Develop. Growth Differ. (2000) **42**, 249–256

Expression and characterization of *Xenopus type I collagen alpha 1 (COL1A1)* during embryonic development

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A cDNA encoding *Xenopus type I collagen alpha 1* (*Xenopus COL1A1*) has been isolated from an ovary cDNA library. The *COL1A1* cDNA is approximately 5.7 kb pairs and encodes 1447 amino acids. The putative COL1A1 polypeptide shares high identities of amino acid sequence with other vertebrate COL1A1 proteins. The level of *Xenopus COL1A1* transcripts was increased markedly in the posterior region of the embryo at the tail-bud stage, then gradually spread to the anterior region. Histological observations of the tail-bud embryos showed that *COL1A1* was mainly expressed in the inner layer of the posterior dorsal epidermis exposed to the somite mesoderm, except for in the dorsal fin. Less intense signals were also detected in the outer layer of the dorsal epidermis and dermatome. The expression of *COL1A1* was increased in posteriorized embryos resulting from treatment with retinoic acid but decreased in hyper-dorsalized embryos resulting from lithium chloride treatment. These results suggest that *COL1A1* is a major component of the dorsal dermis exposed to the somite in *Xenopus* embryos, but its expression is not related to the temporal sequence of somite segregation.

Key words: collagen, dorsal epidermis, retinoic acid, somite, *Xenopus.*

Introduction

Collagen is a major component of the extracellular matrix. To date, 19 collagen types have been identified in mammals. In all these types a major component of the protein is a triple-helical structure of three polypeptide chains (alpha chains). A minimum of 30 genes are needed to code for the constituent chains of these 19 types (Vuorio & de Crombrugghe 1990; Muragaki *et al.* 1991; Yamaguchi *et al.* 1992; Oh *et al.* 1994; Zhang *et al.* 1996; Gatalica *et al.* 1997; Khaleduzzaman *et al.* 1997; Hagg *et al.* 1998; Imhof & Trueb 1999). *Type I collagen*, a heterotrimer of two identical alpha 1(I) chains and one alpha 2(I) chain, is the most abundant member of the *collagen* family genes and a major component of bone and skin. A number of reports have shown that mutations or abnormal splicing of *type I collagen* causes various diseases of osteogenesis and chondrogenesis (Bornstein & Sage 1980).

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Received 22 November 1999; revised 24 December 1999; accepted 24 December 1999.

In *Xenopus*, a few alpha chains of collagen have been reported. Transcripts of *type II collagen alpha 1* began to be expressed at the beginning of the neurula stage and the messenger RNA (mRNA) levels progressively increased afterwards (Su *et al.* 1991). At the neurula stage, *type II collagen* mRNA is localized in the notochord, and its expression expands to the chondrogenic tissues (Su *et al.* 1991; Bieker & Yazdani-Buicky 1992; Seufert *et al.* 1994). In the study of early development of *Xenopus*, *type II collagen* has often been used as a marker for notochord differentiation. Although the type VI collagen gene has not been isolated to date, antibody (3D7) to type VI collagen was used to study the spatial and temporal pattern of type VI collagen expression (Otte *et al.* 1990). According to Otte *et al.* (1990), type VI collagen is already present in the cytoplasm of unfertilized eggs and becomes localized in the peripheral cytoplasm of the superficial cell layer during the cleavage stage. During gastrulation type VI collagen is localized in the presumptive archenteron and antibody against it interferes with the internal involution of mesoderm. Thus, in early developmental stages, type VI collagen seems to play an important role in morphogenic movement. So far, however, the gene for the most general *type I collagen* has not been isolated form *Xenopus*, and mammalian *type*

I collagen expression has not been studied during the early developmental stage. In the present study, we have cloned a *Xenopus COL1A1* gene for the first time and report its expression in the early development of *Xenopus*.

Materials and Methods

Eggs and embryos

Eggs were obtained by injecting *Xenopus laevis* females with 250 units of human chorionic gonadotropin and they were fertilized *in vitro*. Embryos were dejellied with 1.5% sodium thioglycollate and cultured in modified Steinberg's solution. Embryonic stages were determined according to Nieuwkoop and Faber (1967).

Cloning and sequencing

An ovarian cDNA library was constructed as follows. Total RNA was purified from ovaries by the ultra-centrifugation method using CsTFA (Pharmacia, Uppsala, Sweden; Okayama *et al.* 1987) from ovaries

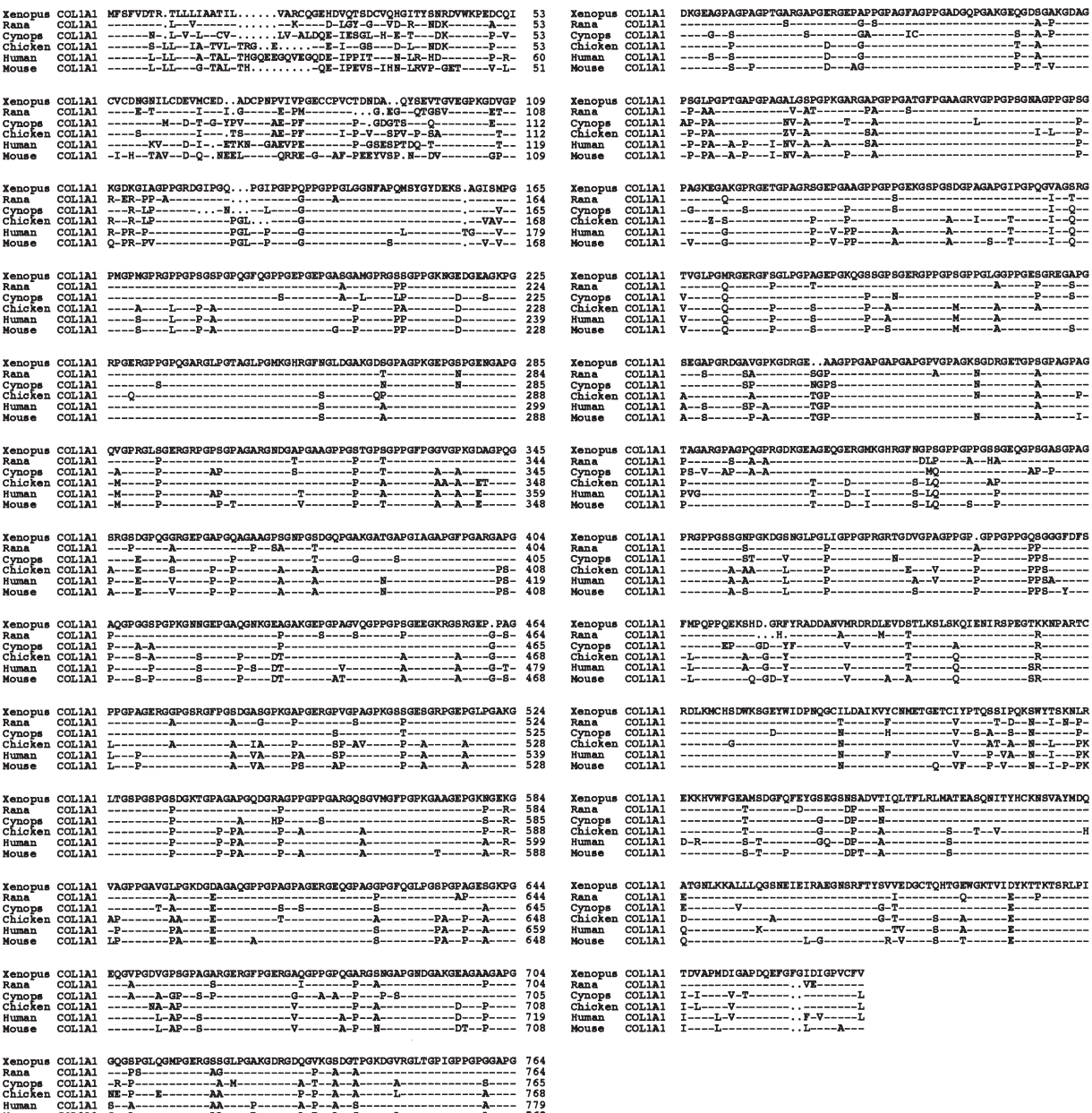

Fig. 1. Alignment of amino acid sequences of COL1A1 of *Xenopus laevis*, *Rana catesbeiana*, *Cynops pyrrhogaster*, chicken, humans and mice. Identical residues are indicated by dashes, and dots represent insertions of gaps for maximal alignment. High identities of amino acids were shared with *Rana* (88.1%), *Cynops* (85.0%), chicken (83.7%), humans (81.7%) and mouse (80.6%).

and mRNA was purified using the polyAtract mRNA Isolation System (Promega, Madison, WI, USA). Double-stranded cDNA was obtained from the purified mRNA using a ZAP-expression cDNA synthesis kit (Stratagene, La Jolla, CA, USA) and cloned into ZAP Express vector digested with *Eco*RI and *Xho*I. A fragment used as a probe for *COL1A1* was obtained as follows. Total RNA was isolated from stage 25 embryos. Double-stranded cDNA was amplified by reverse transcription–polymerase chain reaction (RT-PCR) using degenerate primers. The degenerate primers were designed from the consensus sequences among human, mouse and *Rana catesbeiana COL1A1*: Forward 5′-AA(A/G)ATGTG(C/T)CA(C/T)TC(A/C/T)GA-(C/T)TGGAA(A/G)-3′, reverse 5′-CAT(A/G)TA(A/G/T)-GC(A/C/G/T)AC(A/G)CT(A/G)TT(C/T)TT(A/G)CA-3′. *Xenopus COL1A1* cDNA clone was obtained by screening the ovarian cDNA library and the isolated clone was inserted into the pCS2 + vector. The nucleotide sequence of the cDNA was determined using a BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA).

RT-PCR analysis

Oligo(dT)-primed first-strand cDNA was prepared from 1 µg of total RNA using Superscript II (Gibco BRL, Gaithersburg, MD, USA). One-twentieth of the cDNA obtained was used for each polymerase chain reaction (PCR) reaction. For *Xenopus COL1A1,* 22 amplification cycles were performed under the following conditions: Denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. As an internal loading control, the primers for the ubiquitously expressed histone4 were used under the same PCR conditions as those for *Xenopus COL1A1* except that only 25 amplification cycles were carried out. The sequences of the gene primers used were as follows:

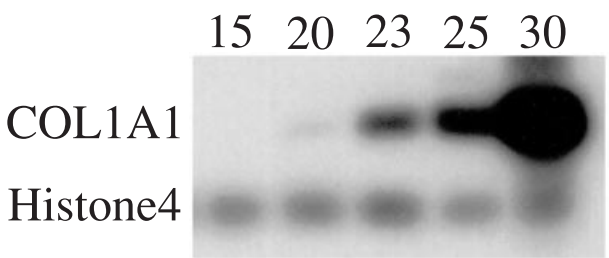

Fig. 2. Temporal expression pattern of *Xenopus COL1A1* during *Xenopus* early development. Quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed using 1 µg of total RNA extracted from *Xenopus* embryos at different stages. Numbers indicate the developmental stages. The levels of transcripts of *Xenopus COL1A1* were increased markedly after the late neurula stage. *Histone4* was included for normalization.

Xenopus COL1A1 forward 5′-ATTCAACGGACCCT-CTGGAC-3′ (Genbank accession number: AB034701; nucleotide (nt) 3396–3415), reverse 5′-ATCTTCAGGTC-ACGGCAGGT-3′ (nt 3845–3826); histone4 forward 5′-CGGGATAACATTCAGGGTATCACT-3′ (Genbank accession number: XELHX1H1; nt 1498–1521), reverse 5′-ATCCATGGCGGTAACTGTCTTCCT-3′ (nt 1686–1663). Aliquots containing one-tenth of the PCR products were electrophoresed on 2% agarose gels and transferred to nylon membranes. The membranes were hybridized to a random-primed isotope-labeled probe for each gene and autoradiographed.

Whole-mount in situ *hybridization*

As a probe for *in situ* hybridization, an antisense probe for *Xenopus COL1A1* was generated by linearizing the vector with *Hin*dIII and transcribing it with T7 RNA polymerase in the presence of digoxigenin-uridine triphosphate (UTP; Boehringer Mannheim, Mannheim, Germany). Whole-mount *in situ* hybridization was performed according to the method of Harland (Harland 1991). After the detection, some embryos were embedded in paraffin (Paraplast Plus; Sigma Chemical Co., St Louis, MO, USA) and cut into serial sections with a thickness of 10 µm.

Retinoic acid and lithium chloride treatments

For the retinoic acid (RA) treatment, stage 9 embryos were incubated in 20 µM all-trans-retinoic acid solution for 2 h. For the lithium chloride treatment, 32-cell stage embryos were incubated in 120 mM lithium chloride solution for 30 min.

Results

Cloning of Xenopus COL1A1

A fragment of *Xenopus COL1A1* was obtained from stage 25 embryos by RT-PCR using degenerate primers corresponding to consensus sequences of other vertebrate *COL1A1* genes (Li *et al.* 1995b; Asahina *et al.* 1999a,b). A full-length *Xenopus COL1A1* cDNA (Genbank accession number: AB034701) was isolated by screening an ovarian cDNA library using this fragment as a probe. The length of the transcript of *Xenopus COL1A1* was about 5.7kb pairs and it encoded a putative polypeptide of 1447 amino acids. This putative *Xenopus* COL1A1 protein contained many proline and glycine residues, as has been reported in other species. Comparison of the amino acid sequences of the *Xenopus* COL1A1 and related genes showed that *Xenopus* COL1A1 was highly homologous to

R. catesbeiana, *Cynops pyrrhogaster*, chicken, human and mouse COL1A1. The identities of shared amino acids were 88.1% with *Rana*, 85.0% with *Cynops*, 83.7% with chicken, 81.7% with humans and 80.6% with mouse (Fig. 1).

Expression of COL1A1

The levels of transcripts of *Xenopus COL1A1* were increased markedly during the neurula stage (Fig. 2). By whole-mount *in situ* hybridization, the transcripts of

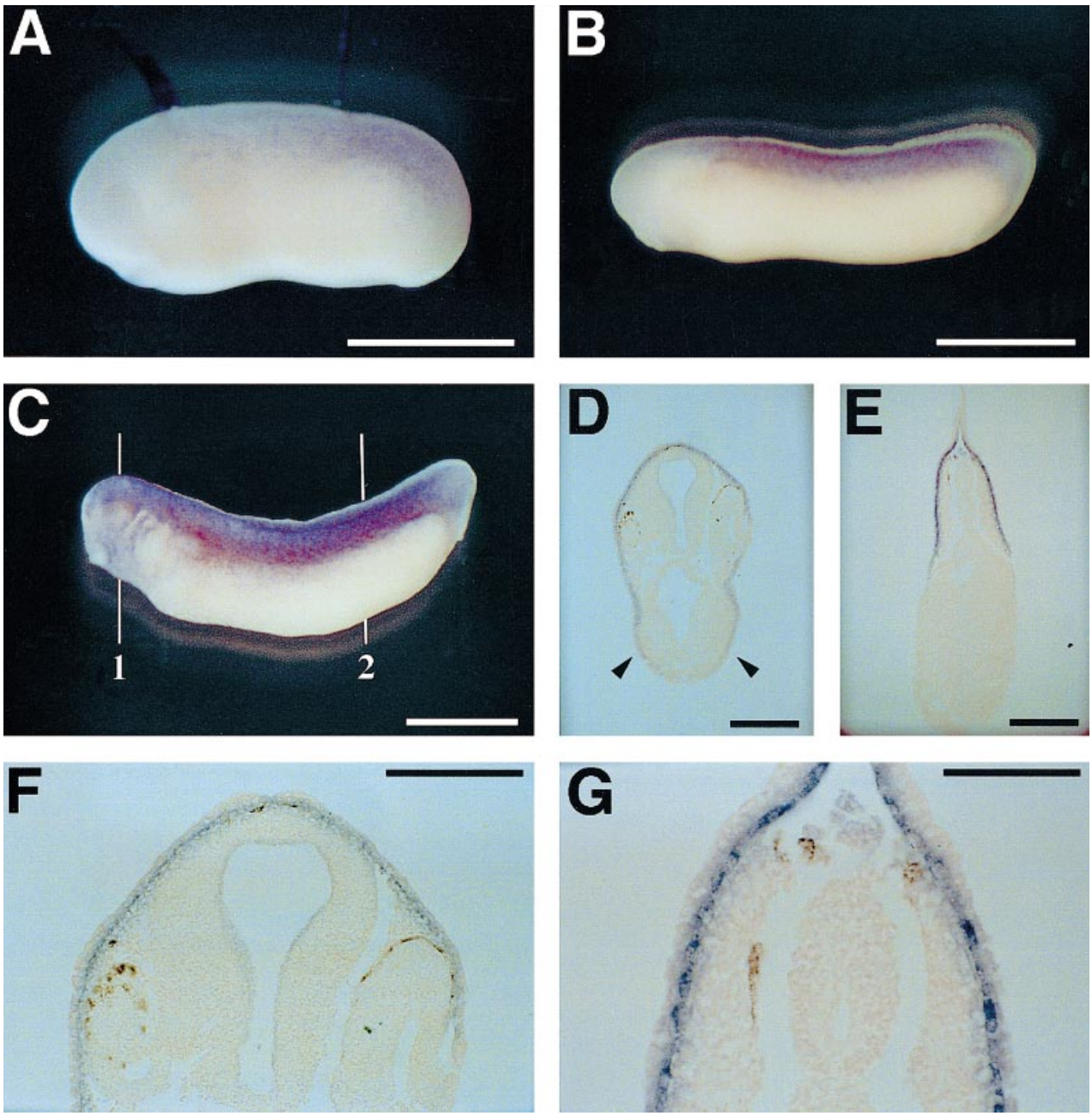

Fig. 3. Spatial expression pattern of *Xenopus COL1A1* shown by whole-mount *in situ* hybridization. (A) Stage 25 embryo. Weak expression of *Xenopus COL1A1* was detected in the posterior dorsal region. (B) Stage 28 embryo. Expression of *Xenopus COL1A1* gradually spread to the anterior region. (C) Stage 30 embryo. Expression of *Xenopus COL1A1* was extended to the head region. (D–G) Transverse sections of the stage 30 embryo at the levels indicated by the white bars in (C). (D,F) A section at the head region (bar 1). The transcripts were detected in the inner layer of epidermis. Note that the signals extended to the ventral region (arrowheads). (E,G) A section at the trunk region (bar 2). Intense staining was detected in the inner layer of the dorsal epidermis. Less intense signals were detected in the outer layer of the dorsal epidermis and dermatome. *COL1A1* was not detected in the ventral epidermis. Bars, 1 mm (A–C), 150 μm (D–F), 75 μm (G).

Xenopus COL1A1 were first detected in the posterior dorsal region at stage 25 (Fig. 3A), and then gradually spread to the anterior region (Fig. 3B), and extended up to the head region by stage 30 (Fig. 3C). Transverse sections of the stage 30 embryos showed that intense signals of *Xenopus COL1A1* transcripts were found in the inner layer of the dorsal epidermis in the trunk and tail regions. *Xenopus COL1A1* mRNA was also detected in the outer layer of the dorsal epidermis and dermatome (Fig. 3E,G). In the head region, *Xenopus COL1A1* transcripts were detected less intensely in the inner layer of the whole epidermis, including the ventral region (Fig. 3D,F). In order to confirm the localization of *Xenopus COL1A1*, we also performed the quantitative RT-PCR and Southern blotting in the dissected embryos. The quantity of *Xenopus COL1A1* transcripts was larger in the posterior region than in the anterior region at all stages examined (Fig. 4). Further, at stage 20, when the segmentation of somites had not yet occurred in the posterior region, *Xenopus COL1A1* mRNA was already present in the posterior region (Fig. 4).

Effects of RA and LiCl on the expression of Xenopus COL1A1

Our data showed that the expression of *Xenopus COL1A1* was concentrated in the posterior region. In order to examine whether modification of the anteroposterior axis affects the expression of *Xenopus COL1A1*, we checked the expression of *Xenopus COL1A1* transcripts in the posteriorized or anteriorized embryos by whole-mount *in situ* hybridization and RT-PCR. Retinoic acid is known to mediate an inductive interaction regulating antero-posterior differentiation in *Xenopus* and to cause posteriorization of embryos in a dose-dependent manner (Durston *et al.* 1989; Sive *et al.* 1990). In contrast, lithium chloride (LiCl) treatment at the 32-cell stage induces hyper-dorsalized embryos that include a large head region (Kao & Elinson 1988, 1989). The expression of *Xenopus COL1A1* was increased by RA treatment (Fig. 5A; lane 2). Whole-mount *in situ* hybridization also confirmed that the expression of *COL1A1* was increased not only in the anterior region, which was posteriorized by RA, but also in the posterior region (Fig. 5B). In contrast, LiCl treatment resulted in reduced levels of *Xenopus COL1A1* transcripts (Fig. 5A; lane 3) and in a lack of detectable levels of *Xenopus COL1A1* transcripts when examined by whole-mount *in situ* hybridization at stage 28 (data not shown).

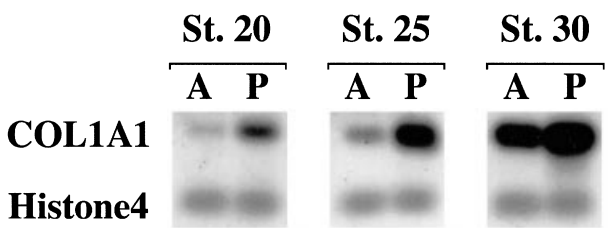

Fig. 4. Spatial expression of *Xenopus COL1A1* during *Xenopus* development. Quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed using 1 µg of total RNA extracted from the anterior (A) or posterior (P) half of the embryos dissected at neural and tail-bud stages. Transcripts of *Xenopus COL1A1* were concentrated in the posterior region at all stages examined. *Histone4* was included for normalization. St., stage.

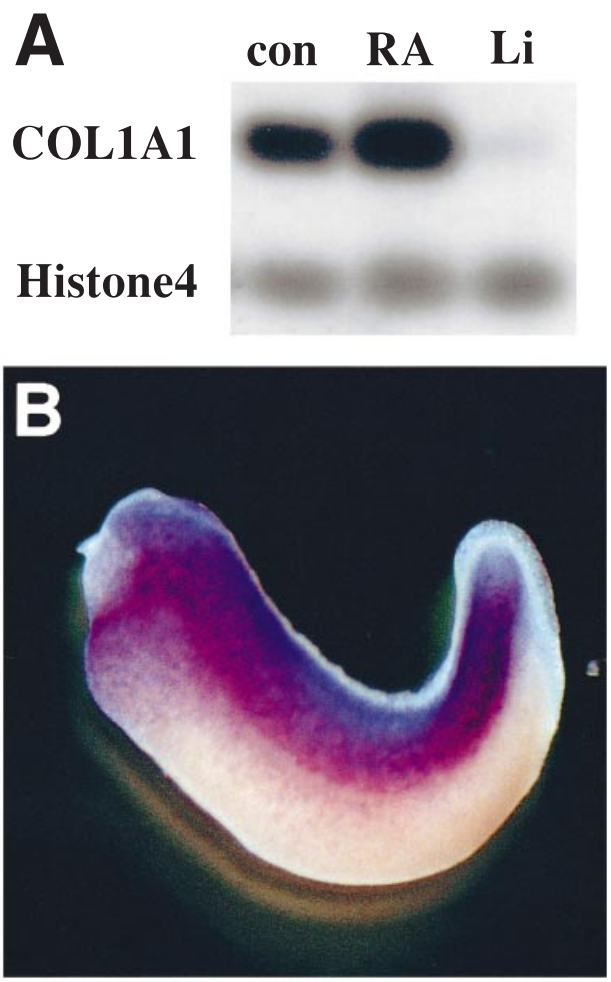

Fig. 5. Effects of retinoic acid and lithium chloride on the expression of *Xenopus COL1A1*. (A) Quantitative reverse transcription–polymerase chain reaction (RT-PCR) was performed using 1 µg of total RNA extracted from the normal, retinoic acid-treated, or lithium chloride-treated embryos. The expression of *Xenopus COL1A1* was increased by retinoic acid and decreased by lithium chloride. (B) Whole-mount *in situ* hybridization of the retinoic acid-treated embryo (stage 28). Expression of *Xenopus COL1A1* was increased not only in the anterior region, which was posteriorized by retinoic acid, but also in the original posterior region.

Discussion

Expression pattern of Xenopus COL1A1

In *Xenopus*, the first indication of somite segregation is found in the anterior region at stage 17. Thereafter the process of somite segregation progresses in a cranio-caudal direction. Simultaneously with somite segregation, somite differentiation proceeds in a cranio-caudal direction. After stage 20, the myocoelic cavities begin to disappear and the myoblasts are spindle shaped in the most anterior somites. Gradual liberation of the sclerotome and the dermatome from the original somite mesoderm also proceeds in a cranio-caudal direction (Nieuwkoop & Faber 1967). As type I collagen is known as a major component of the bone derived from the sclerotome and the skin derived from the dermatome, it was expected that the expression of *COL1A1* would also proceed from anterior to posterior. However, both whole-mount *in situ* hybridization and RT-PCR analysis indicated that the expression of *COL1A1* proceeded from posterior to anterior. This suggests that *COL1A1* is not related to the segregation of somites, although *COL1A1* transcripts were detected close to the somite mesoderm in the trunk and tail regions.

We showed that *COL1A1* is most prominently expressed in the inner layer of the dorsal epidermis and less intensely expressed in the outer layer of the dorsal epidermis, the inner layer of the head epidermis, and the dermatome at stage 30. The spatial pattern of *COL1A1* transcripts suggests that COL1A1 protein is a major component of the basal lamina under the dorsal epidermis and head epidermis at the tail-bud stage. This expression pattern is in sharp contrast to that of type II collagen protein, which is localized in the perinotochordal region, in the intersegmental region of somites, on the ventral side of the neural tube, and in the subnotochordal rod at stage 31 (Su *et al.* 1991). The complementary patterns of *type I* and *II collagen* expression may serve for proximal–distal patterning of the somite mesoderm.

Retinoic acid induces posteriorization of embryos (Durston *et al.* 1989; Sive *et al.* 1990), increases the expression of the posterior marker genes (Cho & De Robertis 1990), and decreases the expression of the anterior marker genes (Pannese *et al.* 1995; Andreazzoli *et al.* 1997; Casarosa *et al.* 1997). In contrast, lithium chloride induces hyper-dorsalization of embryos, resulting in a large head region (Kao & Elinson 1988, 1989) and activates the expression of anterior marker genes (Casarosa *et al.* 1997) including organizer genes (Cho *et al.* 1991; Laurent *et al.* 1997). Our data indicate that the expression of *Xenopus COL1A1* is concentrated in the posterior region, and that its expression was increased by retinoic acid and decreased by lithium chloride. These results suggest that *Xenopus COL1A1* behaves as a posterior marker gene.

Regulation of COL1A1 *expression*

Several transcription factors, such as CBF (Maity *et al.* 1988), IF1 and IF2 (Karsenty & de Crombrugghe 1990), NF-1, Sp1 (Nehls *et al.* 1991, 1992; Li *et al.* 1995a; Artlett *et al.* 1998; Chen *et al.* 1998), SP-3 (Chen *et al.* 1998) and c-krox (Galera *et al.* 1994, 1996), have been shown to bind to the CCAAT motif or GC-rich sites in the promoter region of *COL1A1* in mouse. These factors, except for IF1 and IF2, are transcription activators of *COL1A1*. In contrast to murine c-krox, a POZ/zinc finger transcription factor, human c-krox (hc-krox) represses the expression of *COL1A1* in cultured fibroblast cells (Widom *et al.* 1997). We have recently isolated a novel *Xenopus* POZ/zinc finger transcription factor, *champignon* (*cpg*) (T. Goto *et al.* unpubl. data, 2000). This gene is a member of the c-krox family genes and also represses the expression of *COL1A1* if it is overexpressed in early development. The localization of cpg transcripts in the anterior region, where *COL1A1* expression is very low, suggests that *cpg* is in fact functioning as a transcriptional repressor of *COL1A1* during the late neurula and tail-bud stage. Although *cpg* is the only gene shown to regulate the transcription of *COL1A1* in *Xenopus* to date, many other transcription factors may be involved in the regulation of *COL1A1* transcription, as suggested in mouse.

In cultured human osteosarcoma cells, retinoic acid decreases the levels of *COL1A1* (Mahonen *et al.* 1998). In cultured fibroblast cells, retinoic acid increases the expression of *hc-krox* (Widom *et al.* 1997). These reports suggest that retinoic acid reduces *COL1A1* transcription by elevating the level of hc-krox expression. However, in murine P19 embryonal carcinoma cells, retinoic acid increases the expression of *COL1A1* (Rhodes *et al.* 1996). In the present study, we have demonstrated that the expression of *COL1A1* was also increased by retinoic acid in early development of *Xenopus*. At present we do not know whether the elevation of *COL1A1* expression by retinoic acid is mediated by the reduction of *cpg* levels or not. We now need to examine in detail the regulatory mechanism for *COL1A1* expression in relation to the various transcription factors and retinoic acid.

References

Andreazzoli, M., Pannese, M. & Boncinelli, E. 1997. Activating and repressing signals in head development: The role of Xotx1 and Xotx2. *Development* **124**, 1733–1743.

Artlett, C. M., Chen, S. J., Varga, J. & Jimenez, S. A. 1998. Modulation of basal expression of the human alpha1 (I) procollagen gene (COL1A1) by tandem NF-1/Sp1 promoter elements in normal human dermal fibroblasts. *Matrix Biol.* **17**, 425–434.

Asahina, K., Obara, M. & Yoshizato, K. 1999a. Expression of genes of type I and type II collagen in the formation and development of the blastema of regenerating newt limb. *Dev. Dyn.* **216**, 59–71.

Asahina, K., Utoh, R., Obara, M. & Yoshizato, K. 1999b. Cell-type specific and thyroid hormone-dependent expression of genes of alpha1 (I) and alpha2 (I) collagen in intestine during amphibian metamorphosis. *Matrix Biol.* **18**, 89–103.

Bieker, J. J. & Yazdani-Buicky, M. 1992. Distribution of type II collagen mRNA in *Xenopus* embryos visualized by whole-mount *in situ* hybridization. *J. Histochem. Cytochem.* **40**, 1117–1120.

Bornstein, P. & Sage, H. 1980. Structurally distinct collagen types. *Annu. Rev. Biochem.* **49**, 957–1003.

Casarosa, S., Andreazzoli, M., Simeone, A. & Barsacchi, G. 1997. Xrx1, a novel *Xenopus* homeobox gene expressed during eye and pineal gland development. *Mech. Dev.* **61**, 187–198.

Chen, S. J., Artlett, C. M., Jimenez, S. A. & Varga, J. 1998. Modulation of human alpha1 (I) procollagen gene activity by interaction with Sp1 and Sp3 transcription factors *in vitro*. *Gene* **215**, 101–110.

Cho, K. W., Blumberg, B., Steinbeisser, H. & De Robertis, E. M. 1991. Molecular nature of Spemann's organizer: The role of the *Xenopus* homeobox gene goosecoid. *Cell* **67**, 1111–1120.

Cho, K. W. & De Robertis, E. M. 1990. Differential activation of *Xenopus* homeobox genes by mesoderm-inducing growth factors and retinoic acid. *Genes Dev.* **4**, 1910–1916.

Durston, A. J., Timmermans, J. P., Hage, W. J. *et al.* 1989. Retinoic acid causes an anteroposterior transformation in the developing central nervous system. *Nature* **340**, 140–144.

Galera, P., Musso, M., Ducy, P. & Karsenty, G. 1994. c-Krox, a transcriptional regulator of type I collagen gene expression, is preferentially expressed in skin. *Proc. Natl Acad. Sci. USA* **91**, 9372–9376.

Galera, P., Park, R. W., Ducy, P., Mattei, M. G. & Karsenty, G. 1996. c-Krox binds to several sites in the promoter of both mouse type I collagen genes: Structure/function study and developmental expression analysis. *J. Biol. Chem.* **271**, 21 331–21 339.

Gatalica, B., Pulkkinen, L., Li, K. *et al.* 1997. Cloning of the human type XVII collagen gene (COL17A1), and detection of novel mutations in generalized atrophic benign epidermolysis bullosa. *Am. J. Hum. Genet.* **60**, 352–365.

Hagg, P. M., Muona, A., Lietard, J., Kivirikko, S., Pihlajaniemi, T. 1998. Complete exon—intron organization of the human gene for the alpha1 chain of type XV collagen (COL15A1) and comparison with the homologous COL18A1 gene. *J. Biol. Chem.* **273**, 17 824–17 831.

Harland, R. 1991. *In situ* hybridization: An improved method for *Xenopus* embryos. In *Methods in Cell Biology*, Vol. 36 (Eds B. K. Kay & H. B. Peng), pp. 685–695. Academic Press, San Diego.

Imhof, M. & Trueb, B. 1999. Comparative cytogenetic mapping of COL14A1, the gene for human and mouse collagen XIV. *Cytogenet. Cell Genet.* **84**, 217–219.

Kao, K. R. & Elinson, R. P. 1988. The entire mesodermal mantle behaves as Spemann's organizer in dorso-anterior enhanced *Xenopus laevis* embryos. *Dev. Biol.* **127**, 64–77.

Kao, K. R. & Elinson, R. P. 1989. Dorsalization of mesoderm induction by lithium. *Dev. Biol.* **132**, 81–90.

Karsenty, G. & de Crombrugghe, B. 1990. Two different negative and one positive regulatory factors interact with a short promoter segment of the alpha 1 (I) collagen gene. *J. Biol. Chem.* **265**, 9934–9942.

Khaleduzzaman, M., Sumiyoshi, H., Ueki, Y., Inoguchi, K., Ninomiya, Y. & Yoshioka, H. 1997. Structure of the human type XIX collagen (COL19A1) gene, which suggests it has arisen from an ancestor gene of the FACIT family. *Genomics* **45**, 304–312.

Laurent, M. N., Blitz, I. L., Hashimoto, C., Rothbacher, U. & Cho, K. W. 1997. The *Xenopus* homeobox gene twin mediates Wnt induction of goosecoid in establishment of Spemann's organizer. *Development* **124**, 4905–4916.

Li, L., Artlett, C. M., Jimenez, S. A., Hall, D. J. & Varga, J. 1995a. Positive regulation of human alpha 1 (I) collagen promoter activity by transcription factor Sp1. *Gene* **164**, 229–234.

Li, S. W., Khillan, J. & Prockop, D. J. 1995b. The complete cDNA coding sequence for the mouse pro alpha 1 (I) chain of type I procollagen. *Matrix Biol.* **14**, 593–595.

Mahonen, A., Jukkola, A., Risteli, L., Risteli, J. & Maenpaa, P. H. 1998. Type I procollagen synthesis is regulated by steroids and related hormones in human osteosarcoma cells. *J. Cell Biochem.* **68**, 151–163.

Maity, S. N., Golumbek, P. T., Karsenty, G. & de Crombrugghe, B. 1988. Selective activation of transcription by a novel CCAAT binding factor. *Science* **241**, 582–585.

Muragaki, Y., Jacenko, O., Apte, S., Mattei, M. G., Ninomiya, Y. & Olsen, B. R. 1991. The alpha 2 (VIII) collagen gene: A novel member of the short chain collagen family located on the human chromosome 1. *J. Biol. Chem.* **266**, 7721–7727.

Nehls, M. C., Grapilon, M. L. & Brenner, D. A. 1992. NF-I/Sp1 switch elements regulate collagen alpha 1 (I) gene expression. *DNA Cell Biol.* **11**, 443–452.

Nehls, M. C., Rippe, R. A., Veloz, L. & Brenner, D. A. 1991. Transcription factors nuclear factor I and Sp1 interact with the murine collagen alpha 1 (I) promoter. *Mol. Cell. Biol.* **11**, 4065–4073.

Nieuwkoop, P. D. & Faber, J. 1967. *Normal table of* Xenopus laevis. North Holland Publishing, Amsterdam.

Oh, S. P., Warman, M. L., Seldin, M. F. *et al.* 1994. Cloning of cDNA and genomic DNA encoding human type XVIII collagen and localization of the alpha 1 (XVIII) collagen gene to mouse chromosome 10 and human chromosome 21. *Genomics* **19**, 494–499.

Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T. & Arai, K. 1987. High-efficiency cloning of full-length cDNA; construction and screening of cDNA expression libraries for mammalian cells. *Methods Enzymol.* **154**, 3–28.

Otte, A. P., Roy, D., Siemerink, M. *et al.* 1990. Characterization of a maternal type VI collagen in *Xenopus* embryos suggests a role for collagen in gastrulation. *J. Cell Biol.* **111**, 271–278.

Pannese, M., Polo, C., Andreazzoli, M. *et al.* 1995. The *Xenopus* homologue of Otx2 is a maternal homeobox gene that demarcates and specifies anterior body regions. *Development* **121**, 707–720.

Rhodes, K., Hall, K., Lee, K. E., Razzaghi, H. & Breindl, M. 1996. Correct cell- and differentiation-specific expression of a murine alpha 1 (I) collagen minigene *in vitro* differentiating embryonal carcinoma cells. *Gene Expr.* **6**, 35–44.

Seufert, D. W., Hanken, J. & Klymkowsky, M. W. 1994. Type II collagen distribution during cranial development in *Xenopus laevis*. *Anat. Embryol.* **189**, 81–89.

Sive, H. L., Draper, B. W., Harland, R. M. & Weintraub, H. 1990. Identification of a retinoic acid-sensitive period during primary axis formation in *Xenopus laevis*. *Genes Dev.* **4**, 932–942.

Su, M. W., Suzuki, H. R., Bieker, J. J., Solursh, M. & Ramirez, F. 1991. Expression of two non-allelic type II procollagen genes during *Xenopus laevis* embryogenesis is characterized by stage-specific production of alternatively spliced transcripts. *J. Cell Biol.* **115**, 565–575.

Vuorio, E. & de Crombrugghe, B. 1990. The family of collagen genes. *Annu. Rev. Biochem.* **59**, 837–872.

Widom, R. L., Culic, I., Lee, J. Y. & Korn, J. H. 1997. Cloning and characterization of hcKrox, a transcriptional regulator of extracellular matrix gene expression. *Gene* **198**, 407–420.

Yamaguchi, N., Kimura, S., McBride, O. W. *et al*. 1992. Molecular cloning and partial characterization of a novel collagen chain, alpha 1 (XVI), consisting of repetitive collagenous domains and cysteine-containing non-collagenous segments. *J. Biochem.* **112**, 856–863.

Zhang, X., Zhou, J., Reeders, S. T. & Tryggvason, K. 1996. Structure of the human type IV collagen COL4A6 gene, which is mutated in Alport syndrome-associated leiomyomatosis. *Genomics* **33**, 473–479.