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Genes encoding receptors for insulin and insulin-like growth factor I are expressed in *Xenopus* oocytes and embryos

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ABSTRACT **Insulin and insulin-like growth factor I (IGF-I) initiate their metabolic, growth, and differentiation effects through binding to the insulin receptor and the IGF-I receptor, two members of the tyrosine kinase family of receptors. To study the role of these peptides and receptors in early development, we used the polymerase chain reaction and embryo-derived RNA to generate partial cDNA sequences of the insulin receptor and IGF-I receptor from the amphibian *Xenopus laevis*. Three unique tyrosine kinase-related sequences were obtained. Two of the nucleotide sequences, *XTK 1a* and *XTK 1b*, corresponded to peptides that share 92% amino acid identity, and each is 89% identical to the human insulin receptor. The third sequence, *XTK 2*, corresponds to a peptide that has 92% amino acid identity with the human IGF-I receptor but only 80% identity with *XTK 1a* and *XTK 1b*. On the basis of these similarities, the pattern of conserved amino acids, and the tetraploid nature of the *Xenopus* genome, we suggest that *XTK 1a* and *XTK 1b* most likely represent the product of two different nonallelic insulin receptor genes, while *XTK 2* may be one of the probable two *Xenopus* IGF-I receptor genes. By reverse transcription-polymerase chain reaction and gene-specific hybridization, expression of the three *XTK* sequences was detected in the oocyte, unfertilized egg, and embryos through gastrulation, neurulation, and tailbud stages. Competition binding assays with *Xenopus* membrane preparations demonstrated insulin receptors and IGF-I receptors in older tadpoles. IGF-I receptors were also present in oocytes, eggs, and gastrula embryos. By contrast, insulin binding was present but atypical in oocytes and was barely detected in eggs and gastrula embryos. The expression of receptors for insulin and IGF-I in early *Xenopus* embryos and their apparent distinct developmental regulation suggest that these molecules and their ligands may be important in early *Xenopus* development.**

The *in vivo* roles of insulin and insulin-like growth factor I (IGF-I) have been thought to be confined to late fetal and postnatal stages of development, but evidence for a role in early development has begun to accumulate in studies (reviewed in ref. 1) from *Drosophila* (2), chicken (3, 4), and mouse (5). In *Xenopus laevis* large numbers of oocytes and developing embryos are accessible at all stages, making this organism an excellent model for studying the role of growth factors in development (6). In *Xenopus* oocytes, exogenous insulin and IGF-I stimulate maturation (7), ribosomal protein S6 phosphorylation (8), glucose transport (9), and protein and RNA synthesis (10, 11). Tyrosine and serine protein kinase activities can be stimulated by insulin in oocyte membranes (12). However, the importance of endogenous insulin and IGF-I in the maturing oocyte and developing embryo remains undefined. The cloning and sequencing of *Xenopus* insulin (13) and IGF-I (14, 15) provided powerful tools with which to define the pattern of expression and the role that these ligands may play in early development. However, very little is known about the receptors for these ligands in *Xenopus*.

In mammals, the insulin receptor (IR) and the IGF-I receptor (IGF-IR), distinct but very similar glycoproteins, have heterotetrameric structures ($\alpha_2\beta_2$). In both, the α subunit is extracellular, contains the ligand-binding domain, and is linked to the β subunit by disulfide bonds. The β subunit spans the cell membrane and has a C-terminal cytoplasmic region with a tyrosine kinase domain that is highly conserved. Ligand binding to the α subunit stimulates the tyrosine kinase activity of the β subunit, leading to autophosphorylation of tyrosine residues on the receptor β subunit, further activation of the kinase, and phosphorylation of tyrosine residues of other cellular proteins. Many biological effects following receptor activation seem to require an intact tyrosine kinase (16). The specificity of the two receptor kinases is thought to be based on the amino acid differences between the cytoplasmic domains of the IR and IGF-IR (17-19).

We now report the nucleotide sequences for three tyrosine kinase domains† from *Xenopus* that are homologous to the human IR and IGF-IR tyrosine kinase domains (17-19). We describe the structural features conserved in this region and the expression of mRNA for each of the tyrosine kinases in pre- and postfertilization developmental stages. The developmental patterns of insulin and IGF-I binding to receptors in oocytes and embryos are distinct and independent, suggesting that regulation of their expression may be important in early embryogenesis.

MATERIALS AND METHODS

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Cloning of *Xenopus* IR and IGF-IR Tyrosine Kinase (*XTK*) Domains. *Oligonucleotides.* Synthetic oligonucleotides (Applied Biosystems 381A DNA synthesizer) were used without purification. PCR primers were based on sequences conserved in the tyrosine kinase domains of the IR and IGF-IR from other species but different from other protein kinases. The upstream primer (sense, primer U) was a redundant 20-mer, 5'-TTYGGNATGGTNTAYGARGG-3', and the downstream primer (antisense, primer D) was a redundant 23-mer, 5'-TARTARTCNGTYTCRTADA-TRTC-3' (Fig. 1). The two primers defined a 470-base-pair (bp) sequence encoding part of the tyrosine kinase domain of the IR and IGF-IR. [The same redundant primers were used to amplify and clone sequences corresponding to the IR and IGF-IR of chicken (20).]

RT-PCR amplification, cloning, and sequencing. Xenopus eggs were obtained and fertilized (21); embryos from various stages (22) were harvested and frozen at −70°C until ready for use. Total RNA was isolated from stage 21/22 (≈24 hr) and

Abbreviations: IGF-I, insulin-like growth factor I; IGF-IR, IGF-I receptor; IR, insulin receptor; RT, reverse transcription.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M64659, M64660, and M64661).

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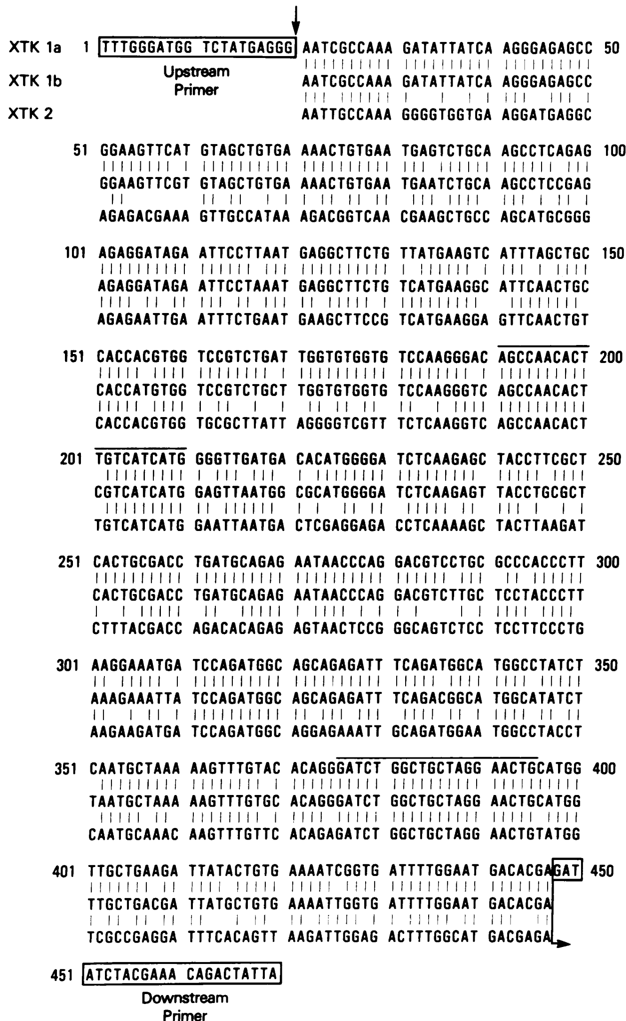

FIG. 1. Nucleotide sequences (sense strand) of the putative *Xenopus* tyrosine kinase clones *XTK 1a, 1b*, and *2*. One representative sequence from the redundant primer regions [upstream (sense) and downstream (antisense)] used for the cloning is shown. Overlined sequences indicate the primers (U′ and D′) used in the expression studies. At position 201 both a T and a C were incorporated.

stage 32/33 (≈48 hr) embryos by the guanidinium thiocyanate method (23). Total RNA (10 μg) from each stage was reverse-transcribed in 50 μl containing NaCl (50 mM), Tris·HCl (50 mM; pH 8.3), dithiothreitol (10 mM), $MgCl_2$ (6 mM), dNTPs (200 μM each), primer D (0.4 μM), RNasin (0.8 unit; Promega) and avian myeloblastosis virus reverse transcriptase (15 units; Promega) at 42°C for 1 hr. The reaction mixture was filtered twice (Centricon 100; Amicon). One-tenth (5 μl) of this mixture was amplified by PCR in a volume of 100 μl, using primers U and D (0.4 μM each), *Taq* polymerase (1.5 units), and the reagents and protocol of the manufacturer (Perkin–Elmer/Cetus). Forty-five cycles were performed, each cycle consisting of denaturation (94°C, 1.5 min), annealing (42°C, 1.5 min), and extension (72°C, 1.5 min), except for the first cycle (denaturation time, 5 min) and the last cycle (extension time, 10 min). The PCR mixture was electrophoresed in a 3.5% agarose composite gel (1.2% SeaKem and 2.3% NuSieve GTG, FMC), and the expected 470-bp amplified product (results not shown) was electroeluted and precipitated with ethanol. The 5′ ends of the gel-purified DNA were phosphorylated using T4 polynucleotide kinase (Boehringer Mannheim), and the DNA was blunt-end-ligated into the dephosphorylated *Sma* I site of pGEM-4Z (Promega).

Escherichia coli DH5α cells were transformed with the ligation mixture (Bethesda Research Laboratories) and plated onto LB agar plates containing ampicillin (100 μg/ml), 5-bromo-4-chloro-3-indolyl β-D-galactoside (2.5 mg per plate surface), and isopropyl β-D-thiogalactoside (25 ng per plate surface). Plasmid DNA was prepared from eight independent white colonies by either the boiling method or the Qiagene method (Qiagene, Studio City, CA). Three unique clones, *XTK 1a, XTK 1b*, and *XTK 2*, were characterized. Both strands of the DNA inserts were sequenced by the dideoxy method (Sequenase 2.0; United States Biochemicals). From the original eight colonies, four corresponded to the same sequence, *XTK 1b*, one corresponded to *XTK 1a*, and one to *XTK 2*. Another set of RT–PCR experiments, with the design described above, was performed with RNA extracted from adult *Xenopus* liver and kidney and from oocytes. Multiple clones for *XTK 1b* and one for *XTK 2* were obtained and their sequences were confirmed.

IR and IGF-IR Gene Expression in *Xenopus* Embryos. *Oligonucleotides*. For further developmental studies, the *Xenopus* sequences were used to design an upstream primer (primer U′; nucleotides 191–210, Fig. 1) and a downstream primer (primer D′; 376–395, Fig. 1), each chosen to hybridize to and amplify equally all three *XTK* sequences. The RT–PCR-amplified pool of 205-bp DNA fragments could then be used for slot blot analysis. To detect selectively each of the three amplified *XTK* sequences, three specific oligonucleotide probes (26-mers) were used: *xtk 1a*, antisense to 291–316 of *XTK 1a*, probe *xtk 1b*, sense to 197–222 of *XTK 1b*, and probe *xtk 2*, antisense to 284–309 of *XTK 2*.

RT–PCR of Xenopus *embryos*. RNA was prepared from single unfertilized oocytes, eggs, or embryos (stages 6.5, 8, 15, 31, and 34) by a microadaptation of the RNAzol method (Cinna/Biotex, Friendswood, TX) and dissolved in 25 μl of sterile water. RT was performed essentially as described above except that the final volume was 20 μl and primer D′ was used rather than primer D. Five microliters of the RT mixture was used directly for PCR in 50 μl with primers U′ and D′; conditions were essentially identical to those described above except that the annealing time was 45 sec and the extension time was 1 min. An aliquot of the PCR mixture was electrophoresed in an agarose composite gel. After ethidium bromide staining and UV transillumination, the expected 205-bp product was observed at all stages (results not shown). To examine which of the *XTK* sequences were represented in this amplified band, ≈80 ng of the RT–PCR products or 300 ng of plasmids containing the *XTK 1a, XTK 1b*, or *XTK 2* sequence were slot-blotted onto Nytran membranes (Schleicher & Schuell) in triplicate and hybridized to 5′-^{32}P-labeled oligonucleotide probe *xtk 1a, xtk 1b*, or *xtk 2*. Hybridization and wash conditions were optimized for gene-specific hybridization of the oligonucleotide probes to their complementary sequences [hybridization in 5× standard saline/phosphate/EDTA (SSPE)/1× Denhardt's solution/0.1% SDS at 60°C; washing in 2× SSPE at 60°C for 2 min, followed by 2× SSPE/0.05% SDS at 65°C for 10 min].

Binding Studies. Oocytes, eggs, or embryos (packed volume, 5 ml) were homogenized in 10 ml of 83 mM NaCl/1 mM $MgCl_2$/10 mM Hepes, pH 7.9, and solubilized membranes were prepared (4). Solubilized receptors were then purified on a 2-ml column of wheat germ agglutinin-agarose and radioreceptor assays were done (4); aliquots from the column eluate were incubated in 150 mM NaCl/50 mM Hepes, pH 7.8/0.1% bovine serum albumin/0.1% bacitracin with ^{125}I-labeled recombinant human IGF-I or insulin (≈30,000 cpm) overnight at 4°C. For competition of binding, unlabeled human IGF-I (Amgen Biologicals), human insulin (Eli Lilly), or human IGF-II (Bachem) was added at various concentrations as indicated. Nonspecific binding was estimated as the

Table 1. Percent identity between amino acid (and nucleotide) sequences

	h.IR	*XTK 1a*	*XTK 1b*	*XTK 2*
XTK 1a	88 (75)	—		
XTK 1b	89 (77)	92 (93)	—	
XTK 2	81 (73)	80 (73)	82 (73)	—
h.IGF-IR	82 (74)	80 (73)	80 (73)	92 (78)

h., Human.

binding of labeled ligand in the presence of IGF-I at 250 ng/ml or insulin at 1000 ng/ml.

RESULTS

Conservation of Functional Domains. Using RT–PCR and redundant oligonucleotides based on known IR receptor and IGF-IR sequences, we amplified the tyrosine kinase region of the putative *Xenopus* homologues. Three distinct but closely related cDNAs, *XTK 1a, XTK 1b,* and *XTK 2*, were cloned and sequenced (Fig. 1 and Table 1). At the nucleotide level *XTK 1a* and *XTK 1b* are remarkably similar to each other (93% identity) and all three sequences are >70% identical to either IR or IGF-IR. At the amino acid level, the relationship between the three *Xenopus* sequences and the human receptors is even more clear (Fig. 2 and Table 1). *XTK 1a* and *1b* are most closely related to the human IR (88% amino acid identity), and *XTK 2* to the human IGF-IR (92%). The two *XTK 1* amino acid sequences share a level of identity with *XTK 2* (80%) that is comparable to that shared between the human receptors for insulin and IGF-I (82%). In the same region, the human IR is related to a number of tyrosine kinases with the following identities: the IR of mouse, 97% (25); the human insulin-related receptor, 80% (26); the IR homologue of *Drosophila*, 64% (2); the *ros* oncogene, 45% (24), and the human receptor for platelet-derived growth factor, 30% (24). In the areas where the human IR and IGF-IR are identical, all three *XTK* sequences are particularly well conserved (Fig. 2). Three motifs in the region amplified have been described in other protein kinases and are perfectly conserved in all the *XTK* sequences. These include the lysine involved in phosphotransfer reactions (b in Fig. 2), two sites implicated in ATP binding (c and d in Fig. 2), and the amino acid sequence believed to be specific for tyrosine kinases (LAAR, also in c in Fig. 2) (24). The amino acids that are unique to the human IR are highly conserved in *XTK 1a* and *1b*, and those that are unique to the human IGF-IR are highly conserved in *XTK 2* (Fig. 2). This reinforces the idea that these amino acids play a functional role in receptor-specific actions, perhaps in the recognition of substrates for phosphorylation (19).

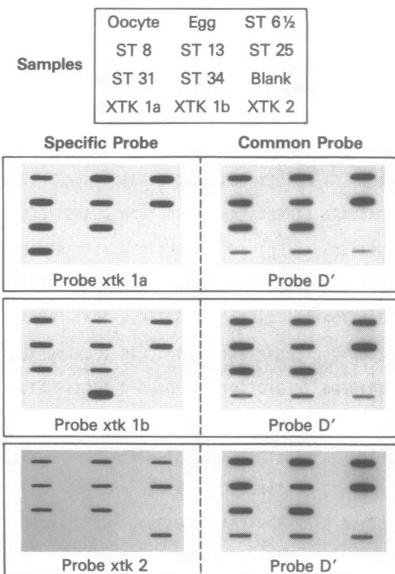

FIG. 3. Specific expression of *XTK 1a, 1b,* and *2* mRNAs. Replicate sets (*a, b,* and *c*) of slot blots of DNA derived from the second RT–PCR experiment were prepared. Scheme at the top indicates the order in which samples were applied: *Xenopus* samples in the first three rows [ST, stage of embryo development according to ref. 22; ST 6½ (morula) is about 4 hr postfertilization; ST 8 (blastula); ST 13 (early neurula); ST 25 (late neurula); ST 31 and 34 (tailbud)] and three plasmid DNAs, each containing one *XTK* sequence, in the fourth row. Each filter was first probed with one of the *XTK*-specific oligonucleotides, *xtk 1a, xtk 1b,* or *xtk 2* (*Left*). The same blots were stripped in 0.01× SSPE at 85°C for 1 hr and then rehybridized with a probe (D') common to all three *XTK* sequences (*Right*). Exposure of the autoradiograms was from several hours to overnight at −70°C.

***XTK* Transcription in *Xenopus* Eggs and Embryos.** It was evident that all three *XTK* sequences were expressed in *Xenopus* embryos at neurula and tailbud stages, since these developmental stages were the sources of RNA used for the cloning. We undertook a more complete study of *XTK* expression, using RT–PCR with new primers that were *Xenopus*-specific and common to all three *XTK* sequences. Total RNA isolated from individual oocytes, from eggs, and from embryos (from morula to tailbud stage) yielded an amplified band of the appropriate size (205 bp; results not shown). No amplification occurred when the starting RNA was digested with RNase prior to RT. To confirm that the amplified sequences represented a mixture of the transcription products of the three *XTK* genes in all developmental stages studied, we probed three replicate slot blots of the amplification products with oligonucleotide highly specific

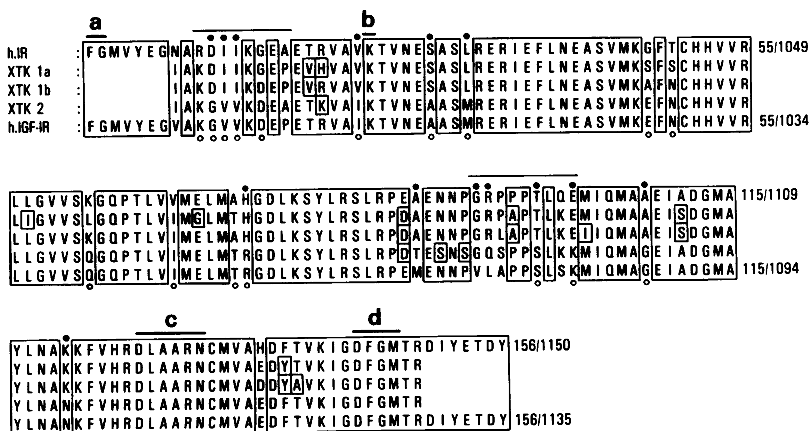

FIG. 2. Derived amino acid sequences of *XTK 1a, 1b,* and *2* compared with the human (h.) IR and IGF-IR. Solid lines surround blocks of amino acids that are identical in the h.IR and h.IGF-IR. Solid circles mark positions where the h.IR and both *XTK 1* sequences are identical. Open circles mark positions where h.IGF-IR and *XTK 2* share identity. Bars a–d represent regions that are highly conserved across protein kinases (24). Lines above amino acids 10–17 and 94–102 represent clusters of amino acids divergent between the h.IR and h.IGF-IR. At right, the number to the left of the slash represents the amino acid number with reference to the first amino acid of the upstream primer sequence, and the number to the right of the slash represents the amino acid number with reference to either the h.IR (17) (top row) or the h.IGF-IR (18) (bottom row).

for one of the three *XTK* sequences (oligonucleotides *xtk 1a*, *xtk 1b*, and *xtk 2*). Control slots, containing the same recombinant plasmids from which the *XTK* sequences were derived, demonstrated that the probes were specific. The stripped blots were reprobed with the downstream primer (D′), which is present in all three *XTK* sequences, and showed that the control slots contained approximately equal copy numbers of the three control sequences (Fig. 3). We concluded from these experiments that the three mRNAs were expressed in oocytes, eggs, and early embryos. While we detected little or no variation in the intensity of the signal at different developmental times, we hesitate to exclude differences in the level of expression among the three *XTK* genes at any given stage, or between various developmental stages.

Differential Expression of the Receptors. To analyze the receptor proteins and the possible physiological relevance of the two types of receptors in developing *Xenopus*, we studied the binding of the labeled peptides to solubilized receptors. Specific binding of ^{125}I-IGF-I (per 8–10 μg of partially purified receptors) was demonstrated in all four stages studied: stage VI oocytes (34.4% of total), unfertilized eggs (12.5%), gastrula (24.6%), and tadpole (74.2%). By contrast, specific binding of ^{125}I-insulin was detectable in stage VI oocytes (5.8%) and tadpoles (33.4%), but it was barely detected in eggs and gastrula embryos. By converting the binding values to receptor number, it appeared that IGF-IRs were ≈12-fold and ≈6-fold more abundant than IRs in oocytes and tadpoles, respectively.

The specificity of the receptors was studied by competition of the binding of each ligand with unlabeled insulin, IGF-I, and IGF-II (Fig. 4). With ^{125}I-IGF-I the binding-competition of IGF-I and the other two peptides showed reactivity that was typical of IGF-IRs in general. The binding of ^{125}I-insulin to receptors from oocytes and tadpoles showed the expected competition with insulin but somewhat greater than expected competition with the two IGFs. In fertilized eggs and gastrula, the specific binding of ^{125}I-insulin was very low, making it difficult to interpret the competition curves. Overall, these data indicate that there is differential regulation of the expression of the two types of cell membrane receptors and that IGF-IRs are clearly dominant in *Xenopus* oocytes and early embryos.

DISCUSSION

We have identified three unique tyrosine kinase domains in *Xenopus* that are expressed in oocytes, eggs, and embryos. The nucleotide sequences and especially the amino acid sequences of *XTK 1a* and *XTK 1b* are most similar to the human IR, whereas the nucleotide and amino acid sequences of *XTK 2* are most similar to the human IGF-IR. The *XTK 1a* and *1b* sequences fall approximately midway between the insulin receptor sequences of three mammals (human, rat, and mouse) and that of an insect (*Drosophila*) (2, 17, 25, 27, 28). Comparable data are not yet available for the IGF-IR. More important, structural features of the receptors are conserved. Specifically, the IR and IGF-IR have stretches of

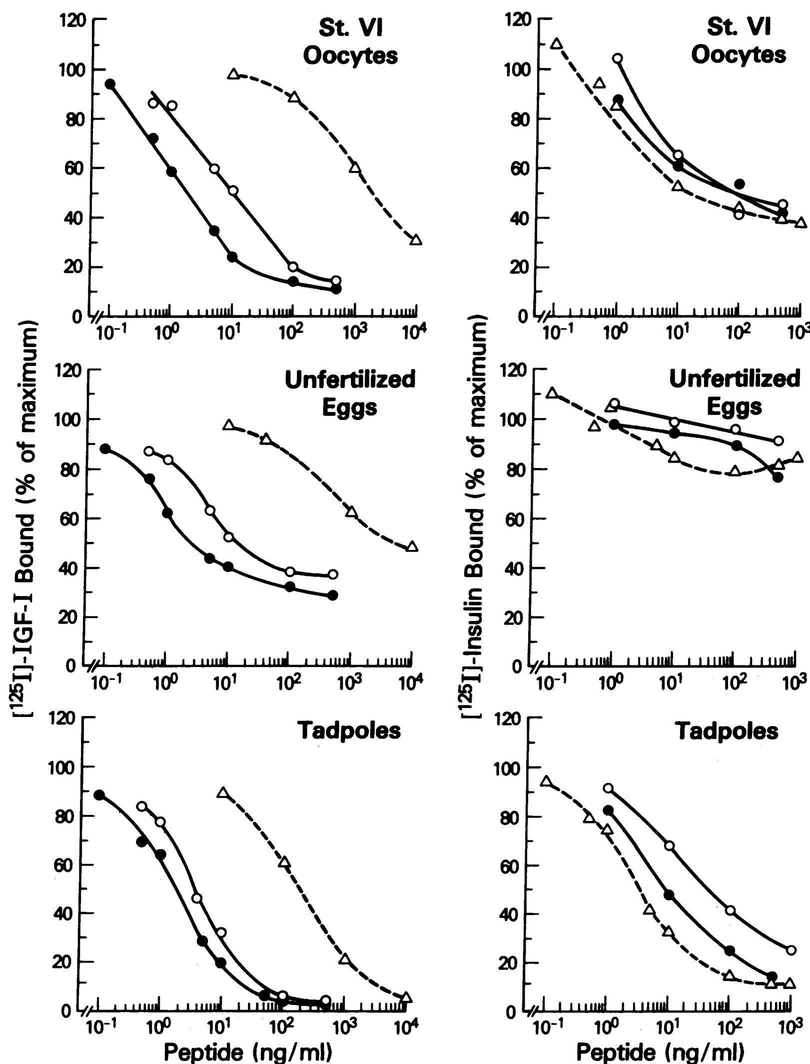

FIG. 4. Binding specificity of IGF-IR and IR in oocytes, eggs, and embryos. Eluates from wheat germ agglutinin columns of stage (st.) VI oocytes, unfertilized eggs, and tadpoles were incubated with ^{125}I-IGF-I (*Left*) or ^{125}I-insulin (*Right*), with increasing concentrations of unlabeled peptides. Binding is expressed as a percentage of the maximum binding obtained in the absence of unlabeled peptides, IGF-I (●), IGF-II (○), or insulin (△). Each point represents the mean of duplicate samples in a representative experiment. In the unfertilized eggs, very low total binding of ^{125}I-insulin accounts for the high percentage of nonspecific binding.

amino acids that are identical; the *XTK* sequences are especially well conserved in these regions. In the divergent regions the amino acids that characterize the human IR are highly conserved in *XTK 1a* and *1b*, and those that characterize the human IGF-IR are highly conserved in *XTK 2*, reinforcing the idea that these amino acids may play a functional role in receptor-specific actions.

X. laevis is a tetraploid organism with two nonallelic copies of many genes (13, 14, 29). Thus, we were not so surprised to find two highly similar IR genes. The apparent absence of a second *Xenopus* IGF-IR gene was, perhaps, more surprising. It is possible that a lack of initial amplification of a second IGF-IR sequence or a problem in the sampling of clones after successful amplification was responsible for not detecting a second IGF-IR mRNA sequence with the initial strategy. In studies to be reported elsewhere, screening of a cDNA embryo library has revealed the existence of a sequence compatible with a second IGF-IR gene. Interestingly, a recent study (30) of the receptor proteins in *Xenopus* oocytes, liver, and muscle has shown heterogeneous β subunits. This is further suggestion that there are two IGF-IRs and two IRs expressed (30).

The presence of all three *XTK* mRNAs throughout development suggests that insulin and IGF-I may have roles early in embryogenesis in *Xenopus*. We have recently found differential expression of the two nonallelic insulin genes during neurulation in *Xenopus* (31). In the chicken embryo, IRs and IGF-IRs are widespread in the blastoderm (4). Insulin and IGF-I stimulate growth (32) and transcription of the lens δ-crystallin gene (33). In *Drosophila* embryos, insulin-sensitive tyrosine kinase phosphorylation has been demonstrated (2) and mRNA for the IR homologue accumulates in the egg and the developing nervous system during neurulation (34). In *Xenopus*, there are reports that suggest that both insulin and IGF-I exert effects on oocyte maturation and glucose transport via IGF-IRs (7, 9). The results presented here indicate that receptors for insulin and IGF-I are transcribed in oocytes and embryos but their posttranscriptional regulation is different. Binding data suggest that the level of mature, membrane-associated IGF-IR molecules is much greater than the level of IR molecules, except in the advanced tadpole. While the specificity of the IR in tadpole membranes was typical, in oocytes the competition by insulin, IGF-I, and IGF-II was unusual in that IGF-I and -II were much more potent competitors of ^{125}I-insulin binding than is typical. The binding characteristics could reflect the presence of hybrid IR/IGF-IR molecules as shown in other systems (35, 36). Alternatively, it could reflect the combination of the two IRs expressed, one or both with unusual binding properties.

In conclusion, we have shown that *Xenopus* expressed mRNAs coding for homologues of the IR and IGF-IR. The tyrosine kinase domains of these receptors are highly conserved in amphibians. The ligand binding data extend previous reports suggesting a low number of IRs and a higher number of IGF-IRs in oocytes and demonstrate that IGF-IRs are prevalent in early stages postfertilization.

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1. de Pablo, F., Scott, L. A. & Roth, J. (1990) *Endocr. Rev.* **11**, 558–577.
2. Petruzzelli, L., Herrera, R., Arenas-Garcia, R., Fernandez, R., Birnbaum, M. J. & Rosen, O. M. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 4710–4714.
3. de Pablo, F., Girbau, M., Gomez, J. A., Hernandez, E. & Roth, J. (1985) *Diabetes* **34**, 1063–1067.
4. Girbau, M., Bassas, L., Alemany, J. & de Pablo, F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5868–5873.
5. Heyner, S., Smith, R. M. & Schultz, G. A. (1989) *BioEssays* **11**, 171–176.
6. Dawid, I. B., Sargent, T. D. & Rosa, F. (1990) *Curr. Top. Dev. Biol.* **24**, 2–16.
7. Sadler, S. E. & Maller, J. L. (1987) *J. Biol. Chem.* **262**, 10644–10650.
8. Maller, J. L., Pike, L. J., Freidenberg, G. R., Cordera, R., Stith, B. J., Olefsky, J. M. & Krebs, E. G. (1986) *Nature (London)* **320**, 459–461.
9. Janicot, M. & Lane, M. D. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 2642–2646.
10. Miller, D. S. (1988) *Science* **240**, 506–509.
11. Miller, D. S. (1989) *J. Biol. Chem.* **264**, 10438–10456.
12. Sakanoue, Y., Hashimoto, E., Nakamura, S. & Yamamura, H. (1988) *Biochem. Biophys. Res. Commun.* **150**, 1176–1184.
13. Shuldiner, A. R., Phillips, S., Roberts, C. T., LeRoith, D. & Roth, J. (1989) *J. Biol. Chem.* **264**, 9428–9432.
14. Shuldiner, A. R., Nirula, A., Scott, L. A. & Roth, J. (1990) *Biochem. Biophys. Res. Commun.* **166**, 223–230.
15. Kajimoto, Y. & Rotwein, P. (1990) *Mol. Endocrinol.* **4**, 217–226.
16. Czech, M. P. (1989) *Cell* **59**, 235–238.
17. Ebina, Y., Ellis, L., Jarnagin, K., Edery, M., Grai, L., Clauser, E., Ou, J., Masiarz, F., Kan, T. W., Goldfine, I. D., Roth, R. A. & Rutter, W. J. (1985) *Cell* **40**, 747–758.
18. Ullrich, A., Gray, A., Tam, A. W., Yang-Feng, T., Tsubokawa, M., Collins, C., Henzel, W., Le Bon, T., Kathuria, S., Chen, E., Jacobs, S., Francke, U., Ramachandran, R. & Fujita-Yamaguchi, Y. (1986) *EMBO J.* **5**, 2503–2512.
19. Lammers, R., Gray, A., Schlessinger, J. & Ullrich, A. (1989) *EMBO J.* **8**, 1369–1375.
20. Scavo, L. M., Serrano, J., Roth, J. & de Pablo, F. (1991) *Biochem. Biophys. Res. Commun.* **176**, 1393–1401.
21. Kinter, C. R. & Melton, D. A. (1987) *Development* **99**, 311–325.
22. Nieuwkoop, P. D. & Faber, J. (1967) *Normal Table of Xenopus laevis (Daudin)* (North-Holland, Amsterdam).
23. Berger, S. L. & Kimmel, A. R. (1987) *Guide to Molecular Cloning Techniques* (Academic, San Diego, CA).
24. Hanks, S. K., Quinn, A. & Hunter, T. (1988) *Science* **241**, 42–52.
25. Flores-Riveros, J. R., Sibley, E., Kastelic, T. & Lane, M. D. (1989) *J. Biol. Chem.* **264**, 21557–21572.
26. Shier, P. & Watt, V. M. (1989) *J. Biol. Chem.* **264**, 14605–14608.
27. Goldstein, B. J. & Dudley, A. L. (1990) *Mol. Endocrinol.* **4**, 235–244.
28. Ludwig, S., Muller-Wieland, D., Goldstein, B. J. & Kahn, C. R. (1988) *Endocrinology* **123**, 594–600.
29. Kobel, H. R. & Pasquier, L. D. (1986) *Trends Genet.* **2**, 310–315.
30. Hairant, P., Kowalski, A., Giorgetti, S., Baron, V. & Van Obberghen, E. (1991) *Biochem. J.* **273**, 673–678.
31. Shuldiner, A. R., de Pablo, F., Moore, C. A. & Roth, J. (1991) *Proc. Natl. Acad. Sci. USA*, in press.
32. Girbau, M., Gomez, J. A., Lesniak, M. A. & de Pablo, F. (1987) *Endocrinology* **121**, 1477–1482.
33. Alemany, J., Zelenka, P., Serrano, J. & de Pablo, F. (1989) *J. Biol. Chem.* **264**, 17559–17563.
34. Garofalo, R. S. & Rosen, O. M. (1988) *Mol. Cell Biol.* **8**, 1638–1647.
35. Treadway, J. L., Morrison, B. D., Goldfine, I. D. & Pessin, J. E. (1989) *J. Biol. Chem.* **264**, 21450–21453.
36. Moxham, C. P., Duronio, V. & Jacobs, S. (1989) *J. Biol. Chem.* **264**, 13238–13244.