation of the developmental effect of lithium is that lithium alters inositol triphosphate (IP_3)-Ca^{2+} signaling pathway through inositol depletion (Maslanski *et al.*, 1992), because lithium inhibits inositol monophospatase (IMPase) *in vitro* (Berrige *et al.*, 1989). Evidences that coinjection of myo-inositol and lithium rescue the embryonic pattern dorsalized by lithium (Busa and Gimlich, 1989), and that injection of anti-IP_3 receptor antibodies rescues the ventralizing pattern (Kume *et al.*, 1997) support this explanation. However, IMPase inhibitor L-690,330, which is 1000 times more potent than lithium, cannot cause the dorsalizing pattern (Klein and Melton, 1996). Thus, inhibition of IMPase cannot completely explain the dorsalizing effect of lithium.

A recent study clarified another target for lithium in the early development of the *Xenopus* embryo (Hedgepeth *et al.*, 1997). Ectopic activation of Wnt signaling causes the similar phenotype to lithium-treated embryos (Moon, 1993). Wnt pathway is a highly conserved signaling pathway that regulates dorsal cell fate decision in the early development of *Xenopus* (Miller and Moon, 1996; Yost *et al.*, 1996). Lithium can cause the upregulation and nuclear

Abbreviations used in this paper: IP_3, inositol triphosphate; IMPase, inositol monophospatase; GSK-3β, beta glycogen synthase kinase-3; MBT, mid-blastula transition; DAI, dorso-anterior index; CEA, carcinoembryonic antigen; IFN-γ, gamma-interferon.

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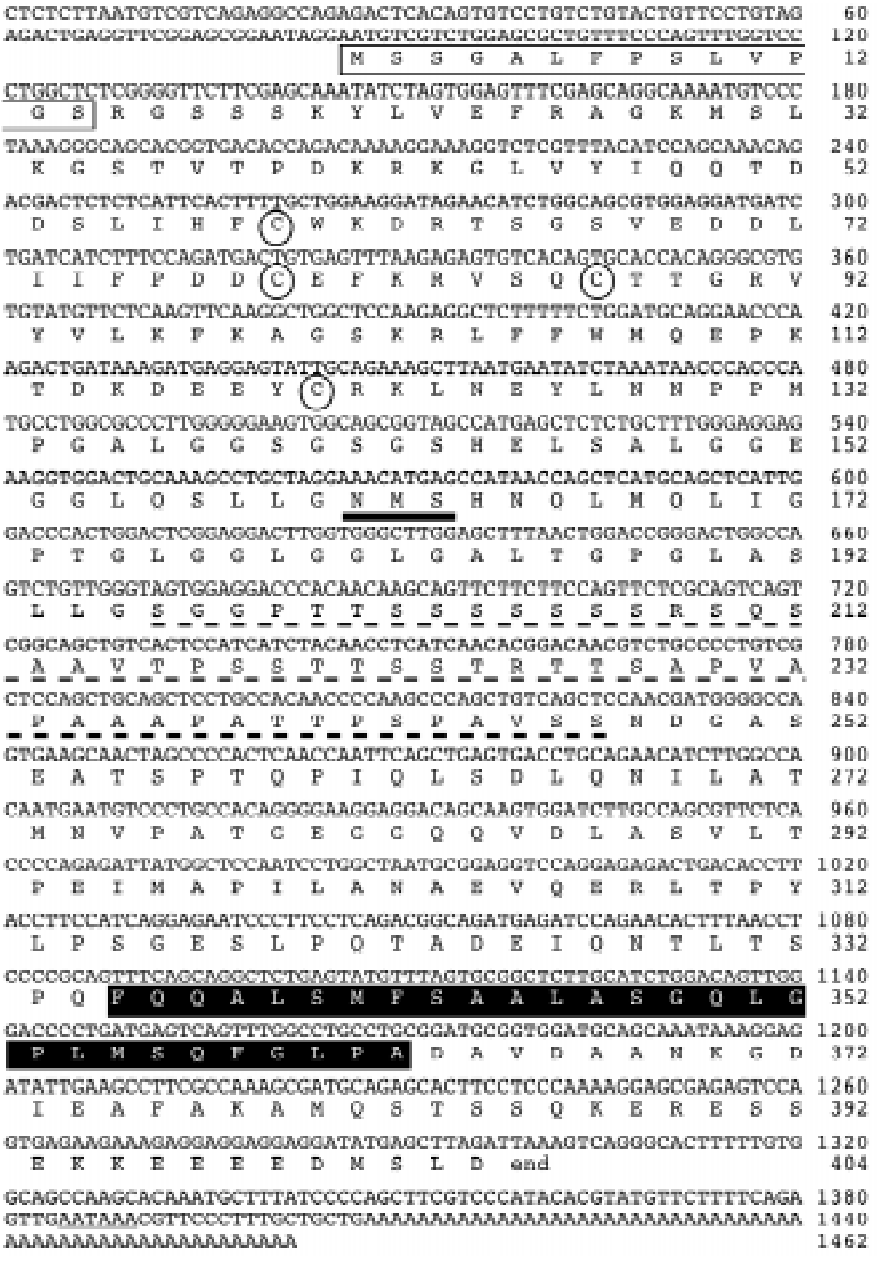

Fig. 1. Nucleotide and predicted amino acid sequences derived from *Xoom* cDNA clone. *The putative signal sequence at the N-terminus and the presumed transmembrane domain are boxed and shadowed, respectively. Cysteine residues are circled. The potential N-glycosylation site is heavily underlined. The serine/threonine-rich region is shown by a dashed line and the polyadenylation signal is underlined.*

translocation of β-catenin by inactivation of glycogen synthase kinase-3β (GSK-3β), which results in activation of Wnt signaling (Schneider *et al*., 1996; Yost *et al*., 1996; Hedgepeth *et al*., 1997). However, ectopic axes formation of dominant negative form of Xgsk-3 is blocked by myo-inositol (Hedgepeth *et al*., 1997). These results suggest a close correlation between Wnt and IP_3-Ca^{2+} signaling pathway, but the molecular mechanism of hyperdorsalization by lithium remains unknown.

In the present study, to find out a clue for molecular mechanism of the dorsoventral patterning, lithium-responsive genes were screened by means of the differential display method. Consequently, we have cloned a novel lithium-responsive cDNA encoding oocyte transmembrane protein, *Xoom*. Gene expression of *Xoom* occurred maternally and its zygotic expression was detected ubiquitously in all adult organs. Antisense *Xoom* RNA injection showed that Xoom protein is a prerequisite for normal gastrulation movement.

Results

Identification of the lithium-responsive transcripts within eggs

Lithium-treatment at cleavage stages causes an expansion of dorsoanterior mesoderm in *Xenopus* embryos. If lithium affects the zygotic transcription through a translational regulation of the maternal message, some changes in mRNA species must occur prior to the onset of zygotic transcription at the mid-blastula transition (MBT). In order to detect some lithium-responsive transcripts before MBT, we compared RNA species between lithium-treated and control embryos using the differential display method at early blastula. Many PCR products showed quantitative differences between both samples. A total of 8 fragments was subcloned and sequenced. Homology searching on databases showed no similarity in all of the clones whose transcripts enriched in lithium-treated embryos. However, within two clones whose transcripts decreased by lithium-treatment, one showed a high similarity with human cDNA clone encoding Mr 110,000 antigen (Shimada *et al*., 1994). In screening of a *Xenopus* oocyte cDNA library using the PCR product as probe, three positive clones were isolated. The longest 1.3 kb clone, C1, contained a complete open reading frame, but both untranslated regions were very short. Then, additional 0.2 kb of sequences were obtained by 5'RACE and 3'RACE. The reconstructed clone resulted in the 1,462bp cDNA (Fig. 1). The first methionine in the longest ORF was assigned as the translational start point. The cDNA encodes a 404 amino acid polypeptide with a predicted molecular mass of 42.2 kDa. Since the predicted amino acid sequence formed a transmembrane protein as described below, this cDNA clone was named *Xenopus laevis* oocyte membrane protein gene, *Xoom*. The cDNA includes a 0.2 kb 3' UTR containing an AATAAA consensus polyadenylation signal at 18bp upstream from the poly A (+) tail, which suggests that the cDNA clone has a complete 3' end. To confirm that the cDNA is full-length, northern blot analysis was performed (Fig. 2). A single transcript was detected at about 1.5 kb from total and poly A (+) RNA prepared from *Xenopus* unfertilized eggs. This size is consistent with a full-length of our cDNA clone (Fig. 1).

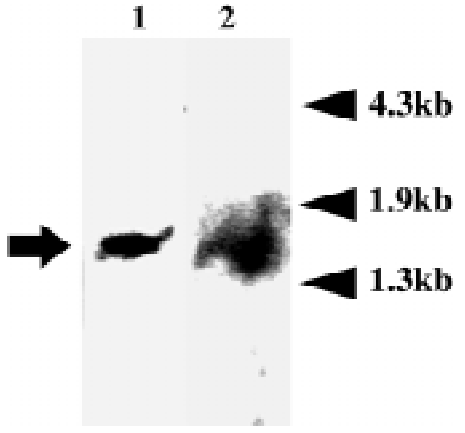

Fig. 2. Northern blot analysis of *Xoom*. *To determine the size of Xoom transcript, 50 μg total RNA (lane 1) and 4 μg poly A(+) RNA (lane 2) from an unfertilized egg were electrophoresed, blotted, and hybridized with digoxigenine-labeled Xoom cDNA. Arrow indicates 1.5 kb size of Xoom mRNA.*

Characterization of Xoom cDNA

To characterize *Xoom* cDNA, we examined hydropathicity plot of the predicted amino acid sequence and searched its homology on GenBank and DDBJ databases. The results showed that the predicted Xoom protein showed a high homology to a transmembrane protein reported as a human Mr 110,000 antigen (Fig. 3) (Shimada *et al.*, 1994). A comparison of Xoom with human Mr 110,000 antigen revealed a 71.9% similarity in nucleotides and 86.9% identity and 98.7% similarity in amino acid sequence. Both the predicted Xoom protein and the Mr 110,000 antigen had none of the already known domains.

However, a cysteine-rich region containing four completely conserved cysteine residues, N-glycosylation site and a serine/threonine-rich region were highly conserved (Figs. 1 and 3). The second candidate of the closest known gene was cosmid C56G2.7 from chromosome III of *C. elegans* (Wilson *et al.*, 1994), but characterization of the product remains to be examined.

Expression pattern of Xoom mRNA during development

To clarify the expression level of *Xoom* during development, we quantitatively carried out RT-PCR analysis. Expression of *Xoom* was recognized from oocyte and the maternal RNA level remained constant to the blastula stage (Fig. 4A). Transcription of *Xoom* was very low until the early stage of neurula. The remarkable zygotic expression occurred from the late stage of neurula and reached to the peak at tadpole stage.

Expression pattern of *Xoom* was examined by whole-mount *in situ* hybridization (Harland, 1991). At first, *Xoom* mRNA showed uniform distribution in stage I and II oocyte, and was thereafter gradually localized to the animal hemisphere at stages IV to VI (Fig. 5A). During cleavage stages, the maternal *Xoom* RNA was detected in the animal hemisphere (Fig. 5B). However, *Xoom* transcript was not detected in the gastrula stage embryo (Fig. 5C). Zygotic expression was first detected in the dorsoanterior region of neural plate stage embryos (Fig. 5D). At late neurula, localized expression of *Xoom* was observed in both sides of the neural tube and upper space of the eye vesicles (Fig. 5E,F). Transverse section of the stained embryo showed that the expression of *Xoom* preferentially occurs in neural crest cells beside the neural tube (Fig. 5G, arrows). The histological section showed that *Xoom* expression of upper space of the eye vesicles is restricted in neural crest cells participating in trigeminal nerve formation (data not shown) (O'Rahilly and Muller, 1994). In the tailbud stage embryo, broad and weak expression of *Xoom* was observed in the somite and head region (Fig. 5H). However, clearing procedure of the stained embryos revealed that *Xoom* expression occurs in the optic and otic vesicles (Fig. 5I).

In order to examine the gene expression of *Xoom* in the adult organs, we carried out RT-PCR analysis with various RNAs extracted from ovary, testis, brain, eye, heart, lung, stomach, liver, spleen and kidney. Expression of *Xoom* was detected ubiquitously in all adult organs tested. However, expression level was different depending on organs; very high expression of *Xoom* was observed in the eye, relatively high in heart, liver and kidney, and lower in the others (Fig. 4B). The high expression in the adult eye was consistent with that of the tadpole eye detected by whole-mount *in situ* hybridization (Fig. 5I).

Effect of lithium on Xoom transcript

To examine a relation of *Xoom* to the dorsoventral axis formation, quantitative analyses of maternal *Xoom* transcript were performed in the lithium-treated and UV-irradiated embryos. The irradiation of 2×10^4 ERG/mm^2 UV light for 4 min at 40 min after fertilization efficiently induced the ventralizing embryos (average 3.2DAI). Although the typical hyperventralization occurred in these embryos, the maternal transcripts of *Xoom* kept the same levels as the control embryos (Fig. 6A). In contrast to the ventralized

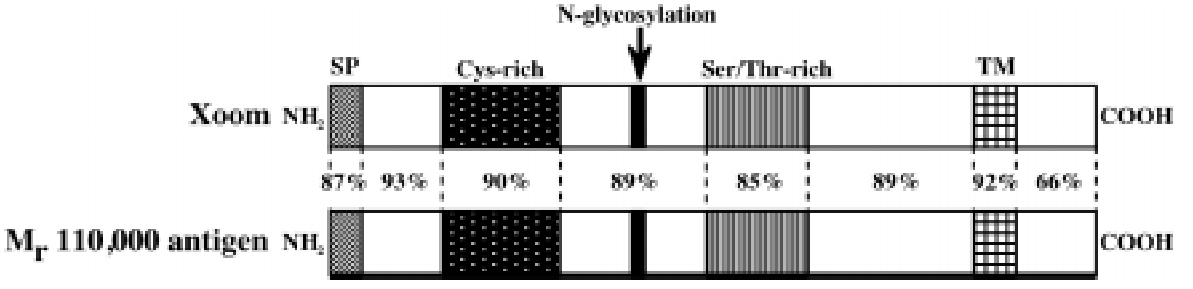

Fig. 3. Comparison of molecular structure between Xoom and human Mr 110,000 antigen. *The percentage of sequence homology in each domain is indicated in the space between the two proteins. Xoom protein consists of 404 amino acids (accession number in DDBJ: AB026995). Human Mr 110,000 antigen consists of 407 amino acids (accession number in DDBJ: D64154).*

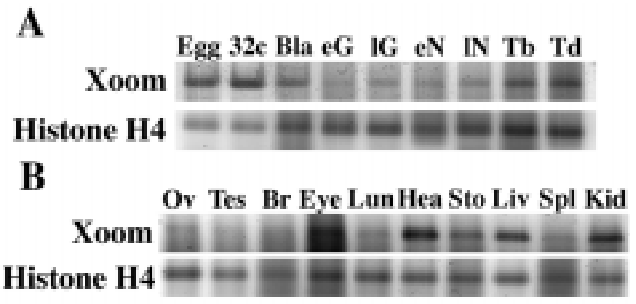

Fig. 4. Expression profiles of *Xoom*. *Gene expression was examined by quantitative RT-PCR in developing whole embryos* **(A)** *and in adult organs* **(B)**. *Total RNAs for RT-PCR were isolated from unfertilized egg (Egg), 32-cell stage embryo (32c), stage 8 blastula (Bla), stage 10 early gastrula (eG), stage 11.5 late gastrula (lG), stage 14 early neurula (eN), stage 21 late neurula (lN), stage 27 tailbud (Tb) and stage 38 tadpole (Td) during normal development, and from ovary (Ov), testis (Tes), brain (Br), eye (Eye), lung (Lun), heart (Hea), stomach (St), liver (Liv), spleen (Spl) and kidney (Kid) of 2 years-old female. Histone H4 was used as an internal marker for standard.*

embryos induced by UV-irradiation, the hyperdorsalized embryos induced by 0.3M LiCl (average 8.5DAI) showed a remarkable decrease in the *Xoom* transcripts (Fig. 6A). The same result was obtained repeatedly even when changing the cycle number of RT-PCR. These results show that lithium-treatment, but not UV-irradiation, induces depletion of *Xoom* transcripts prior to MBT.

Lithium-induced hyperdorsalization can be rescued by myo-inositol (Busa and Gimlich, 1989). To examine whether or not *myo*-inositol rescues the depletion of *Xoom* transcripts, we performed quantitative RT-PCR analysis of *Xoom* transcripts in lithium- and/or *myo*-inositol- injected embryos. Microinjection with 0.2M LiCl caused dorsalized embryo (average 7.8DAI), and reduced *Xoom* transcripts (Fig. 6B). Microinjection with 0.5M *myo*-inositol showed no effect both on dorso-ventral patterning (average 5.0DAI) and on *Xoom* transcripts. However, coinjection with *myo*-inositol rescued the dorsalization induced by lithium. These rescues occurred in dependence on dose of *myo*-inositol; 0.1M *myo*-inositol resulted in average 7.5DAI, 0.2M in 6.6DAI and 0.5M in 6.1DAI. In these embryos, *myo*-inositol also reversed the RNA level of *Xoom* in a dose-dependent manner (Fig. 6B). These results suggest that depletion of *Xoom* transcripts induced by lithium is closely related to IP_3-Ca^{2+} signal pathway.

Functional analysis of Xoom

To examine the function of *Xoom* during *Xenopus* development, we injected *Xoom* RNAs into both blastomeres of a 2-cell stage embryo. Although sense *Xoom* RNA was injected, both invagination and gastrulation in the injected embryos occurred normally as control *β-galactosidase*-injected embryos (Fig. 7 A,B,D,E) and morphological defects were not observed (Table 1). When, antisense *Xoom* RNA was injected, the invagination initiated normally at the early-gastrula stage (Fig. 7C). However, antisense *Xoom* RNA-injected embryo failed to close the blastopore and large yolk plug was observed even at the mid- and late-gastrula stage (Fig. 7F, Table 1). These results suggest that Xoom is a prerequisite protein for normal gastrulation movement.

Discussion

Xoom encodes a novel transmembrane protein

In the present study, gene screening with the differential display method succeeded in a cloning of *Xoom*. The *Xoom* cDNA encoded a novel transmembrane protein that is a homolog of a human Mr 110,000 antigen (Shimada *et al*., 1994). The amino acid sequence of *Xoom* was highly conserved with that of Mr 110,000 antigen in the whole region. Four cysteine residues within the cysteine-rich region of the extracellular domain were completely conserved, but the intracellular domain had a slightly lower similarity. The Mr 110,000 antigen has been found initially in human gastric carcinoma cells by its cross reactivity with anti-carcinoembryonic antigen (CEA) (Shimada *et al*., 1991). Molecular weight of the antigen is 110 kDa, but its cDNA encodes a 42 kDa polypeptide with a N-glycosylation site (Shimada *et al*., 1994). Similar to this cDNA, the predicted polypeptide of *Xoom* was 42.2 kDa and had a N-glycosylation site. Therefore, Xoom as well as Mr 110,000 antigen probably forms the transmembrane glycoprotein *in vivo*.

Expression of Xoom occurs during both periods of oogenesis and embryogenesis

The first phase of *Xoom* expression occurred in the early stages of oogenesis. Since its transcripts localized to the animal hemisphere of full-grown oocytes and were recruited into the blastomeres in the animal half, Xoom protein may participate in ectoderm and/or mesoderm formation (Dale and Slack, 1987). Zygotic expression of *Xoom* was first detected at the dorsoanterior region of the neural plate stage embryo and specifically localized in the neural crest cells in both sides of the neural tube and trigeminal nerve. Both cell types in the presumptive ectoderm and in the neural crest show the active migratory movement, suggesting that Xoom protein may relate to cell movement. However, *Xoom* expression has been detected in various organs and maintained even in the adult body. Judging from the expression pattern, Xoom protein may be used ubiquitously in morphogenesis and maintenance of multicellular tissues.

Xoom may function on the downstream of the IP_3-Ca^{2+} signaling pathway

In the present study, cDNA of *Xoom* was cloned as one of the lithium-responsive genes. Evidently, it was demonstrated that depletion of *Xoom* transcripts was induced by treatment with lithium, but rescued by coinjection of lithium and *myo*-inositol prior

TABLE 1

EFFECTS OF *XOOM* RNAs ON GASTRULATION

	Number ofembryos (%)		
sample	normal	large yolk plug	total
No injection	24 (100)	0 (0)	24
β-galactosidase RNA	28 (100)	0 (0)	28
sense Xoom RNA	27 (100)	0 (0)	27
antisense Xoom RNA	5 (20.0)	20 (80)	25

Embryos were injected with sense *Xoom* RNA and antisense *Xoom* RNA at the 2-cell stage and scored at st.11.5. Siblings were injected with *β-galactosidase* RNA as control.

to MBT. This rescue is consistent with the previous reports that lithium inhibits the IP_3-Ca^{2+} signaling pathway by inhibition of IMPase (Berrige *et al.*,1989; Busa and Gimlich, 1989; Kume *et al.*, 1997). Furthermore, it has been reported that the expression level of human Mr 110,000 antigen is promoted by gamma-interferon (IFN-γ) (Shimada *et al.*, 1994). Since INF-γ increases inositol phosphate and intracellular calcium concentration in neutrophilic glanulocytes (Rotnes *et al.*, 1994), thyroid cells (Kung *et al.*, 1995) and renal tubular cells (Hansen *et al.*, 1996), transcriptional control of Mr 110,000 antigen may be performed through the IP_3-Ca^{2+} signaling pathway. If this is the case, one possibility is that depletion of *Xoom* transcripts in the lithium-treated embryos may come from the downregulation of gene expression by inhibition of the IP_3-Ca^{2+} signaling pathway. In many embryonic genes, however, the zygotic transcription is quiescent prior to MBT (Newport and Kirschner, 1982; Almouzni and Wolffe, 1995). Therefore, it is more likely that post-transcriptional regulation of *Xoom* may be controlled on the downstream of the IP_3-Ca^{2+} signaling pathway.

Injection of antisense *Xoom* RNA into *Xenopus* embryos produced severe gastrulation defects, probably by inhibiting translation of *Xoom* transcripts. This evidence shows that Xoom is a prerequisite protein for normal gastrulation movement. Since lithium causes abroad invagination at the whole marginal zone, not restricted at the dorsal side (Kao and Elinson, 1988), active translation and consumption of *Xoom* transcripts may cause a remarkable depletion of *Xoom* transcripts in the lithium-treated embryos.

Materials and Methods

Embryo and oocyte manipulations

Unfertilized eggs were obtained by intracoalomic injection with gonadotropic hormone (Gestron, Denkaseiyaku, Japan) into *Xenopus laevis* female and artificially fertilized *in vitro* (Asashima *et al.*, 1990). The fertilized eggs were chemically dejellied with 1% thioglycorate (pH11) and cultivated in 0.1×modified Barth's solution (MBS) at 20°C (Moon and Christian, 1989). Embryos were staged according to Nieuwkoop and Faber (1967). For treatment with lithium, 32-cell stage embryos were incubated in 0.1× MBS containing 0.3M LiCl for 5 min at 20°C. Irradiation of UV was performed by essentially the same method as Scharf and Gerhart (1983). The fertilized eggs at 40 min after the insemination were irradiated with 2×10^4 ERG/mm^2 UV light with mini-transilluminator (Funakoshi, Japan) for 4 min. Effect of lithium or UV-irradiation was evaluated according to the Dorsoanterior Index (DAI) of Kao and Elinson (1988). Oocytes were obtained by defollicling ovary in 1×MBS containing 1% collagenase. Staging of oocytes was according to Dumont (1972).

Probe preparation and screening

To compare mRNA species between normal and lithium-treated embryos, total RNAs were extracted from embryos by guanidinium thiocyanate-phenol-chloroform and the LiCl precipitation method (Sargent *et al.*, 1986). The cDNA synthesis and PCR were performed by using a commercially available differential display kit (GenHunter, USA). The specific cDNA fragments were detected and subcloned by using our modified silver staining method (Shiraishi and Kinoshita in preparation). The synthesized cDNA probes were labeled with digoxigenin-dUTP by random labeling kit (Roche Diagnostics, USA). These probes were used

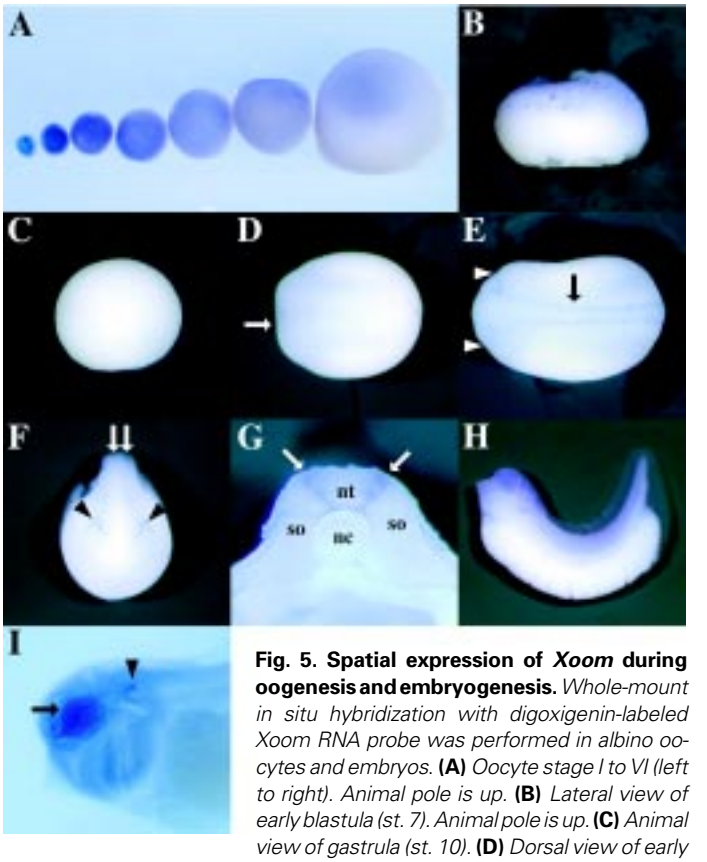

Fig. 5. Spatial expression of *Xoom* during oogenesis and embryogenesis. *Whole-mount in situ hybridization with digoxigenin-labeled Xoom RNA probe was performed in albino oocytes and embryos.* **(A)** *Oocyte stage I to VI (left to right). Animal pole is up.* **(B)** *Lateral view of early blastula (st. 7). Animal pole is up.* **(C)** *Animal view of gastrula (st. 10).* **(D)** *Dorsal view of early neurula (st. 15). Zygotic expression occurs in the dorsoanterior region (arrow).* **(E)** *Dorsal view of late neurula (st. 19). Expression of Xoom is localized in both sides of the neural tube (arrow) and upper space of the eye vesicles (arrowheads).* **(F)** *Anterior view of late neurula (st. 19). Expression of Xoom is localized in both sides of neural tube (arrow) and upper space of eye vesicles (arrowheads).* **(G)** *Transverse section at the trunk region of late neurula (st. 19). nt, neural tube; nc, notochord; so, somite. Xoom specifically expresses in neural crest cells around the neural tube.* **(H)** *Lateral view of tadpole (st. 35).* **(I)** *High magnification of cleared tadpole head. Intense expression of Xoom is observed in the optic vesicle (arrow) and the otic vesicle (arrowhead).*

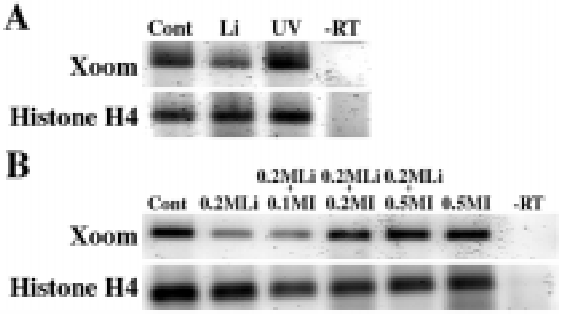

Fig. 6. Effect of lithium and *myo*-inositol on *Xoom* transcripts. (A) *Depletion of Xoom transcripts induced by lithium. Normal (cont), lithium-treated (Li) and UV-irradiated embryos (UV) were cultured and total RNAs were extracted at stage 7. Quantitative RT-PCR analyses were performed with a reference of histone H4 as an internal marker.* **(B)** *Rescue of Xoom transcript depletion by myo-inositol. Quantitative analyses of Xoom transcripts were performed in embryos injected with 0.2M LiCl (0.2M Li), 0.2M LiCl and 0.1M myo-inositol (0.2M Li+0.1M I), 0.2M LiCl and 0.2M myo-inositol (0.2M Li+0.2M I), 0.2M LiCl and 0.5M myo-inositol (0.2M Li+0.5M I) and 0.5M myo-inositol (0.5M I) or none (Cont). Total 5 nl of the solution was injected into each of the two ventral blastomeres at 4-cell stage.*

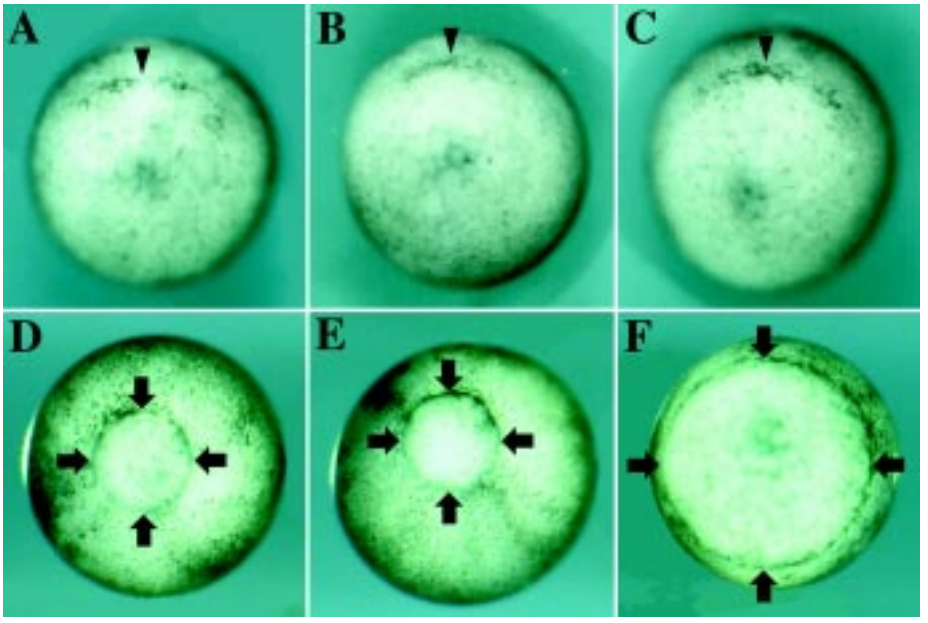

Fig.7 Effect of *Xoom* RNAs on gastrulation movement. *Xenopus embryos were injected with β-galactosidase RNA* **(A,D)**, *sense Xoom RNA* **(B,E)** *or antisense Xoom RNA* **(C,F)**. *In every case, normal invagination was observed on the vegetal view of stage 10 embryo (arrowhead of A,B,C). Gastrulation of the embryo injected with the sense Xoom RNA* **(E)** *proceeded normally as control embryo* **(D)**. *In contrast, gastrulation movement was inhibited at the initial phase in the embryo injected with antisense Xoom RNA* **(F)**. *Arrows of D, E and F shows the boundary of the blastopore on the vegetal view at st. 11.5.*

to screen the λgt10 oocyte cDNA library (gifted from Dr. D. Melton) (Rebagliati *et al.*, 1985). Approximately 5×10^7 plaques were screened and three positive clones were isolated. The longest clone, C1, contained a 1.3 kb insert and subcloned into pBK-CMV vector (Stratagene, USA) with *Eco*RI site. Clone C1 contained a complete open reading frame, but its untranslated regions were very short. To obtain a full-length of clone C1, 5'UTR and 3'UTR were cloned by the RACE method (Frohman *et al.*, 1988) from λZapII oocyte cDNA library (gift from Dr. S. Miyatani). The longest 5'RACE product was subcloned between *Eco*RI and *Sac*I site of the clone C1, and the 3'RACE product was between *Eco*NI and *Xho*I site. Thus, 1.5 kb clone was reconstructed as a full length of *Xoom* cDNA. DNA sequence was determined by ALFred DNA sequencer (Amersham Pharmacia, USA) using thermosequenase kit and Cye5-labeled primers. Homology search was carried out by using BLAST research program on GenBank database.

RNA isolation and northern blot

Total RNA was isolated from unfertilized eggs, embryos and adult organs by the LiCl precipitation method (Sargent *et al.*, 1986). For northern blotting, 4 μg of poly A (+) RNA were purified from unfertilized eggs and electorophoresed in denaturing 0.8% agarose gel containing 7% formaldehyde, then transferred to Hybond N^+ nylon membrane (Amarsham Pharmacia, USA) with 20×SSC. Hybridization with digoxigenin-dUTP labeled cDNA probe was carried out for 16 h at 50°C in high SDS buffer, containing 50% formamide, 5×SSC and 7% SDS according to the manufacturer's protocol (Roche Diagnostics, USA). Hybridized membrane was washed and treated with the anti-digoxigenin AP fab fragment and with Attophos substrate kit (Roche Diagnostics, USA). Bands hybridized with the probes were detected by using a FluorImager SI (Moleculer Dynamics, USA).

Whole-mount in situ hybridization

Whole-mount *in situ* hybridization was carried out according to the conventional method (Harland, 1991). Antisense RNA probe was synthesized using a full length of the cDNA as a template and T7 RNA polymerase. If necessary, the stained embryos were cleared in benzyl benzoate/benzyl alcohol mixed in ratio 2:1.

RT-PCR analysis

Since copy number of *Xoom* transcripts was very low, the quantitative RT-PCR method was used to monitor the gene expression. RT-PCR was carried out following essentially the same manner as Goto and Kinoshita (1999), using 5'-CAG GAA CCC AAG ACT GAT AAA GAT GAG-3' as upprimer and 5'-CGA GAA CTG GAA GAA GAA CTG CTT GTT GTG-3' as downprimer. The histone H4 primers were described previously (Goto and Kinoshita, 1999). Cycle number of PCR was 35 for *Xoom* and 25 for histone H4, respectively. These cycle numbers locate within the linearity of growth curve prior to saturation.

RNA production and microinjection

Full-length *Xoom* was subcloned into pBK-CMV, and sense RNA transcript was produced by T3 RNA polymerase. Clone C2, which encodes 1.1 kb Xoom RNA truncated with the initial 54 amino acid, was subcloned into pBluescript II(+) (Stratagene, USA), and antisense RNA transcript was produced by T3 RNA polymerase. Capped mRNA was made using mCAP RNA synthesis kit (Gibco BRL, USA) according to manufacturer's instructions. *β-galactosidase* RNA was produced from pCMV-SPORT β-gal (Stratagene, USA).

Fertilization, culture and microinjection were described previously (Moon and Christian, 1989, Asashima *et al.*, 1990). Five ng sense *Xoom* RNA, antisense *Xoom* RNA or *β-galactosidase* RNA was injected into each blastomeres of a 2-cell stage embryo.

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