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Expression of a mRNA related to c-*rel* and dorsal in early *Xenopus laevis* embryos

(amphibia/development/oncogene)

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ABSTRACT **We describe a *Xenopus* mRNA, Xrel1, that is related to the avian protooncogene c-*rel*, the embryonic pattern gene dorsal of *Drosophila*, and the mammalian transcription factor NK-κB/KBF1. The sequence of Xrel1 is homologous to the other rel-related proteins in the large amino-terminal region that defines this class of transcriptional regulators, but the carboxyl-terminal part of the protein is quite different. Xrel1 mRNA is present throughout oogenesis and during early embryogenesis at 4 × 10^5 transcripts per oocyte or embryo. Xrel1 transcripts are present in all of the dissected parts of early embryos that we have examined. They are enriched in the animal hemisphere compared to the vegetal hemisphere of oocytes and blastulae.**

The rel family of proteins is a group of transcriptional regulators involved in cell differentiation and development (for review, see ref. 1). The founding member of the family v-*rel* (2–4) is the oncogene of reticuloendotheliosis virus strain T, which causes rapid and fatal leukemia in juvenile birds (5). The cellular counterparts of v-*rel* have been cloned in birds (4, 6) and in mammals (7, 8). The other members of the rel family are the ubiquitous mammalian transcriptional activator NF-κB/KBF1 (9–11) and dorsal, which is believed to be the morphogen whose distribution determines the state of differentiation of cells along the dorso-ventral axis of the *Drosophila* embryo (ref. 12; for review, see ref. 13).

All members of the rel family are very similar in an amino-terminal region of about 300 amino acids. The remaining carboxyl-terminal parts vary in size and their sequences are completely divergent. An unusual feature of the rel family is that the activity of its members appears to be regulated post-translationally: the activity of rel-related proteins is correlated with their nuclear localization. Two well-illustrated examples are the translocation of NF-κB protein to the nuclei of B lymphocytes during B-cell differentiation (for review, see ref. 14), and the pattern of nuclear localization of the dorsal protein along the dorsal-ventral axis of *Drosophila* embryos. In quiescent B cells, inactive NF-κB protein is retained in the cytoplasm by the inhibitor IκB. When B cells are induced to differentiate, IκB dissociates, allowing NF-κB to travel to the nucleus and bind to the κ light chain enhancer (15). During *Drosophila* embryogenesis, dorsal mRNA and protein are initially present uniformly in the egg, but soon after fertilization the protein localizes only to the nuclei of the ventral cells, where it is required for development of the mesoderm and other ventral tissues (16–18).

The widespread presence of rel proteins and their apparent roles as tissue-specific regulators of gene expression in cultured cells and in *Drosophila* embryos suggest that they may have important functions in vertebrate embryogenesis. This paper reports the sequence FN* and expression of a *rel*-related gene, Xrel1, in embryos of the frog *Xenopus laevis*. Xrel1 encodes a protein that shares the same conserved amino-terminal domain as all of the rel family of proteins but has a different carboxyl-terminal region. Xrel1 mRNA is present in oocytes and early embryos and in all tissues of the early embryo.

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MATERIALS AND METHODS

Library Screening. A *Xenopus* oocyte cDNA library in λgt10 (19) was screened at low stringency using a random-primed probe (20) made from the *Xba* I fragment of clone pTG7, which contains the complete v-*rel* gene (4). Hybridization and washing of filters were done as described (21).

Characterization of Clones. DNA stocks were maintained in pBS and pBluescript II vectors (Stratagene). The rl17 cDNA (see *Results*) was subcloned into M13 vectors and sequenced using the dideoxynucleotide method (22, 23) on overlapping templates generated by digestion with exonuclease III (24). Gaps in the sequence were filled using oligonucleotide primers, and the sequence was compiled using the DB programs (25). Each nucleotide was sequenced at least twice on each strand.

Oocytes and Embryos. Staged oocytes (26) and embryos (27) were obtained by standard means (28, 29). Embryos were reared at 23°C in 1/10 MBS (29). Dissections were carried out with fine forceps in MBS or, for neurulae, in MBS containing collagenase (Sigma C-2139; ≈1 mg/ml).

Analysis of RNA. RNA from embryos or dissected tissues was prepared as described (30). Northern blots were made as described (21) and washed to 0.1× SSPE/0.1% SDS at 65°C (1× SSPE = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA). Probes were made by random priming of the 3′ *Hin*dII–*Eco*RI fragment of rl17 (nucleotides 1324–1998, see Fig. 1), representing the 3′ untranslated region and coding sequences outside the region of homology to *rel*.

RNase protection analysis was performed (31) using RNase T1 (4 ng/ml) and RNase A (2 ng/ml). Xrel1 probes were made from a *Hin*dII–*Pst* I fragment of rl17 cloned in pBS, using T3 RNA polymerase after linearization with *Eco*RI. This fragment contains 231 base pairs of coding sequence outside the region of homology to *rel* (nucleotides 1321–1558, see Fig. 1*A*). Probes for 5S rRNA representing 120 base pairs of somatic-type 5S DNA were made as described (32). A fraction of each of the RNA samples was diluted 1:500 for analysis with the 5S probe to show the relative amounts of total RNA extracted. The number of Xrel1 transcripts in oocytes and embryos was determined by Northern blot analysis as described (21) and confirmed by RNase protection. Quantitative comparisons of the relative amounts of Xrel1 transcripts were carried out using densitometry of appropriately exposed autoradiographs.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M60785).

RESULTS

A *Xenopus* cDNA Related to *rel*. A *Xenopus* oocyte cDNA library (19) was screened at low stringency with a v-*rel* probe (4). Forty phage were purified from a large number of hybridizing plaques. The clones were sorted into two groups based on their ability to cross-hybridize at high stringency on Southern blots (data not shown). rl17, the longest member of one of the groups, was selected for further analysis.

The rl17 cDNA was 2.0 kilobases (Fig. 1*A*), similar to the length of the transcripts to which it hybridized on Northern blots (see below). At position 1961 there was a polyadenylylation signal, which was followed by a poly(A) tail 14 nucleotides downstream. Conceptual translation of the rl17 sequence in all reading frames revealed a single long open reading frame that would encode a protein of ≈58 kDa.

This reading frame was preceded upstream by two in-frame stop codons (at nucleotides 103 and 169) and also by an out-of-frame ATG codon (nucleotide 20). When synthetic RNA made from rl17 was translated *in vitro*, a product of about 69 kDa was detected by SDS/PAGE (data not shown).

The predicted amino acid sequence of rl17 was compared to those of c-rel from birds and mammals (4–8), mammalian NF-κB/KBF1 (9–11), and *Drosophila* dorsal (12) (Fig. 1*B*). There was a high degree of similarity in the amino-terminal parts of all of these proteins, but the carboxyl-terminal regions were quite different. Within the amino-terminal region of similarity (residues 18–305 of Xrel1 in Fig. 1*B*), the rl17 protein was 66–72% identical to the avian and mammalian c-rel proteins, 55% identical to dorsal, but only 36% identical to NF-κB/KBF1 (Table 1). No similarity was found when the Xrel1 carboxyl-terminal region was compared at the protein and nucleic acid levels to sequences in several data bases. The rl17 protein is thus most closely related to c-rel and dorsal. Insufficient information is available to decide whether or not it is a *Xenopus* homologue of c-rel or dorsal. Because there may be other *rel*-related genes in *Xenopus*, we name this gene Xrel1.

Xrel1 mRNA Was Present in Oocytes and Embryos. RNA was extracted from staged oocytes and embryos and analyzed by Northern blotting. Random-primed probes detected transcripts of a single size (about 2.1 kilobases) on blots of all stages of oocyte and embryo RNA when hybridized at high stringency (Fig. 2). Total RNA was visualized on the RNA gels stained with ethidium bromide before blotting, to ensure that the same amount of total RNA had been loaded per embryonic stage (Fig. 2*B*).

Xrel1 transcripts were in oocytes of all stages (Fig. 2*A*). The number of transcripts changed through oogenesis, in contrast to 18S rRNA, which accumulated steadily with oocyte growth (Fig. 2*A*). The transcript number was minimal in stage I oocytes, rising to a peak at around stage IV. The number of transcripts at stage VI was 5×10^5 per oocyte. The concentration of Xrel1 mRNA during oogenesis was similar to that of the mRNAs encoding the protooncogenes c-*fos* (33) and *ras* (34), but about 10 times less than the unusually high level of c-myc mRNA (35).

Xrel1 transcripts were also present in embryos of all developmental stages examined (Fig. 2*B*). The number of transcripts gradually decreased from fertilization to the end of neurulation (stage 20), after which there was a marked increase in the tailbud embryo. The average number of transcripts per embryo from fertilization to neurulation was 4×10^5. These observations indicate that Xrel1 mRNA is present as a maternal mRNA in eggs and is maintained during embryogenesis.

A

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GGCCAGGGATCGTCTCTCAATGTCTGTAAATGCGAAGAAGGGGGCGGATATCTATTAGGGAAGGAAGAAGAAGAGGGAGAGCGAAACCGT    90
GAAGTGGAGAAGTAAAAGAGTATCGGTGGCGAAAATATTGTATACATTTCTGAAACTGCAAGGGGAGCTAGCCCCTGTTAAAGGCCAGCG   180
      M  D  G  F  H  W  T  D  I  V  S  S  M  P  P  S  I  P  P  V  E  I  I  E  Q  P  K  Q      28
TTGAACATGGATGGATTCCATTGGACAGACATAGTTTCAAGCATGCCTCCCTCCATCCCACCTGTGGAAATTATAGAGCAGCCAAAGCAG   270
 R  G  M  R  F  R  Y  K  C  E  G  R  S  A  G  S  I  P  G  E  R  S  T  D  T  S  K  T  H  P      58
CGAGGGATGCGATTTCGTTACAAGTGTGAAGGGCGCTCTGCTGGAAGCATTCCAGGTGAACGAAGCACGGACACATCAAAAACTCACCCC   360
 T  I  K  I  N  N  Y  Q  G  P  A  R  I  R  I  S  L  V  T  K  D  S  P  H  K  P  H  P  H  E      88
ACTATTAAGATCAACAATTACCAGGGCCCAGCTCGTATTCGGATATCTCTAGTTACAAAGGATTCTCCACATAAACCTCACCCTCATGAG   450
 L  V  G  K  D  C  K  D  G  Y  Y  E  A  E  L  S  P  D  R  S  I  H  S  F  Q  N  L  G  I  Q      118
TTGGTTGGAAAGGACTGCAAGGATGGATATTATGAAGCTGAACTTTCCCCAGATCGGAGTATCCACAGCTTCCAGAACCTGGGCATCCAG   540
 C  V  K  K  R  E  V  E  D  A  V  A  H  R  I  R  T  N  N  N  P  F  N  V  S  P  E  E  L  K      148
TGTGTGAAAAAGAGAGAGGTGGAAGATGCCGTCGCCCATCGCATTCGGACAAATAATAACCCCTTTAATGTGTCTCCTGAGGAGCTGAAG   630
 A  D  Y  D  L  N  T  V  C  L  C  F  Q  V  F  I  P  D  Q  A  A  G  R  M  L  P  L  P  F  V      178
GCCGATTACGACCTCAACACGGTTTGCCTGTGCTTCCAGGTTTTCATTCCTGATCAGGCTGCTGGTCGCATGCTTCCATTACCATTTGTG   720
 V  S  Q  P  I  Y  D  N  R  A  P  N  T  A  E  L  K  I  C  R  V  N  K  N  S  G  S  C  L  G      208
GTATCCCAGCCCATCTATGACAACAGAGCCCCTAATACTGCCGAACTGAAGATATGCAGAGTGAACAAAAATTCCGGGAGCTGCTTGGGA   810
 G  D  E  I  F  L  L  C  D  K  V  Q  K  E  D  I  E  V  I  F  G  L  G  N  W  E  A  R  G  I      238
GGAGATGAAATTTTCTTACTTTGTGATAAAGTACAAAAAGAAGATATCGAGGTGATATTCGGTTTGGGCAACTGGGAAGCTCGTGGGATA   900
 F  S  Q  A  D  V  H  R  Q  V  A  I  V  F  R  T  P  A  F  Q  D  T  K  I  R  Q  S  V  K  V      268
TTTTCCCAAGCAGATGTTCACCGCCAGGTGGCTATTGTCTTCCGAACCCCTGCTTTTCAAGACACAAAAATACGTCAGTCTGTCAAAGTG   990
 Q  M  Q  L  R  R  P  S  D  K  E  V  S  E  P  M  E  F  Q  Y  L  P  D  E  G  D  P  H  H  I      298
CAAATGCAGCTGAGGCGGCCATCAGACAAAGAAGTCAGTGAGCCTATGGAGTTTCAGTATCTTCCAGATGAAGGAGACCCCCACCATATT  1080
 D  E  K  R  K  R  T  L  D  N  F  K  H  Y  V  K  N  N  P  F  A  G  G  E  T  R  P  Q  R  R      328
GATGAAAAACGAAAAAGGACCCTTGACAATTTCAAGCATTATGTGAAAAATAATCCTTTTGCAGGAGGAGAAACGCGACCCCAACGCAGA  1170
 I  A  V  A  N  R  N  V  P  T  K  S  E  P  I  R  P  S  I  P  V  P  N  P  V  V  S  C  L  P      358
ATTGCTGTGGCAAATCGTAATGTGCCAACAAAGTCAGAGCCTATAAGACCCAGCATACCCGTTCCAAATCCAGTGGTATCCTGTCTTCCA  1260
 F  S  M  P  V  L  K  A  E  N  V  T  S  P  S  T  L  L  S  T  V  N  I  S  D  F  S  N  L  G      388
TTTTCCATGCCCGTTTTAAAGGCTGAGAATGTGACTTCACCTTCGACTCTTCTGTCCACCGTCAACATCAGCGACTTCAGCAATTTAGGA  1350
 F  S  S  Q  P  P  S  Q  S  D  H  D  R  L  E  S  M  L  N  Y  P  S  F  P  G  D  A  N  L  D      418
TTTTCATCACAGCCTCCCTCTCAGTCTGATCACGACCGCTTAGAATCCATGTTAAACTACCCCTCATTCCCAGGCGATGCAAATCTGGAT  1440
 L  V  E  M  L  P  H  E  N  E  S  R  C  T  S  L  S  S  I  D  N  S  D  F  S  Q  L  L  S  E      448
TTAGTAGAAATGTTGCCACATGAGAACGAAAGCCGATGTACCAGTTTGAGTTCTATTGACAATAGTGATTTCAGCCAACTTCTCAGCGAG  1530
 S  Q  S  S  G  T  L  S  A  A  L  Q  E  P  G  T  S  Q  G  T  F  M  A  Y  P  E  S  I  A  R      478
TCACAATCATCTGGGACACTCTCTGCAGCTTTACAAGAGCCTGGGACTAGTCAAGGCACGTTCATGGCTTATCCTGAATCCATTGCTCGA  1620
 L  M  T  N  R  P  N  E  D  E  G  G  E  R  I  D  S  G  L  I  N  G  M  F  D  M  Q  Q  G  G      508
CTTATGACCAACCGGCCCAATGAGGATGAGGGTGGAGAGAGAATTGACAGTGGCCTGATTAATGGGATGTTTGATATGCAGCAGGGAGGA  1710
 D  S  L  T  S  L  F  E  L  D  F  S  S  L  L  S  N  M  K  *                                  527
GATTCATTAACCTCCTTATTTGAGCTTGACTTTTCTTCTTTATTAAGTAACATGAAATAAATTGCTTTATTAATATGAGCAATAAGACTA  1800
AGCATGAGACGTGCCTAAATTGCTGTGGTTTCTGGATGTTTGTTCAAATGCAGTAGCCAGAAATGTGTGCATGATCACTTCTTCAACCTC  1890
TTAAGACTCCTGTAGATACTTTTATCAAGATATTTTTAAAGTTATATTTCACCTCTGCTACTATCATGCAGAATAAAATAAGCATGGTCT  1980
TTTA15
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FIG. 1. (*Figure continues on the opposite page.*)

FIG. 1. (*A*) cDNA sequence of Xrel1. The poly(A) addition signal is boxed and the stop codon is denoted by an asterisk. The region of homology to the other members of the rel family is underlined. (*B*) Comparison of the predicted protein sequence of Xrel1 with those of other members of the rel family. The Xrel1 sequence was aligned with those of rel-related sequences from turkey (c-rel; ref. 4), mouse (mc-rel; ref. 7), human (hc-rel; ref. 8), *Drosophila* dorsal (12), and NF-κB (9), in a region spanning the amino-terminal domain of conservation (amino acids 18–305 of Xrel1). Identical and conservatively substituted amino acid residues are shown in boldface type.

Distribution of Xrel1 mRNA in Eggs and Early Embryos. The relatively low level of Xrel1 transcripts suggests that *in situ* hybridization would be impracticable. A major advantage of using *Xenopus* embryos, however, is that they are large enough to dissect in fine detail. Therefore, to determine the spatial distribution of Xrel1 transcripts, RNA was extracted from dissected pieces of eggs, blastulae, and neurulae and analyzed by RNase protection.

Table 1. Comparison of the rel proteins in the amino-terminal region of homology

	% sequence identity					
	Xrel1	hc-rel	c-rel	mc-rel	dorsal	NF-κB
Xrel1	—	72 (79)	69 (75)	66 (74)	55 (60)	36 (45)
hc-rel	72 (79)	—	78 (88)	80 (86)	48 (60)	39 (49)
c-rel	69 (75)	78 (88)	—	89 (92)	47 (59)	44 (52)
mc-rel	66 (74)	80 (86)	89 (92)	—	47 (58)	42 (50)
dorsal	55 (60)	48 (60)	47 (59)	47 (58)	—	45 (52)
NF-κB	36 (45)	39 (49)	44 (52)	42 (50)	45 (52)	—

Data are presented as percent sequence identity (conservatively substituted amino acid residues in parentheses) over the region spanning amino acid residues 18–305 of Xrel1, based on the alignment shown in Fig. 1*B*. Refer to Fig. 1*B* for the sources of the sequence data.

Frozen unfertilized eggs were fractured along the equator and tested for the presence of Xrel1 mRNA (Fig. 3A). Xrel1 transcripts were in both animal and vegetal hemispheres, and when compared using the level of 5S transcripts as a reference, there was about a 2-fold enrichment of Xrel1 mRNA in the animal hemisphere.

Stage 8 midblastulae were dissected to find the transcript distribution in the tissues that form the ectoderm, mesoderm, and endoderm (Fig. 3*B*). Xrel1 transcripts were present in the animal caps (prospective ectoderm), the equatorial region (mainly prospective mesoderm), and the vegetal piece (prospective endoderm). When compared relative to the amount of 5S rRNA, Xrel1 mRNA was 6–8 times more concentrated in animal tissue and twice as concentrated in the equatorial region as in vegetal pieces. Xrel1 transcripts were, therefore, present in all parts of the blastulae we examined but were enriched in the animal cells. This enrichment, however, was less marked than that of other mRNAs localized in the animal cells (19).

Embryos that had just formed a neural tube (stage 22) were also dissected to determine the distribution of Xrel1 transcripts after the formation of the embryonic axes (Fig. 3*C*). There was no preferential localization to head, middle, or posterior parts, indicating that there was no anterior–posterior difference in the presence of Xrel1 mRNA (Fig.

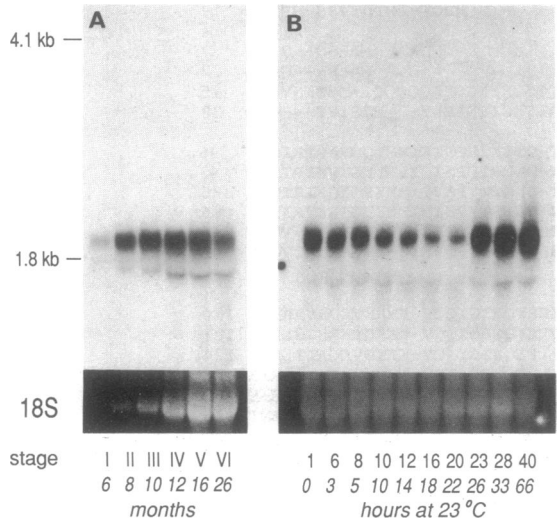

FIG. 2. Northern blot analyses of developmental expression of Xrel1. (*A*) Accumulation of Xrel1 transcripts during oogenesis. RNA from five oocytes was loaded per lane. Ethidium bromide staining of the 18S rRNA shows the accumulation of total RNA with increasing size of oocyte. The time in months is the approximate time oogonia take to reach each oocyte stage. kb, Kilobases. (*B*) Accumulation of Xrel1 transcripts during embryogenesis. RNA from five embryos was loaded per lane. Approximately equal amounts of total RNA were loaded as shown by ethidium bromide staining of 18S rRNA.

3*C*). The dorsal-to-ventral and germ-layer distribution of Xrel1 mRNA was determined by dissecting trunk tissue. Xrel1 transcripts were in all tissues tested, including notochord, neural tube, somites, dorsal and ventral endoderm, ventral mesoderm, and epidermis. We conclude, therefore, that Xrel1 transcripts are present in most, if not all tissues of late neurulae.

DISCUSSION

We have described a mRNA in *Xenopus* oocytes and embryos that encodes a member of the rel family. The predicted Xrel1 protein shares with the other members of the family the amino-terminal domain that defines this class of proteins, but as with the other rel-related proteins, the rest of the Xrel1 protein is quite different. Within the region of homology, Xrel1 is more similar to avian c-rel and what are considered its mammalian counterparts and dorsal than to the other members of this class, the mammalian NF-κB/KBF1. Xrel1 is therefore not a *Xenopus* homologue of NF-κB/KBF1 but may be frog c-rel. If so, then the carboxyl termini of all of the vertebrate c-rel proteins show an unusual degree of divergence.

Our reason for cloning Xrel1 was to find a gene related to *rel* with a role in early *Xenopus* development that is, perhaps, like that played by dorsal in *Drosophila*. One function that dorsal has in flies is to activate the genes twist and snail, which are required for the formation of ventral tissues, including the mesoderm (for review, see ref. 13). Xrel1, therefore, could activate the genes Xtwi (36) and Xsna (37),

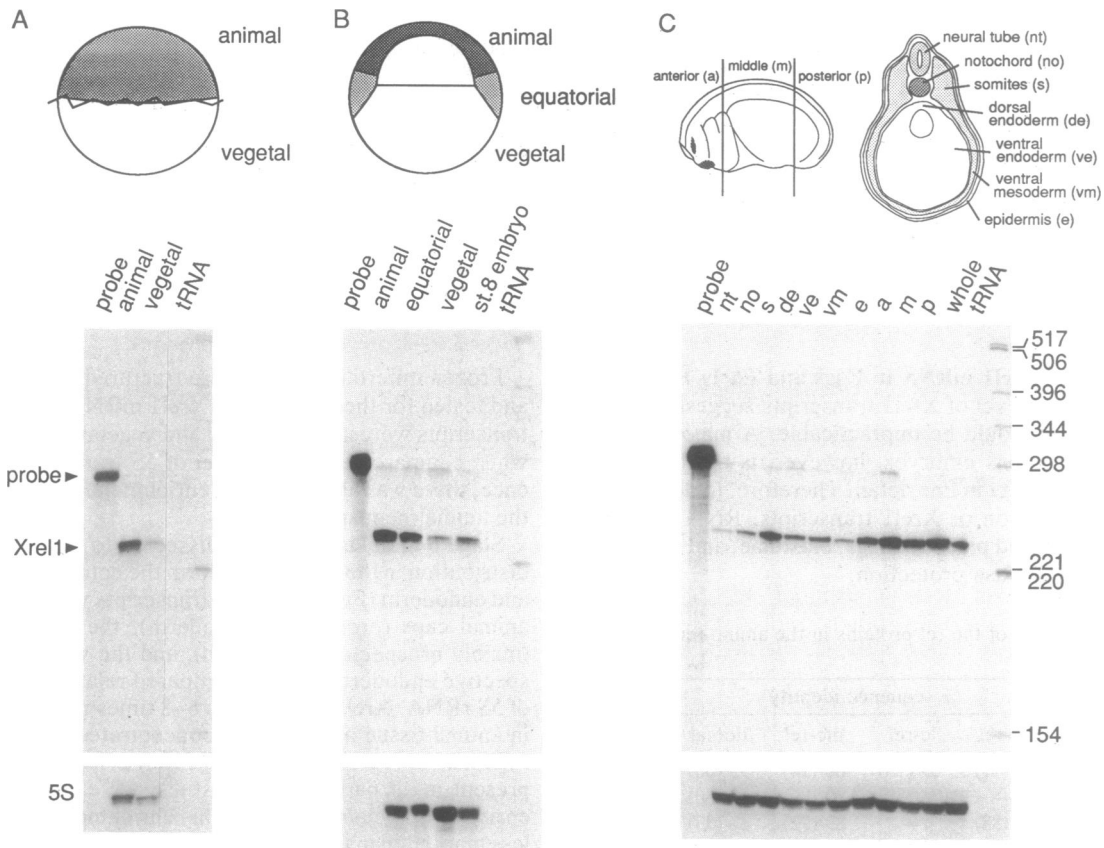

FIG. 3. Distribution of Xrel1 transcripts in early development. In *A*, 6 animal and 6 vegetal hemispheres of unfertilized eggs, in *B*, 15 animal, 10 equatorial, 25 vegetal pieces, and three whole embryos of mid-blastulae, and in *C*, 20–50 neurula trunk parts, six anterior, six middle, and six posterior parts, and three whole embryos of late neurulae were analyzed by RNase protection for Xrel1 and 5S rRNA transcripts. Any differences in the concentration of Xrel1 transcripts in the various fractions are not more than a fewfold. The neural tube, which, in the assay shown, appears to contain very little Xrel1 RNA, did not reproducibly have a lower concentration than in the other tissues. Yeast tRNA (5 μg) was used as a hybridization control (tRNA lane). The numbers on the right hand side of this figure are the sizes in bases of the end-labeled *Hin*fI fragments of pBR322 used as molecular size markers.

which are probably frog homologues of twist and snail. Xtwi is expressed specifically in the early mesoderm (but later also in the neural crest; ref. 36), which contrasts with the widespread accumulation of Xrel1 mRNA that we observe. Nonetheless, the activity of Xrel1 protein could be regulated by nuclear localization, like that of dorsal (16–18) and other rel-related proteins (1), and hence the pattern of accumulation of its mRNA does not exclude a role in the embryonic activation of Xtwi. The work we have reported makes possible the further study of a vertebrate member of the rel family in an embryo that is particularly suited to classical embryological analysis.

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