We isolated a cDNA clone for a novel member of the S-II family of transcription elongation factors from Xenopus laevis. This S-II, named XSII-K1, is assumed to be the Xenopus homologue of mouse SII-K1 that we reported previously (Taira, Y., Kubo, T., and Natori, S. (1998) Genes Cells 3, 289–296). Expression of the XSII-K1 gene was found to be restricted to mesoderm-derived tissues such as liver, kidney, and skeletal muscle. Contrary to the general S-II gene, expression of the XSII-K1 gene was not detected in embryos at stages earlier than 11. The animal cap assay revealed that activin A, but not basic fibroblast growth factor, induced expression of the XSII-K1 gene and that it participated in the expression of mesoderm-specific genes such as Xbra and X-aetin. This is the first demonstration that the regulation at the level of transcription elongation is included in the development of mesoderm-derived tissues.

Transcription is a crucial step of gene expression. Various factors that participate in the process of transcription have been identified, and many of them are transcription initiation factors (1–4). These factors are essential for the modulation of RNA polymerase II, so that it can recognize the transcription initiation site on a specific gene and initiate correct transcription. These transcription initiation factors are divided into two groups as follows: general initiation factors, which are commonly required for transcription, and sequence-specific initiation factors, which initiate the transcription of a specific gene(s).

Regulation of transcription elongation is assumed to be simpler than that of transcription initiation because, once RNA synthesis has begun, it is assumed to continue until RNA polymerase II reaches the transcription termination site. The number of transcription elongation factors is much lower than that of transcription initiation factors, but several have already been identified, such as S-II (TFIIS) (5–10), elongin (SII) (11–14), TFIIF (15–18), P-TEFb (19, 20), and ELL (21). Among them, transcription elongation factor S-II is known to make RNA polymerase II readthrough intrinsic blocks within the transcription units of eukaryotic genes by promoting cleavage of the 3′-end of the nascent RNA by RNA polymerase II (22–32). S-II is unique in two respects. First, in various eukaryotic organisms, it forms a protein family with well conserved sequences of about 40 and 170 residues at their N and C termini, respectively, whereas each of the intervening sequence of about 50 residues is unique (10, 33–36). Second, multiple S-II molecules are present in a single organism, as has been demonstrated in the mouse. One form of these molecules is general S-II (10), which is ubiquitous in various cells, and the other forms are tissue-specific (37–40). The above sequence rule is applicable to all these S-II molecules.

Previously, we identified mouse SII-K1, which is expressed exclusively in heart, liver, skeletal muscle, and kidney (40). Contrary to general S-II mRNA, SII-K1 mRNA was not detected in embryos at an early developmental stage but became detectable in 15- and 17-day-old embryos, suggesting a functional difference between general S-II and SII-K1 (40). To gain further insight into the function of SII-K1 in embryonic development, we cloned the cDNA for Xenopus laevis SII-K1 and examined the expression of the Xenopus SII-K1 gene during development.

MATERIALS AND METHODS

cDNA Cloning for Xenopus SII-K1—To isolate Xenopus SII-K1 cDNA, we performed RT-PCR using Xenopus kidney poly(A)− RNA and two degenerate primers corresponding to Pro-178 to Asp-184 and Glu-339 to Cys-347 of SII-K1, followed by a nested PCR with degenerate primers corresponding to Asp-188 to Leu-194 and Asn-286 to Glu-293 (40). A DNA fragment of 327 bp was amplified in this way. On the basis of the sequence of this DNA fragment, we performed 5′-rapid amplification of cDNA ends to obtain more complete Xenopus SII-K1 cDNA, and we cloned a PCR product of 1320 bp. This cDNA contained part of the N-terminal consensus region, a subsequent unique region, and the C-terminal consensus region of the S-II family protein. To isolate the full-length Xenopus SII-K1 cDNA, we performed plaque hybridization using a DNA fragment corresponding to nucleotides +2 to +349 of this cDNA as a probe. This probe corresponded to the junction region of the N-terminal consensus region and the subsequent unique region. In this way, we finally isolated a full-length Xenopus SII-K1 cDNA consisting of 2,250 bp.

Northern Blot Hybridization—To detect Xenopus SII-K1 mRNA, poly(A)− RNA was extracted from Xenopus embryos at stage 0, 7, 8, 11, 13, 18, 21, 26, 33, and 39 or adult Xenopus tissues (heart, brain, spleen, lung, skeletal muscle, kidney, and testis) and subjected to Northern blot hybridization. The probe used was the PCR product corresponding to nucleotides +335 to +683 of the Xenopus SII-K1 cDNA (2 × 10^6 cpm/μg). The same filter was rehybridized with part of the Xenopus EF1-α cDNA (41) to assess the efficiency of RNA extraction. Each lane contained a

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Participation of XSII-K1 in Tissue Development

RESULTS

Identification and Characterization of Xenopus SII-K1 cDNA—SII-K1 is a unique S-II family transcription factor whose expression is known to be developmentally regulated during the embryonic development of the mouse (40). To investigate the function of SII-K1 in embryonic development, we isolated a cDNA for the Xenopus homologue of SII-K1 on the basis of the sequence of mouse SII-K1. As shown in Fig. 1, this cDNA encoded a novel S-II family protein of 645 amino acid residues. The partial sequence of this cDNA was found to have significant sequence similarities to that of mouse SII-K1 using RNA prepared from various tissues of Xenopus adults. As shown in Fig. 3A, an intense signal corresponding to a 2.5-kb RNA was detected in liver, skeletal muscle, and kidney, but no appreciable signal was detected in spleen, brain, and testis. This tissue-specific expression was very similar to that of mouse SII-K1 (40), except that significant expression was not detected in the heart. Analyzing the cDNA with the aid of the probe RNA was visualized by incubating the embryos with 75 mg/ml nitro blue tetrazolium and 50 mg/ml 5-bromo-4-chloro-3-indolyl phosphate for 14 h at 4 °C. Antibody bound to the probe RNA was visualized by incubating the embryos with 75 mg/ml RNase A and 2 mg/ml RNase T1 for 30 min and then treated with 10 μg/ml proteinase K for 10 min, 0.1 M triethanolamine twice for 5 min each time. The embryos were washed in a washing solution (10 mM phosphate buffer, pH 7.4, 150 mM NaCl, 15 mM MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 3.7% formalin) for 15 min at room temperature. They were then successively treated with 100% methanol and 0.1% Tween 20) three times for 5 min each time. The embryos were fixed in MEMFA (100 mM MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 3.7% formalin) for 15 min at room temperature. Whole Mount in Situ Hybridization—Whole mount in situ hybridization of Xenopus neurula and tail bud embryos was performed essentially as described previously (42). Embryos were fixed in MEMFA (100 mM MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 3.7% formalin) for 15 min at room temperature. Whole Mount in Situ Hybridization—Whole mount in situ hybridization of Xenopus neurula and tail bud embryos was performed essentially as described previously (42). Embryos were fixed in MEMFA (100 mM MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 3.7% formalin) for 15 min at room temperature. Whole Mount in Situ Hybridization—Whole mount in situ hybridization of Xenopus neurula and tail bud embryos was performed essentially as described previously (42). Embryos were fixed in MEMFA (100 mM MOPS, pH 7.4, 2 mM EGTA, 1 mM MgSO4, and 3.7% formalin) for 15 min at room temperature.
Expression of the XSII-K1 Gene during Embryogenesis—We located the expression of XSII-K1 by in situ hybridization using embryos at stage 13 (neurula) and stage 33 (tail bud). The XSII-K1 gene was clearly expressed in the head, somites, and tail of embryos at the neurula stage (Fig. 4A), whereas it was expressed in mesoderm-derived tissues such as head mesenchyme, pharynx (branchial arches), and somites in embryos at the tail bud stage (Fig. 4B). No significant staining was detected with the sense RNA probe. These results suggest that XSII-K1 is involved in the development of dorsal mesoderm-derived tissues.

During Xenopus development, activin A is known to induce dorsal mesoderm tissues (49), whereas bFGF induces ventral mesoderm tissues (50). Therefore, using the animal cap assay, we investigated whether activin A or bFGF was able to induce expression of the XSII-K1 gene. Animal caps from embryos at the neurula stage were incubated with or without 10 ng/ml activin A or bFGF. Then total RNA was extracted, and expression of the XSII-K1 gene was examined by RT-PCR. As is evident from Fig. 5, mRNA for XSII-K1 was detected only in the activin A-treated samples, although expression of the Xbra gene was induced by both activin A and bFGF (Fig. 6). Thus, it is clear that the XSII-K1 gene is expressed in the dorsal mesoderm and not the ventral mesoderm. These results are in accord with those of in situ hybridization.

Induction of Mesoderm by Injection of XSII-K1 mRNA—It is obvious that the XSII-K1 gene is activated during the course of induction of the dorsal mesoderm by activin A. As XSII-K1 is a transcription elongation factor, it is assumed to participate in the transcription of mesoderm-specific genes, such as Xbra (45). Therefore, using animal cap assay, we examined whether XSII-K1 alone is able to activate mesoderm-specific genes. For this, we injected XSII-K1 mRNA into the animal poles of stage 1 (one-cell stage) embryos. Animal caps were dissected from embryos at the neurula stage and incubated without activin A for 24 h. Then total RNA was extracted from the embryos, and expression of the Xbra gene was examined by RT-PCR. If XSII-K1 mRNA was injected in advance, the Xbra gene was found to be activated in the animal caps even in the absence of activin A, as shown in Fig. 6. Therefore, using the animal cap assay, we examined whether XSII-K1 alone is able to activate mesoderm-specific genes. For this, we injected XSII-K1 mRNA into the animal poles of stage 1 (one-cell stage) embryos. Animal caps were dissected from embryos at the neurula stage and incubated without activin A for 24 h. Then total RNA was extracted from the embryos, and expression of the Xbra gene was examined by RT-PCR.

If XSII-K1 mRNA was injected in advance, the Xbra gene was found to be activated in the animal caps even in the absence of activin A, as shown in Fig. 6. Therefore, using the animal cap assay, we examined whether XSII-K1 alone is able to activate mesoderm-specific genes. For this, we injected XSII-K1 mRNA into the animal poles of stage 1 (one-cell stage) embryos. Animal caps were dissected from embryos at the neurula stage and incubated without activin A for 24 h. Then total RNA was extracted from the embryos, and expression of the Xbra gene was examined by RT-PCR. If XSII-K1 mRNA was injected in advance, the Xbra gene was found to be activated in the animal caps even in the absence of activin A, as shown in Fig. 6. Therefore, using the animal cap assay, we examined whether XSII-K1 alone is able to activate mesoderm-specific genes. For this, we injected XSII-K1 mRNA into the animal poles of stage 1 (one-cell stage) embryos. Animal caps were dissected from embryos at the neurula stage and incubated without activin A for 24 h. Then total RNA was extracted from the embryos, and expression of the Xbra gene was examined by RT-PCR.

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induces their expression. This is the first report to suggest that mesoderm induction is regulated at the level of transcription elongation.

**DISCUSSION**

In this study, we showed that *Xenopus* SII-K1 participates in the development of mesoderm-derived tissues. As SII-K1 is a transcription elongation factor that belongs to the S-II family (40), this is the first clear demonstration that tissue development is regulated at the level of transcription elongation. As well as in dorsal mesoderm-derived tissues such as head mesenchyme, somites, and branchial arches are indicated by blue, white, and black arrowheads, respectively. Illustration represents a tail-bud stage embryo.

**FIG. 4.** Whole mount *in situ* hybridization of XSII-K1 mRNA. Embryos at the neurula stage (A), and tail bud stage (B) were treated with the antisense or sense probe of XSII-K1. For hybridization-positive tissues, head mesenchyme, somite, and branchial arches are indicated by blue, white, and black arrowheads, respectively. Illustration represents a tail-bud stage embryo.

**FIG. 5.** Activation of the XSII-K1 gene by activin A. RNA was extracted from animal caps treated with activin A or bFGF and subjected to RT-PCR to detect XSII-K1 (top), Xbra (middle), or XEF1-α mRNA (bottom). NT, embryos incubated without activin A or bFGF; WE, control embryos at stage 21. RT(+) and RT(−) indicate experiments with or without RT reaction.

**FIG. 6.** Induction of expression of the mesoderm marker genes (Xbra and Xα-actin) by injection of XSII-K1 mRNA. XSII-K1 mRNA was injected into the animal pole of embryos at stage 1 (one-cell stage). Animal caps were dissected at the neurula stage and incubated for 24 h. Total RNA was extracted from the animal caps, and expression of the Xbra (top), α-actin (middle), and XEF1-α (bottom) genes was detected by RT-PCR. NT, animal caps from non-treated embryos. RT(+) and RT(−) indicate experiments with or without RT reaction.
termination codons were identified upstream of the first Met codon. However, we assigned this codon as the first Met codon by comparison with the sequences of other S-II family proteins. Moreover, this Met codon satisfied the Kozak’s rule (51).

It is noteworthy that the unique region of XSII-K1 is quite different from those of other S-II family proteins. XSII-K1 contained two repeated sequences in this region consisting of 18 and 50 amino acid residues. Although the biological significance of these sequences is unknown, we assume that there is a protein(s) that interacts with this region in mesoderm-derived tissues. Identification and characterization of such a protein(s) may be essential to elucidate the way in which XSII-K1 regulates the transcription of mesoderm-specific genes such as Xbra (45) and Xo-actin (46).

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