Volume 303, number 1, 81–84 FEBS 11066 May 1992
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Multiple genes for *Xenopus* activin receptor expressed during early embryogenesis

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Received 1 April 1992

Four distinct cDNAs for activin receptor designated as XSTK2, 3, 8 and 9 have been cloned from a *Xenopus laevis* cDNA library. The protein structures deduced from the cDNAs have shown that they all have a putative extracellular ligand-binding domain, a single transmembrane domain and cytoplasmic Ser/Thr kinase domain, except that XSTK2 is extremely similar to the XSTK3 gene but lacks a carboxyl-terminal part of the kinase motif. Northern blot analysis showed that all transcripts are maternally inherited. The levels of transcript for XSTK2, 3 and 8 appeared to fluctuate during early development while those for XSTK9 maintain constant.

Activin; Transforming growth factor-β (TGF-β); Receptor; Serine/theorine kinase; *Xenopus laevis*

1. INTRODUCTION

Activin, a member of the transforming growth factor-β (TGF-β) family, was originally purified from gonadal fluids and defined as a stimulator of follicle-stimulating hormone (FSH) secretion from anterior pituitary cells [1,2]. Activin was later found to be identical to erythroid differentiation factor (EDF) which stimulates the differentiation of F5-5 friend leukemia cells into hemoglobin synthesizing cells [3]. It is now well recognized that functions of activin are not limited to hormonal regulation but closely correlated with cell differentiation (reviewed in [4]). One of the most interesting activities discovered for activin is related to the regulation of early development of *Xenopus laevis*. Two groups [5,6] have reported independently that mammalian activin is a potent mesoderm-inducing factor (MIF), which is a predicted key factor responsible for the cell–cell interaction between two populations of cells, namely endoderm and ectoderm to yield mesoderm. The findings have prompted many developmental biologists to find the endogenous activin or activin-like factor in early *Xenopus* embryos. To date, homologous genes to mammalian activins [6,7] and an activin-like activity [8] have been identified in early *Xenopus* embryos. Subsequently, it was speculated that receptor(s) must also be present in the embryos because presumptive ectoderm (animal cap) responds to mammalian activin and induces remarkable changes in morphology as well as activation of the muscle specific α-actin gene. Although the presence of activin receptors on a variety of mammalian cells was reported (reviewed in [4]), it was just recently that mouse activin receptor gene had been isolated and found to encode a serine/theorine (Ser/Thr) kinase [9].

In this study, we report the cloning of a family of *Xenopus* activin receptor genes and the temporal expression of the genes in early embryos. We purposed to examine at molecular level how activin receptors are contributing to the regulation of the embryonic induction.

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2. MATERIALS AND METHODS

Complementary DNA for mouse activin receptor [9] was cloned by reverse transcription of mRNA from mouse AtT20 cells followed by PCR using specific primers: 5′-TAGCTAGCGAGAACTTCC-3′ and 5′-TAGGAGCTCCAGTTCAGA-3′. The amplified cDNA was digested with *Kpn*I to prepare probes for cytoplasmic Ser/Thr kinase domain and for extracellular domain. Approximately 1×10^6 recombinants of the *Xenopus* cDNA library of stage 5–6 embryos [10] kindly donated by Dr. K. Cho were first screened with the probe for the Ser/Thr kinase domain under the conditions previously described [8]. All DNA sequencing procedures were carried out using the dideoxy chain termination sequencing method using a Sequenase kit (USB, USA), and [α-^{32}P]dCTP (Amersham, UK). Sets of the nested deletions were prepared with ExonucleaseIII and Mung Bean nuclease and both strands of the DNA were sequenced. Embryos of *Xenopus laevis* were obtained as described previously [8]. Total RNA was extracted from the embryos and Northern blot analysis was performed as previously described [8].

3. RESULTS AND DISCUSSION

Specific primers designed according to the nucleotide sequence of mouse activin receptor [9] enabled us to obtain by PCR an approximately 1.6 kb mouse cDNA

Fig. 1. Southern blot analysis of *Xenopus* genomic DNA with mouse activin receptor cDNA as a probe. Ten micrograms of the genomic DNA were digested by *Eco*RI (lanes 1 and 3) and *Hin*dIII (lanes 2 and 4). Lanes 1 and 2 were hybridized with the probe for the extracellular domain, and lanes 3 and 4 with the probe for the Ser/Thr kinase domain.

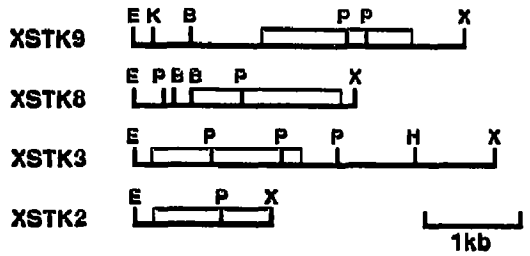

Fig. 2. Restriction map of isolated cDNA clones. Open boxes represent the translated regions of the cDNAs. Letters denote the restriction enzymes: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; X, *Xho*I.

which covered the entire translated region of the receptor. Southern hybridization that used two probes, one for the kinase domain and the other for the extracellular domain has suggested that there are multiple genes which are related to activin receptor (Fig. 1). The first screening of a *Xenopus* cDNA library was performed by hybridization with the probe for kinase domain. After screening of 1×10^6 phages, 21 positive clones were isolated. Subsequently they were classified into 4 groups by restriction enzyme mapping (Fig. 2) and based on the intensity of hybridization signals with the probe for the extracellular domain. One representative cDNA clone was chosen from each group (XSTK2, 3, 8 and 9) for nucleotide sequence analysis. The intensity of hybridization signal was XSTK9 > 8 > 3 > 2. The amino acid sequence deduced from each cDNA structure showed that XSTK 9, 8, 3 and 2 each encodes a 512, 510, 510 and 386 amino acid protein, respectively that has putative extracellular ligand-binding domain, a transmembrane domain (underlined) and a Ser/Thr kinase domain (indicated by arrows in Fig. 3). An unexpected finding is that a gene for a truncated form of the receptor has been cloned. It was found that XSTK 2 and 3 are extremely similar to each other but the former lacks the carboxyl-terminal half of the kinase domain as a result of an interuption by a stop codon. It is not clear at present whether or not XSTK 2 is generated by alternative splicing. Nevertheless, the novel receptor structure impaired at the kinase domain suggests that it may play a role as a loss-of-function regulator of activin effects. It is thus intriguing to see whether or not the function of the kinase is indeed lost. Regarding the extracellular domain, it was noted that the position of 10 cysteine residues in the putative ligand-binding domain (shaded in Fig. 3) are perfectly conserved among the four *Xenopus* receptors and the mammalian receptor [9]. The high conservation implies that these cysteine residues are essential for maintaining the conformation of the binding site for activin. Two potential *N*-glycosylation sites in the extracellular domain are also highly conserved (double underlined in Fig. 3). Another remarkable conservation of primary structure was found in the Ser/Thr kinase domain, especially in subdomain VIB and VIII (wavy underlined) [11]. The kinase activity has recently been confirmed for the TGF-β receptor [12] which was found to be structurally related to activin receptor. The homology of functional domains of *Xenopus* activin receptors to mouse receptor is schematically indicated in Fig. 4. Among four receptors, XSTK9 shows the highest homology to mouse activin receptor [9] and is identical to XAR5 [13]. The extracellular domain of XSTK2/3 and 8 were found to be most similar to the receptor recently reported by Attisano et al. [14].

In order to examine how receptor genes are regulated in embryogenesis, poly(A)$^+$ RNA was purified from oocytes and embryos, and analyzed by Northern blotting. The result shows that there are several transcripts with different sizes for each gene and that all transcripts are maternally encoded and the levels are sustained during development (Fig. 5A-C). It is noted, however, that the level of XSTK 2/3 and XSTK8 transcripts appears to fluctuate somewhat during embryogenesis.

The present study purposed to correlate the function of the activin receptor with early induction events observed in *Xenopus* embryos. Is activin is an endogenous MIF, its receptor should also exist when mesoderm induction takes place. In addition, experiments that used the animal caps indicated that the competence for activin or the endoderm factor is known to be acquired after stage 8 and lost after stage 11 [15]. Northern blot analysis showed that mRNAs for all receptors are maternally present in infertilized eggs and remain throughout embryogenesis, supporting the hypothesis that activin receptor is involved in mesoderm induction. However, rather stable expression of these receptor genes does not explain the loss of competence after stage 11. Possible explanations are that synthesis of functional activin receptor is regulated at the translational or post-translational level, or that inhibitory substance, such as an activin-binding protein, follistatin [16] or

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XSTK9                                    MGAATKLAFAVFLIFCSSGAILGRLETKECIYYNANWEK  39
XSTK8                                    MGASVALTFLLLLATFRAGSGHDEVETRECIYYNANWEL  39
XSTK3                                    MGAAVPLTLALLLATFRAGSGHDEVETRECIYYNANWEL  39
XSTK2  MLRLLPESSLLLLRAADPRGNMGAAVPLTLALLLATFRAGSGHDEVETRECIYYNANWEL  60

XSTK9  DKTNSNGTEPCYGDNDKRKHCFATWKNTSGSIEIVKQGCWLDDVNCYNKNECIEKKESPD  99
XSTK8  EKTNOSGVESCEGEKDKRLHCYASWRNNSGFIELVKKGCWLDDFNCYDRQECIAKEENPQ  99
XSTK3  EKTNOSGVESCEGEKDKRLHCYASWRNNSGFIELVKKGCWLDDFNCYDRQECIAKEENPQ  99
XSTK2  EKTNOSGVESCEGEKDKRLHCYASWRNNSGFIELVKKGCWLDDFNCYDRQECIAKEENPQ 120

XSTK9  VFFCCCEGNACNERFYHSPEMEVTQPTSNPVPKPPLFNTLLYSLVPIIVVAVIVLFLFWM 159
XSTK8  VFFCCCEGNYCNKKFTHLPEVETFDPKPQP--SASVLNILIYSLLPIVGLSMAILLAFWM 157
XSTK3  VFFCCCEGNYCNKKFTHLPEVETFDPKPQP--MPSVLNILIYSLLPIAGLSMVILLAFWM 157
XSTK2  VFFCCCEGNYCNKKFTHLPEVETFDPKPQP--MPSVLNILIYSLLPIAGLSMVILLAFWM 178

XSTK9  YRHHKLGYPPELVPTQDPGPPPPSPLLGLKPLQLLEVKARGRFGCVWKAQLLNETVAVKI 219
XSTK8  YRHRKPPY-GHVEINEDPGLPPPSPLVGLKPLQLLEIKARGRFGCVWKARLLNEYVAVKI 216
XSTK3  YRHRKPPY-GHVDLNEDPGPTPPSPMVGLKPLQLLEIKARGRFGCVWKARLLNEYVAVKI 216
XSTK2  YRHRKPPY-GHVDLNEDPGPSPPSPMVGLKPLQLLEIKARGRFGCVWKARLLNEYVAVKI 237

XSTK9  FPIQDKLSWQNEYEIYSLPGMKHENILHFIGAEKRGTNLDTDLWLITTFHEKGSLTDFLK 279
XSTK8  FPVQDKQSWQCEKEIFTTPGMKHENLLEFIAAEKRGSNLEMELWLITAFHDKGSLTDYLK 276
XSTK3  FPVQDKQSWQCEKEIFNTPGMKHENLLEFIAAEKRGSNLEMELWLITAFHDKGSLTDYLK 276
XSTK2  FPVQDKQSWQCEKEIFNTPGMKHENLLEFIAAEKRGSNLEMELWLITAFHDKGSLTDYLK 297

XSTK9  ANIVSWNELCHIAETMARGLSYLHEDIPGLR-DGHKPAVAHRDIKSKNVLLKNNLTACIA 338
XSTK8  GNLVSWNELCHITETMARGLAYLHEDVPRCKGEGHKPAIAHRDFKSKNVLLRNDLTAILA 336
XSTK3  GNLVNWNELCHITETMARGLSYLHEDVPRCKGEGHKPAIAHRDFKSKNVLIRNDLTAILA 336
XSTK2  GNLVNWNELCHITETMARGLSYLHEDVPRCKGEGHKPAIAHRDFKSKNVLIRNDLTAILA 357

XSTK9  DFGLALKFEAGKSAGDTHGQVGTRRYMAPEVLEGAINFQRDAFLRIDMYAFGLVLWELAS 398
XSTK8  DFGLAVRFEPGKPPGDTHGQVGTRRYMAPEVLEGAINFQRDSFLRIDMYAMGLVLWEIVS 396
XSTK3  DFGLAVRFEPGKPPGDTHGQVGTRRYMAPEVLEGAINFQRDSFLRIDMYAMGLVLWEIVS 396
XSTK2  DFGLAVRFEPCKPPGDTHGQVITCAAINL                                 386

XSTK9  RCTAADGPVDEYMLPFEEEAGQHPSLEDMQEVVVHKKKRPILRECWQKHAGMAMLCETIE 458
XSTK8  RCTAADGPVDEYLLPFEEEIGQHPSLEDLQEVVVHKKIRPVFKDHWLKHPGLAQLCVTIE 456
XSTK3  RCTAADGPVDEYLLPFEEEIGQHPSLEDLQEVVVHKKMRPVFKDHWLKHPGLAQLCVTIE 456

XSTK9  ECWDHDAEARLSAGCVEERIIQMQKLTNIITTEDIVTVVTMVTNVDFPPKESSL         512
XSTK8  ECWDHDAEARLSAGCVEERISQIRKSVNGTTSDCLVSIVTSVTNVDLPPKESSI       510
XSTK3  ECWDHDAEARLSAGCVEERISQIRKSVNGTTSDCLVSIVTSVTNVDLPPKESSI       510
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Fig. 3. Alignment of *Xenopus* activin receptor amino acid sequences. Potential *N*-linked glycosylation sites are indicated by double underlines, the transmembrane domain by a single underline, the Ser/Thr kinase domain by two arrows, and the subdomains VIB and VIII of the kinase by wavy underline. The conserved 10 cysteine residues in the extracellular domain are shaded.

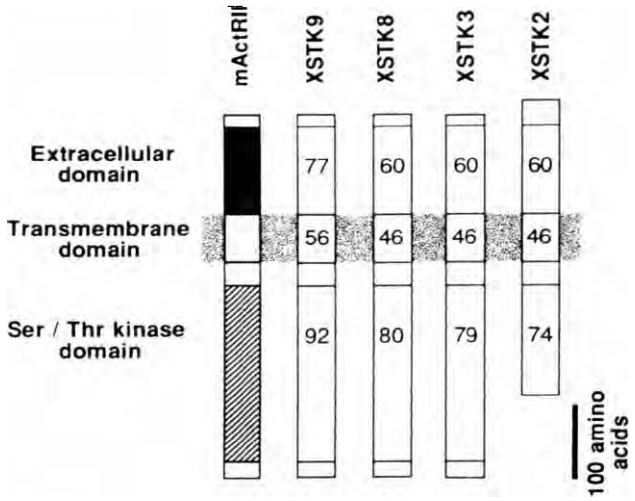

Fig. 4. Structural comparison of activin receptors between *Xenopus* and mouse. The numbers represent percent amino acid identity to mouse activin receptor (mActRII) [9].

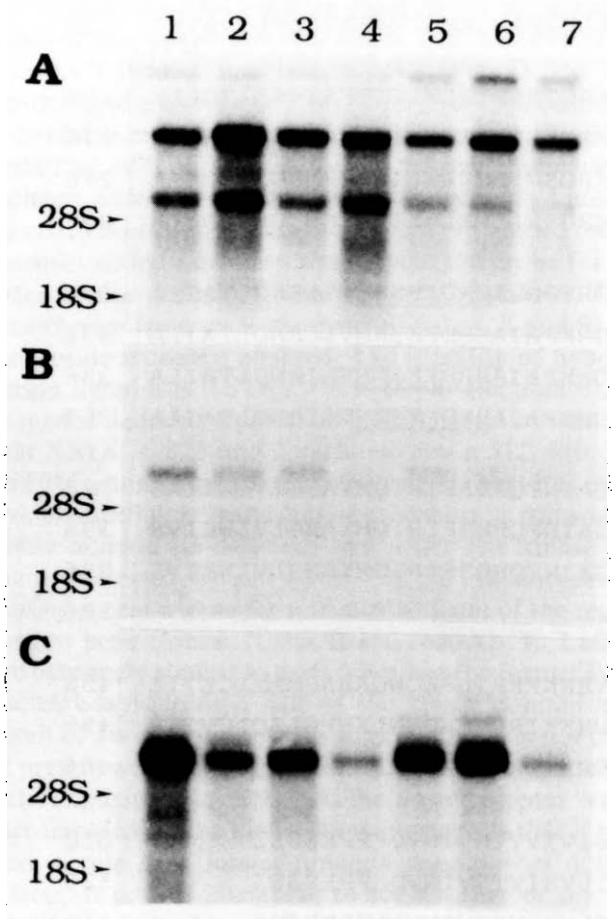

Fig. 5. Northern blot analysis of *Xenopus* activin receptor mRNAs during early embryogenesis. Ten micrograms each of poly(A)$^+$RNA from staged embryos were hybridized with the specific probes for XSTK9 (A), XSTK8 (B) and XSTK2/3 (C). Lane 1, oocyte; lane 2, morula; lane 3, blastula; lane 4, gastrula; lane 5, neurula; lane 6, tailbud; lane 7, tadpole.

phosphatase, controls activin action in *Xenopus* embryos.

We hope that the present molecular analysis on *Xenopus* activin receptor will provide useful tools to elucidate the mechanism of the embryonic inductions first described by Spemann and Mangold [17] and which has been attracting many developmental biologists for almost 70 years.

Acknowledgements: We wish to thank Dr. Nakayama for mRNA from AtT20 cells and Dr. Cho for the generous gift of a *Xenopus laevis* cDNA library. This study was supported in part by grants from Chichibu Cement Co. and Ministry of Education, Science and Culture, Japan (03833001).

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