The Rel family of transcriptional activators forms a large diverse group of proteins that are involved in the activation of genes involved in immunity, development, apoptosis and cancer. So far, none of the rel genes cloned in mammals appear to be required for embryonic development. We have cloned and characterized a cDNA from an embryonic cDNA library that encodes a novel Xenopus Rel protein, called Xrel3. Xrel3 is a member of the cRel subfamily and is most closely related to but distinct from other Xenopus Rel members. The expression of Xrel3 mRNA was investigated using Northern analysis, RNase protection assay, reverse transcriptase-linked polymerase chain reaction and in situ hybridization. Messages are present maternally and are slightly enriched in the equatorial region of the blastula stage embryo. At gastrulation, the accumulation of Xrel3 messages declines to undetectable levels but then increases after neurulation. In situ RNA hybridization was used to determine the spatial location of Xrel3 messenger RNA in embryos. Messages are localized to the developing forebrain, dorsal mid-hindbrain region, the inner ear primordium, or otocyst, and in the notochord. Overexpression by microinjection of Xrel3 RNA induced tumors in the developing embryo that appeared after gastrulation. The location of the tumors depended on the location of the injection site. These results suggest that Xrel3 might have a generalized role in regulation of cell differentiation in the embryo.

The Rel family of transcriptional activators is a group of higher eukaryotic proteins that participate in cell differentiation (for reviews, see Refs. 1–7). The family includes the c-Rel oncoprotein, RelB, NF-κB (both p65 or RelA, and p50 subunits), and the DORSAL patterning morphogen of Drosophila. They are expressed in a wide variety of tissues and transactivate genes that are involved in the immune response, apoptosis, cancer, and development. Rel transcription complexes exist as homo- or heterodimers, but only specific dimer pairs have been demonstrated in vivo.

Although there are different levels of rel gene regulation in different cells, the activity of all of the proteins is determined by their subcellular localization. Thus, Rel proteins are active in the nucleus, but are retained in the cytoplasm in an inactive state. Depending on the cell type, Rel complexes activate transcription from target genes in either a constitutive or inducible manner. Inducible complexes are retained in the cytoplasm by a regulatory subunit (1–3). Upon induction, the regulatory subunit releases the Rel complex and allows it to translocate to the nucleus where it binds to target DNA sequences.

Rel proteins share motifs required for their similar functions. The amino terminus contains the highly conserved Rel Homology Domain (RHD) of about 300 amino acids, which is required for DNA binding, dimerization with other Rel proteins, and a nuclear localization signal. The remainder of the protein is variable and is necessary for transcriptional activation (8). In some cases, such as the p50 subunit of NF-κB, the activation domain is absent so that the protein is essentially an RHD.

The DORSAL protein of Drosophila is localized to the nuclei of ventral cells that form the embryonic body axis (9). Genetic and biochemical studies indicate that DORSAL activates body patterning genes such as twist and snail (6). In mice, Rel/NF-κB activity is detected at the fetal stage and in the developing brain (10). Mice deficient for c-rel and relB develop defects in their immune systems while NF-κB(p50)-defective mice die of liver failure before birth (for review, see Ref. 11). These fetuses develop apparently normal bodies suggesting that rel genes are not required for embryogenesis. It is possible, however, that redundant pathways exist to compensate for the loss of specific rel gene function. Furthermore, there are likely other undiscovered vertebrate embryonic patterning rel-related genes since homologues to twist and snail are expressed in axis-forming tissues of the postimplantation mouse embryo (12–13) and since human twist is associated with congenital defects of the mesoderm (14).

Xenopus homologues of twist (15) and snail (16) are expressed in tissues that form the body axis. None of the Xenopus genes, Xrel2 (17), XrelA (18–20), and XrelB (21), have significant spatiotemporal restriction of mRNA expression in embryos. The lack of an embryonic patterning phenotype in Rel-defective mice is paradoxical since both twist and snail homologues are expressed and/or required for correct body patterning in frogs, mice, and humans.

In this paper, we identify a new Xenopus rel family member, Xrel3, that is a version of c-rel, but unlike any other rel gene, messenger RNA encoding Xrel3 protein is expressed in two phases of early development. Xrel3 messenger RNA is present in embryos up to the late blastula stage followed by a dramatic decline to undetectable levels at gastrulation. Messages then accumulate in the forebrain and otic placode of neurula-stage embryos and in the dorsal part of the mid-hindbrain, the forebrain, the otocyst, and finally the notochord of tailbud and later stage embryos. This novel expression pattern indicates a new role for rel genes in vertebrate embryonic development.

Interestingly, overexpression of Xrel3 mRNA in the animal
hemisphere of 2-cell embryos results in the formation of tumors. The size of the tumors is proportional to the dosage of RNA injected, and their position on the embryo depends on the location of injection: epidermal tumors develop from animal pole injection (prospective ectoderm) while tumors in the endoderm (digestive system) develop from vegetal pole injection. Recent evidence indicates that Rel protein overexpression directly causes oncogenesis in mammalian cells. Our results support this observation and suggest that Xrel3 might function in the decision for cells to differentiate or proliferate in the embryo.

EXPERIMENTAL PROCEDURES

Library Screening—Hybridization and washing of filters at high stringency were performed as described previously (18).

Clone Characterization—cDNAs retrieved from library screening were subcloned into pBluescript SK+ (Stratagene) and sequenced on both strands at least twice by the dideoxynucleotide method as described (22) using Sequenase (Amersham Pharmacia Biotech) and oligonucleotide primers corresponding to overlapping regions of sequence.

Embryos—Wild-type and albino Xenopus embryos were obtained by in vitro fertilization as described (23). Embryos were raised at room temperature and dissected in normal amphibian medium (24).

Analysis of RNA—RNA from embryos was prepared as described (15). Northern blots and RNase protection assays were performed as described (19, 25). For RNase protection assays, an EcoRI-DraI fragment from the entire 5'-nontranslated region of d10 that protects a 210-base pair region in Xrel3 was subcloned into pBluescript. As a control, an XrelA probe was added to the hybridization mix, which protected a 229-base pair region (19).

Reverse transcriptase-linked polymerase chain reaction (RT-PCR) was performed as described with 32P-ATP trace labeling (24, 26) and gene-specific primers for Xrel3 (forward primer, 5'-TCCTGTAGATTTTGCTGCGG-3'; reverse primer, 5'-TTTAAAACCGCCAATGTTGATG-3'), Vg1 (Ref. 27; forward primer, 5'-GAGGATATCTACACCAAAAAGC-3'; reverse primer, 5'-CGTCTCTATCGACACATTAC-3'), and histone (Ref. 28). The cycling parameters were as follows: 94 °C for 5 min, followed by 26 cycles for Xrel3 and Vg1 (19 for histone) with the program: 30 min at 94 °C, 30 min at 62 °C, and 30 min at 72 °C. Reactions were then extended at 72 °C for 5 min.

Whole mount in situ hybridization with digoxigenin-UTP (Boehringer Mannheim) incorporated probes was carried out as described (29). In situ hybridization was detected by alkaline phosphatase-linked anti-digoxigenin antibodies using nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate as a chromogenic reagent.

Embryo Microinjection—Microinjection of embryos was done as described (24) using capped, synthetic Xrel3 mRNA made using the Ribomax kit (Promega). The Xrel3 coding domain was subcloned into the CS21 expression vector, linearized with EcoRI, and used as a template. Control RNA was made using the D10 cDNA subcloned into CS21 and linearized with NotI. D10 lacks a significant region (~30 amino acids) of the carboxyl terminus which represents the transcriptional activation domain.

RESULTS

Identification and Sequence of a Novel Rel mRNA—We searched for rel genes in Xenopus by screening a neurula stage λ cDNA library (a gift from Dr. H. Sive, Massachusetts Institute of Technology) with XrelA cDNA sequences at high stringency. Several of the largest cross-hybridizing clones were used as templates for RNA probes for whole mount in situ hybridization on neurula stage embryos. One of the probes, clone d10, was subcloned into the CS21 expression vector, linearized with EcoRI, and used as a template. Control RNA was made using the D10 cDNA subcloned into CS21 and linearized with NotI. D10 lacks a significant region (~30 amino acids) of the carboxyl terminus which represents the transcriptional activation domain.

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The translation codon does not appear to be within the context of a typical Kozak consensus, but it predicts a protein size in line with in vitro synthesized protein made from this clone (not shown), putative translation of upstream sequences reveals multiple stops in all three reading frames, and it is in the same position as that of other related Rel family members.
The sequence of Xrel3 is most closely related to Xrel2 at 64% identical amino acids (Fig. 2A). Within the RHD, the two proteins share 81% identity. Throughout their respective coding regions, there are significant divergent stretches of coding sequence (Fig. 2A). The acid-rich region in the carboxyl terminus of Xrel3 contains a high level of proline and serine residues, consistent with the proposed role of this region as a transactivation domain (8). At the nucleotide level, the sequences between Xrel2 and Xrel3 are completely different outside their respective coding regions.

Temporal Patterning of Expression of Xrel3—To determine the temporal pattern of Xrel3 message accumulation during early embryonic development, we probed Northern blots of total RNA extracted from embryos at various stages. Probes made from d10 cDNA hybridized to a message of about 3 kilobase pairs in length. The signal was present in RNA extracted from pregastrula embryos but declined rapidly at gastrulation (Fig. 3). Because embryonic transcription is considered to begin at the 12th cleavage division (about stage 8, Ref. 33), we expect that the early accumulation of Xrel3 mRNA must be maternal in origin. Limited hybridization was observed in later stages, but the resolution was poor on our Northern analyses, perhaps due to increasing genomic DNA levels in our preparations that caused the signal to smear.

The more sensitive RNase protection assay using an RNA probe made from a unique 5'-untranslated sequence of Xrel3 confirmed its later expression. The probe protected a single fragment of the expected 229 nucleotide size upon digestion of hybrids with RNase A and RNase T1 (Fig. 4). These assays confirmed the presence of message in pregastrula embryos. The message level drops significantly during gastrulation (stages 10 and 11) to nil but then increases once again during late neurula through to the early tadpole stage (stage 33). Probes made against XrelA (19), whose RNA is present throughout development, were added to the hybridization mix to determine the levels of Xrel3 mRNA relative to this known message. Comparison of the mRNA levels between these two genes indicates that the maximal level of Xrel3 mRNA is about one-third that of XrelA mRNA.

Spatial Patterning of Xrel3 Transcript Accumulation—In previous studies with XrelA (19) and Xrel2 (17), in situ hybridization of mRNA was not performed because dissected embryo pieces showed roughly equivalent distribution throughout the embryo. Our original strategy for obtaining Xrel3 was based on screening according to spatial restriction of mRNA expression. In this sense, Xrel3 represents a distinctly different gene from other Xenopus rel genes.

The whole mount analysis was extended to a range of stages to determine more finely the progression of mRNA localization during development. Early cleavage embryos show digoxigenin counterstaining...
staining in all cells (Fig. 5A). As expected, late gastrula stage embryos do not stain for digoxigenin, confirming the low level or absence of message at these stages (Fig. 5B). Postgastrula embryos earlier than stage 23 did not stain using our probes even though RNase protection assays indicated presence of message, albeit at a low level prior to this stage. This may be due to differences in detection sensitivity between the two methods. Also, RNA equivalent to five embryos was used in the protection assays, greatly increasing the potential number of transcripts in the assay compared with in situ analysis.

At about stage 23, message accumulation appears in the forebrain and in a small region adjacent to the mid-brain/hindbrain junction (Fig. 5C). We believe this latter staining to correspond to the prospective ear vesicle, or otocyst, based on its anatomical position, and because in later stages (30+), the otocyst is clearly labeled, along with the forebrain, the dorsal part of the mid- and hindbrain, and the notochord (Fig. 5D). Accumulation of messages in the notochord progresses gradually with staining absent in stage 30 embryos but is apparent in embryos a few hours older (Fig. 5E). Probes made from sense templates do not stain embryos, further confirming the specificity of our probe (Fig. 5F).

Whole mount RNA analysis of pregastrula embryos is relatively unreliable due to significant amounts of yolk that may hinder the hybridization and staining process. To confirm expression in blastula-stage embryos, we applied RT-PCR analysis using oligonucleotide primers specific to Xrel3 mRNA on total RNA extracted from dissected blastulae. PCR of RNA alone did not amplify a product, indicating that the products we observed were not due to genomic DNA. Our analysis confirmed the in situ, Northern, and RNase protection assays, showing amplification of Xrel3 cDNA in pregastrula and post-neurula embryos, with a decline in expression during gastrulation (Fig. 6A). Animal, vegetal, and equatorial zones all express Xrel3 mRNA in stage 8 blastulae, indicating that it is present all along the animal-vegetal axis (Fig. 6B). There is a modest (2–3-fold) increase in Xrel3 mRNA accumulation in the equatorial zone as compared with the vegetal and animal zones (Fig. 6B), based on laser densitometric analysis normalized using histone mRNA levels.

Overexpression of Xrel3 mRNA by Microinjection Induces Embryonic Tumors—Microinjection of up to 500 pg of Xrel3 synthetic mRNA into the animal or vegetal pole did not prevent embryos from gastrulating normally. However, after gastrulation, the embryos develop abnormal growths or tumors whose location and size depend on the site of injection. Tumors (Fig. 7, arrowheads) generally develop in the epidermis from animal pole injection (Fig. 7, A–D). They appear at the neurula stage first as a patch of pigmented tissue on the ventral side (Fig. 7A), and the region quickly enlarges into a tumor (Fig. 7B, arrowhead). Abnormal head and facial development is also associated with animal pole injection, as might be expected since these structures also develop from animal cells (data not shown). In some cases, the tumors are so large, they inhibit normal development, for instance, anterior (Fig. 7E, arrow) head structures. Injection into the vegetal pole or endoderm (prospective digestive system) also caused tumor formation, but these tumors were below the surface of the embryo after having been covered over by ectoderm during gastrulation (Fig. 7E). Control embryos injected with 1 ng of a nonfunctional truncated Xrel3 message develop normally (Fig. 7F).

The size of tumors also correlated with the dose of injection. The minimum concentration that gave any indication of tumor formation was 100 pg. At this dosage, only a small percentage of embryos developed tumors (8%, n = 88), and these appeared only in the epidermis, covering less than 1% of the surface of the embryo in the ventral region. At 200 or 300 pg, the tumors were larger, covering about 5–10% of the embryo surface (Fig. 7D) and appeared in every embryo injected (n = 121). Many tumors (n = 82) appeared in the anterior ventral epidermis (Fig. 7D, lower two embryos), and the remainder appeared on the head (n = 20) or ventral posterior epidermis (n = 9). At 500 pg, the tumors appeared in every embryo examined (n = 255) and covered most of the ventral epidermis (Fig. 7A). The tumors that form are very large and typically interfere with the development of normal axial tissues when examined histologically.

**DISCUSSION**

We have identified a novel rel gene, Xrel3, whose RNA is expressed in a spatiotemporally restricted manner. The Xrel3 predicted protein is most similar to the previously identified Xrel2 protein. The differences in nucleotide sequences outside their respective coding regions suggest that Xrel3 and Xrel2 are two separate genes with different regulatory regions. At the protein level, in the most highly conserved amino-terminal RHD, Xrel2 has a unique insert very close to the start of translation (Fig. 2A, positions 5–22), whereas at positions 37 to 58 of Xrel3 (Fig. 2A), there is a stretch of residues that are only found in Xrel3 and not shared by any other Rel member. These two regions span the putative DNA binding and dimerization domain. These differences suggest that Xrel3 and Xrel2 might dimerize with different partners to form a transcription complex, and/or they bind to different DNA targets. Outside of the RHD, Xrel2 and Xrel3 also vary considerably in amino acid residue sequence. There are numerous substitutions with a large stretch of unique residues spanning positions 435 to 474 of Xrel3 corresponding to the predicted transcriptional activation domain of Rel proteins (9). Thus, the combination of nucleotide and amino acid differences indicates that Xrel3 and Xrel2 are distinct genes.
The mRNA expression data also indicates that Xrel3 is distinct from other Xenopus genes. Xrel3 mRNA is expressed in all cells maternally but is restricted to anterior neural structures after gastrulation. The DORSAL morphogen of Drosophila shows limited spatio-temporal expression during development. Although its RNA and protein are present throughout the entire embryo, DORSAL protein is localized only to ventral nuclei of early Drosophila embryos (8). XrelA protein may also be localized to nuclei of blastula and neurula embryos but not gastrula stage embryos (18). This observation correlates with the temporal expression pattern of Xrel3 mRNA, and it will be interesting to determine the interaction between XrelA and Xrel3. We are presently raising antibodies against Xrel3 to determine if, like Dorsal and XrelA, it is localized to nuclei of specific structures of the embryo.

The formation of tumors by Xrel3 overexpression provides a convenient model to study mechanisms of neoplasia. Similar tumors can be induced by overexpression of Gli1 (35), a zinc-finger transcription factor that is also involved in neural differentiation in Xenopus (36) and is up-regulated in human basal cell carcinoma (35). We are presently testing whether Xrel3 up-regulates Gli1, but there is no precedent linking Gli and rel gene activity. The effectiveness of tumor induction by Xrel3 mRNA, however, is much greater than that of Gli1 since much lower doses of Xrel3 (200–500 pg) as compared with Gli1 mRNA (2000 pg, Ref. 35), induce much larger tumors. Also, Xrel3 mRNA induced tumors when it was injected into the vegetal pole (prospective digestive system), suggesting that it may have a more generalized role in tumor formation.

Rel protein overexpression also has been shown to be associated with or directly cause mammalian tumors. For instance, the oncogenic tyrosine kinase p210BCR-ABL is involved in leukemia and activates the p65 subunit of NF-κB (RelA) in leukemic cells (37). A constitutively active form of NF-κB (Lyt-10) causes tumors to form in murine fibroblasts (38) and RelA expression is significantly up-regulated and required for the transformed phenotype of thyroid carcinoma cell lines (39). Our results are not inconsistent with these observations. However, tumor formation by Xrel3 overexpression in embryos is likely similar to the formation of teratocarcinomas, which result from prevention of differentiation. In this sense, our results indicate that Xrel3 may be involved in regulation of the decision for embryonic cells to undergo differentiation.

Finally, if we hypothesize that Xrel3 is involved in regulating differentiation, then perhaps its role in selected tissues in which it is expressed normally is to allow limited proliferation of those tissues before they differentiate. This hypothesis is consistent with Xrel3 mRNA expression in the pregastrula embryo when it is undergoing cleavage. In any case, the formation of tumors by Xrel3 overexpression in embryos provides a useful model to study the earliest steps in neoplastic transformation.

Acknowledgments—We thank Corinne Mercer, Blue Lake, Terry-Lynn Young, and Ann Ryan for technical assistance, Dave Turner for providing the CS2 plasmid, and Kim Mowry for providing the histone plasmid.

REFERENCES

1. Verma, I. M., Stevenson, J. K., Schwarz, E. M., Antwerp, D. V., and Miyamoto, S. (1995) Genes Dev. 9, 2723–2735.
2. Baeuerle, P. A., and Baltimore, D. (1996) Cell 87, 13–20.
3. Baldwin, A. S. (1996) Annu. Rev. Immunol. 14, 649–681.
4. Huguet, C., Crepieux, P., and Laudet, V. (1997) Oncogene 15, 2965–2974.
5. Ip, Y. T., Kraut, R., Levine, M., and Rushlow, C. A. (1991) Cell 64, 439–446.
6. Wasserman, S. A. (1995) Mol. Biol. Cell 4, 767–771.
7. Barinaga, M. (1996) Science 273, 735–736.
8. Schmitz, M. L., and Baeuerle, P. A. (1991) EMBO J. 10, 3805–3817.
9. Stein, D., Roth, S., Vogelsang, E., and Nusslein-Volhard, C. (1991) Cell 65, 725–735.
10. Schmidt-Ullrich, R., Memet, S., Lilienbaum, A., Feuillard, J., Raphael, M., and Israel, A. (1996) Development 122, 2117–2128.
11. Attar, R. M., Caamano, J., Carrasco, D., Iotsova, V., Ishikawa, H., Ryseck, R. P., Weih, F., and Bravo, R. (1997) Semin. Cancer Biol. 8, 93–101.
12. Smith, D. E., Del Amo, F. F., and Gridley, T. (1992) Development 116, 1033–1039.
13. Stoeckel, C., Weber, B., Bourgeois, R., Bolcato-Bellemain, A. L., and Perrin-Schmidt, F. (1995) Mech. Dev. 51, 251–263.
14. Dixon, M. (1997) Nat. Genet. 15, 3–4.
15. Hopwood, N. D., Pluck, A., and Gurdon, J. B. (1989) Cell 59, 893–903.
16. Sargent, M. G., and Bennett, M. (1990) Development 109, 967–973.
17. Tannahill, D., and Wardle, F. (1995) *Int. J. Dev. Biol.* **39**, 549–558
18. Bearer, E. (1994) *Eur. J. Cell Biol.* **63**, 255–268
19. Kao, K., and Hopwood, N. (1993) *Proc. Natl. Acad. Sci.* **88**, 2697–2701
20. Richardson, J. C., Garcia-Estrabot, A. M., and Woodland, H. R. (1994) *Mech. Dev.* **45**, 173–189
21. Suzuki K., Yamamoto, T., and Inoue, J. (1995) *Nucleic Acids Res.* **23**, 4664–4674
22. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. (U. S. A.)* **74**, 5463–5467
23. Kao, K. R., and Elinson, R. P. (1988) *Dev. Biol.* **127**, 64–77
24. Kao, K., and Lockwood, A. (1996) *Mech. Dev.* **58**, 129–139
25. Krieg, P., and Melton, D. (1984) *Nucleic Acids. Res.* **12**, 7057–7070
26. Hemmati-Brivanlou, A., and Melton, D. (1994) *Cell* **77**, 273–281
27. Rebagliati, M. R., Weeks, D. L., Harvey, R. P., and Melton, D. A. (1985) *Cell* **42**, 769–777
28. Niehrs, C., Steinbesser, H., and DeRobertis, E. M. (1994) *Science* **263**, 817–820
29. Harland, R. (1991) in *Methods in Cell Biology*, Vol. 36, pp. 685–695
30. Gillespie, L. L., Chen, G., and Paterno, G. (1995) *J. Biol. Chem.* **270**, 22758–22763
31. Brownell, E., Mittereder, N., and Rice, N. (1988) *Oncogene* **4**, 935–942
32. Rice, N. R., Mackichan, M. L., and Israel, A. (1992) *Cell* **71**, 243–254
33. Newport, J., and Kirschner, M. (1982) *Cell* **30**, 675–686
34. Richardson, J. C., Gatherer, D., and Woodland, H. R. (1995) *Mech. Dev.* **52**, 165–177
35. Dahmene, N., Lee, J., Robins, P., Heller, P., and Ruiz i Altaba, A. (1997) *Nature* **389**, 876–881
36. Lee, J., Platt, K. A., Censullo, P., and Ruiz i Altaba, A. (1997) *Development* **124**, 2537–2552
37. Hamdane, M., David-Cordonnier, M-H., D’Halluin, J. C. (1997) *Oncogene* **15**, 2267–2275
38. Ciana, P., Neri, A., Cappellini, C., Cavallo, F., Pomati, M., Chang, C-C., Maiolo, A. T., and Lombardi, L. (1997) *Oncogene* **14**, 1805–1810
39. Visconti, R., Cerutti, J., Battista, S., Fedele, M., Trappasso, F., Zeki, K., Miano, M. P., deNigris, F., Casalino, L., Curcio, F., Santoro, M., and Fusco, A. (1997) *Oncogene* **15**, 1987–1994