8-1994

# A Thyroid Hormone-Regulated Gene in Xenopus Laevis Encodes a Type III Iodothyronine 5-Deiodinase.

Donald L. St Germain
*Dartmouth College*

Robert Schwartzman
*Carnegie Institution of Washington*

Walburga Croteau
*Dartmouth College*

Akira Kanamori
*Carnegie Institution of Washington*

Zhou Wang
*Carnegie Institution of Washington*

*See next page for additional authors*

*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 7767–7771, August 1994
Developmental Biology

# A thyroid hormone-regulated gene in *Xenopus laevis* encodes a type III iodothyronine 5-deiodinase

DONALD L. ST. GERMAIN*†, ROBERT A. SCHWARTZMAN‡, WALBURGA CROTEAU*†, AKIRA KANAMORI‡, ZHOU WANG‡, DONALD D. BROWN‡, AND VALERIE ANNE GALTON†

Departments of *Medicine and †Physiology, Dartmouth Medical School, Lebanon, NH 03756; and ‡Department of Embryology, Carnegie Institution of Washington, Baltimore, MD 21210

*Contributed by Donald D. Brown, April 18, 1994*

**ABSTRACT** **The type III iodothyronine 5-deiodinase metabolizes thyroxine and 3,5,3′-triiodothyronine to inactive metabolites by catalyzing the removal of iodine from the inner ring. The enzyme is expressed in a tissue-specific pattern during particular stages of development in amphibia, birds, and mammals. Recently, a PCR-based subtractive hybridization technique has been used to isolate cDNAs prepared from *Xenopus laevis* tadpole tail mRNA that represent genes up-regulated by thyroid hormone during metamorphosis. Sequence analysis of one of these cDNAs (XL-15) revealed regions of homology to the mRNA encoding the rat type I (outer ring) 5′-deiodinase, including a conserved UGA codon that encodes selenocysteine in the mammalian enzyme. We report here that the protein encoded by the XL-15 cDNA efficiently catalyzes the (inner ring) 5-deiodination of 3,5,3′-triiodothyronine with a $K_m$ value of 2 nM and is resistant to inhibition by propylthiouracil and aurothioglucose. Our analysis confirms that the UGA codon encodes a selenocysteine that is critical for the catalytic activity of the enzyme. In addition, the direct induction of XL-15 mRNA levels by thyroid hormone in *X. laevis* tadpole tail tissue and cultured cell lines correlates closely with increases in 5- (but not 5′-) deiodinase activity. These findings indicate that the XL-15 cDNA encodes a type III 5-deiodinase and underscores the importance of the trace element selenium in thyroid hormone metabolism.**

Amphibian metamorphosis requires thyroid hormone (TH) for the coordinate maturation of a number of organ systems and metabolic processes (1). Essential to the regulation of TH action in both adult and larval forms are the iodothyronine deiodinases that metabolize TH to active and inactive metabolites (2). The type III 5-deiodinase (5DIII), which catalyzes the removal of an iodine from the inner ring of thyroxine and 3,5,3′-triiodothyronine ($T_3$) to form the inactive metabolites 3,3′,5′-triiodothyronine ($rT_3$) and 3,3′-diiodothyronine, respectively, has been postulated to play a protective role in selected tissues by preventing their exposure to inappropriately timed or excessive levels of active TH (3). This enzyme is expressed in a tissue-specific pattern during particular stages of fetal growth in amphibia, birds, and mammals, suggesting that it plays an important role in coordinating the developmental effects of TH (1, 4–6).

Little is known about the biochemical characteristics of the 5DIII or the mechanism of its regulation during development because, like the other deiodinases, it has yet to be purified, and cDNAs for the enzyme have not been isolated. Recently, the type I 5′-deiodinase (outer ring, 5′DI) was demonstrated to be a selenoprotein and to contain selenocysteine at its catalytically active site (7). Results of site-directed mutagenesis studies of the 5′DI suggest that the presence of the selenocysteine renders the enzyme sensitive to inhibition by 6*n*-propyl-2-thiouracil (PTU) and aurothioglucose (AThG) (8). Based on this and other evidence, the 5DIII and the type II 5′-deiodinase, both of which are insensitive to PTU and AThG inhibition, have been assumed to be unrelated structurally to the 5′DI and to not contain selenocysteine (9–11).

In *Xenopus laevis*, TH has been demonstrated to alter directly the expression of a number of genes involved in programmed metamorphosis (12, 13). A subset of these genes are up-regulated rapidly in *X. laevis* tadpoles after the administration of $T_3$ (13). We report here the characterization of the protein product of one such early response gene (XL-15)§ (13). We demonstrate that this gene product, which is a selenoprotein bearing significant sequence homology to the mammalian 5′DI, is the enzyme 5DIII.

## MATERIALS AND METHODS

**Isolation and Sequencing of the XL-15 cDNA.** The XL-15 (gene 15) cDNA was isolated as described (13) from a directionally cloned λ phage cDNA library prepared from *X. laevis* tail mRNA (ZapII; Stratagene) using a probe obtained from a PCR-based cDNA fragment library (14). The cDNA was sequenced on both strands using nested deletions (Erase-a-Base system; Promega) and an automated sequencing system using fluorescent dye terminators (Applied Biosystems).

**Expression of XL-15 in *X. laevis* Oocytes.** Stage 5 to 6 *X. laevis* oocytes were microinjected with *in vitro*-synthesized, capped RNA transcripts in amounts that maximized deiodinase expression (XL-15 transcripts at 10 ng per oocyte, rat G21 5′DI mRNA at 1 ng per oocyte), and incubated for 4 days in L-15 medium [for determination of 5′-deiodinase (5′D) activity] or Barth's medium [for determination of 5-deiodinase (5D) activity]. Oocytes were then harvested, membrane fractions were prepared as described (15), and deiodinase activity was determined according to published methods (15, 16). In kinetic studies, 5′D activity was determined using 1.5–1000 nM [$^{125}$I]$rT_3$ as substrate and 20 mM dithiothreitol as cofactor, and 5D activity was determined using by 1–20 nM [$^{125}$I]$T_3$ and 50 mM dithiothreitol. Kinetic constants were determined from Eadie–Hofstee plots. In some experiments, glutathione (5 or 50 mM) or a reconstituted thioredoxin system consisting of thioredoxin (42 μM final concentration; Calbiochem), thioredoxin reductase (42 nM; provided by James A. Fuchs, University of Minnesota), and NADPH (0.5 mM) were used as cofactors in the deiodination assays. [$^{125}$I]iodothyronines used as substrates were obtained from DuPont and were purified by chromatography using Sephadex LH-20 (Sigma) before use.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: AThG, aurothioglucose; PTU, 6*n*-propyl-2-thiouracil; $rT_3$, 3,3′,5′-triiodothyronine; TH, thyroid hormone; $T_3$, 3,5,3′-triiodothyronine; 5D, 5-deiodinase; 5′D, 5′-deiodinase; 5DIII, type III 5-deiodinase; 5′DI, type I 5′-deiodinase.

§The sequence reported in this paper has been deposited in the GenBank data base (accession no. L28111).

In other experiments, the induced XL-15 and G21 deiodinase activities in oocyte membrane preparations were determined in the absence or presence of PTU (10–1000 μM) or AThG (0.01–10 μM). 5'D activity was measured using 67 nM [$^{125}$I]rT$_3$ as substrate and 20 mM dithiothreitol as cofactor. The 5D assay used 1 nM [$^{125}$I]T$_3$ and 50 mM dithiothreitol. Proteins were determined by the method of Bradford (17) with reagents obtained from Bio-Rad. The G21 cDNA was provided by M. Berry and P. R. Larsen (Brigham and Women's Hospital, Boston).

**Functional Analysis of the XL-15 cDNA by Mutation and Deletion.** Site-directed mutagenesis was done by using the Altered Sites mutagenesis system according to the manufacturer's instructions (Promega). Partial deletions of the 3'-untranslated region were prepared using the XL-15 cDNA cloned into the Bluescript vector. XL-15/Bluescript plasmids were isolated from transformed *Escherichia coli* and digested with *Xho* I and *Kpn* I. The 3'-untranslated region of the cDNA insert was subjected to exonuclease III digestion for various periods of time followed by S1 nuclease treatment. Plasmids were then recircularized using Klenow and T4 DNA ligase and used to transform bacteria. All procedures were done according to the manufacturer's instructions (Erase-a-Base system; Promega). Plasmids containing the deletion mutants were then subjected to PCR using the Bluescript forward and reverse sequencing primers. The PCR products, containing the T3 RNA polymerase promoter site upstream from the cDNA inserts, were then used for the synthesis *in vitro* of capped RNA transcripts. All mutations and deletions were confirmed by sequence analysis. For each construct, oocytes were injected with 50 ng of RNA transcripts and 4 days later 5D and 5'D activities were determined as described above. Activity was compared with that obtained in oocytes injected with an equivalent amount of RNA synthesized from the wild-type cDNA in either the pAlter vector (for mutational analysis) or Bluescript (for deletional analysis).

**Animal Experiments.** *X. laevis* tadpoles were maintained in deionized water. Developmental stages were determined according to Nieuwkoop and Faber (18). Tails were obtained from stage 54, 60, and 63 tadpoles, some of which had been injected with T$_3$ (1 nmol/g of body weight, i.p.) 2–4 days before sacrifice. Tissue was homogenized in 3 vol of 0.25 M sucrose/0.02 M Tris·HCl, pH 7.6, for determination of 5D activity or 0.25 M sucrose/0.02 M Tris·HCl, pH 7.0/1 mM EDTA for determination of 5'D activity. Assays were done as described above, except that the 5'D assay used 1.5 nM [$^{125}$I]rT$_3$ as substrate. The 50-μl reaction mixture contained 10–50 μg of tail homogenate protein.

**Cell Culture Experiments.** Cultured XTC, XLA, and XL58 *X. laevis* cells were grown and maintained as described in 70% (vol/vol) Leibovitz medium/10% (vol/vol) fetal bovine serum (GIBCO/BRL) that had been depleted of TH by incubation with AG1-X8 resin (Bio-Rad) (19), gentamycin sulfate (100 μg/ml), and 10 mM Hepes (pH 7.5) at 25°C (20).

```
   1 CGGAGGGGGTGAGGGCTGAGCACCATGTTGCACTGCGCGGGACCCCACACCGGTAAACTT
  61 GTGAAACAGGTGGCCGCCTGCTGCCTGCTGCTGCCCCGCTTCCTGCTCACGGGGCTGATG
 121 CTGTGGCTGCTGGATTTCCAGTGTATCAGGAGGAGGGTCCTGTGACCGCCAGGGAGGAGA
 181 GCACCGCCGAGCACGAAGACCCCCCGCTGTGCGTGTCCGACTCCAACCGAATGTGCACCG
                                                       M  C  T  V
 241 TGGAGTCGCTGCGAGCCGTGTGGCACGGGCAGAAGCTGGACTACTTCAAGTCGGCGCACC
       E  S  L  R  A  V  W  H  G  Q  K  L  D  Y  F  K  S  A  H  L
 301 TGGGCTGCTCGGCGCCCAACACGGAGGTGGTGATGCTGGAAGGGCGCAGGCTGTGCAAGA
       G  C  S  A  P  N  T  E  V  V  M  L  E  G  R  R  L  C  K  I
 361 TCCTGGACTTCTCCCAGGGCAAGAGACCGCTGGTTGTCAATTTCGGCAGCTGCACCTGAC
       L  D  F  S  Q  G  K  R  P  L  V  V  N  F  G  S  C  T  SC P
 421 CCCCGTTCATGGCTCGCCTGCAAGCCTATCGCCGCCTGGCAGCCCAGCACGTTGGCATCG
       P  F  M  A  R  L  Q  A  Y  R  R  L  A  A  Q  H  V  G  I  A
 481 CGGATTTCCTGCTGGTGTACATAGAAGAAGCGCACCCGTCAGACGGCTGGCTCAGCACCG
       D  F  L  L  V  Y  I  E  E  A  H  P  S  D  G  W  L  S  T  D
 541 ACGCCTCCTACCAAATCCCCCAGCACCAGTGCCTGCAGGACCGCCTGGCCGCGGCTCAGC
       A  S  Y  Q  I  P  Q  H  Q  C  L  Q  D  R  L  A  A  A  Q  L
 601 TCATGCTCCAAGGGGCGCCCGGCTGCCGGGTGGTGGTGGACACCATGGACAACTCCTCCA
       M  L  Q  G  A  P  G  C  R  V  V  V  D  T  M  D  N  S  S  N
 661 ACGCCGCCTACGGTGCCTACTTTGAGAGACTTTACATCGTCCTGGAGGGCAAAGTGGTGT
       A  A  Y  G  A  Y  F  E  R  L  Y  I  V  L  E  G  K  V  V  Y
 721 ACCAGGGGGGTCGGGGGCCGGAGGGCTACAAGATCTCTGAACTGAGGATGTGGCTGGAGC
       Q  G  G  R  G  P  E  G  Y  K  I  S  E  L  R  M  W  L  E  Q
 781 AGTACCAGCAGGGCTTGATGGGGACCAAGGGCAGCGGCCAAGTGGTCATTCAAGTGTAAT
       Y  Q  Q  G  L  M  G  T  K  G  S  G  Q  V  V  I  Q  V  *
 841 TGTCATCAGCAGCAGCAGCACCAAGGCAACGGGACACAATAACCACCACCAGCAGCAGCA
 901 GCAGTATTATTACTATTGTTATTATTATTGTCATTATTATAGAGGCAGGTGGAACCTGTT
 961 AGGTGAAGTGACTGAAAGTACACAAAAAGTGCGACCAAACGACTCCTTTCTTTAAATCCC
1021 AGTGCGACAAATAGTAGTAAACTGCAACAAGGGAAAGGCATCCCATCTGCGCACCTCGGG
1081 CTCAATCGCAACTTCCAACACGTCCAGTCCCCCCGACTCATCAGGGAGTTGCCATTGAAC
1141 AAATGCCGGAGGGTCGCGGTTCAGATGTCATTGCGAGAATAATAAGCTACAGTGGCTGTC
1201 TGTCTGTCCCCAGCTGTGTGTGCGACTAAGCCGCTGTGTGAAGTGGGGCGGGAGTACAAG
1261 GTGCGTGTGACTGGAGCCACCCACTCCGACTCTGCAGGTGTTTGCAAATGACGACCGATT
1321 TTGAAATGGTCTCACGGCCAAAAACTCGTGTCCGACATCAACCCCCTTCCTCAAACTACC
1381 TGCAGGCTCCGGTGTCGGGCTCCTCCAGTCCAGTAGATTTATTTATGTGATTTTTGTAAG
1441 CAGACTTTTATATAAAGGATTTTTTACGATTAAAAACATGACCACATAAAAAAAAAAAAA
1501 AAAAAAAAAA
```

FIG. 1. Nucleotide and deduced amino acid sequence of the XL-15 cDNA. Underlined nucleotide sequences in the open reading frame represent five regions with significant homology to the rat G21 5'DI cDNA (7). Underlined amino acid residues have been conserved between the XL-15 and G21 proteins. The underlined nucleotides in the 3'-untranslated region between 1306 and 1351 are similar to the selenocysteine insertion element required to translate the UGA codon into a selenocysteine residue (22). The double-underlined nucleotides are identical to the eukaryotic consensus sequences of this element (22).

Cells were treated with 5 nM $T_3$ for 48 hr before harvesting. For Northern analysis, RNA was extracted from cells, electrophoresed, blotted, and probed as described (20). For deiodinase determinations, cells were harvested, washed, and sonicated according to published methods (21). Protein concentrations in the sonicated preparations were 14.1–15.2 mg/ml. 5D and 5'D activities were assayed as described above using undiluted cell sonicates or dilutions of the sonicates of 1:5 to 1:200 depending on the relative amount of activity. XTC and XL-58 cells were from I. Dawid (National Institutes of Health) and R. Steele (University of California, Irvine), respectively.

## RESULTS

The nucleotide sequence of the 1.5-kb *X. laevis* XL-15 cDNA is shown in Fig. 1. Comparison of this sequence with that of the rat G21 5'DI cDNA isolated by Berry *et al.* (7) reveals five regions with 53–75% nucleotide identity. The first region contains an in-frame TGA triplet which in the G21 cDNA encodes for selenocysteine. Assuming a similar function for this codon in XL-15, an open reading frame of 606 nt encoding a protein of 202 amino acids with a molecular mass of 22 kDa is predicted from the sequence. Amino acid conservation with the G21-encoded protein within the 162-residue central region of the XL-15 protein is 50%, including two histidine residues encoded by codons at nt 513 and 564. These histidines have been shown previously to be critical for the activity of the rat 5'DI (23). Nucleotide sequences within the 3'-untranslated region of XL-15 (nt 1306–1351) are similar to consensus sequences required in eukaryotes to translate the UGA codon with the amino acid selenocysteine (22).

The protein encoded by XL-15 was characterized by expression in *X. laevis* oocytes. The 5'DI encoded by the G21 cDNA was expressed in other oocytes in the same experiments, so that the properties of these two proteins could be compared directly. Results of kinetic analyses of the expressed activities are shown in Table 1. As we and others have demonstrated previously, the injection into oocytes of G21-derived RNA transcripts induces 5'D activity with a $K_m$ value for $rT_3$ of 0.1–0.2 $\mu$M when assayed in the presence of 5–20 mM dithiothreitol (7, 15). No 5D activity was detected in these oocytes when $T_3$ was used as substrate. In contrast, oocytes injected with XL-15-derived RNA transcripts contained abundant 5D activity with a $K_m$ for $T_3$ of 2 nM. Maximal 5D activity was noted at a dithiothreitol concentration of 200 mM, whereas 5 or 50 mM glutathione did not stimulate 5D activity (data not shown). Although 5'D activity could also be demonstrated in concentrated microsomal preparations from these oocytes, this reaction was catalyzed much less efficiently than 5-deiodination, as reflected by the 700-fold greater value of the $V_{max}/K_m$ ratio for the latter process. Unlike the G21-derived 5'DI (15), glutathione (5 or 50 mM) or a reconstituted thioredoxin cofactor system did not support a significant level of 5'-deiodination by the XL-15-encoded deiodinase (data not shown).

The sensitivity of the G21- and XL-15-encoded deiodinases to inhibition by PTU and AThG was compared. As shown in Fig. 2, and consistent with previous studies by Berry *et al.* (7), the G21-encoded 5'D activity was sensitive to inhibition by these two compounds, a property demonstrated to require a selenocysteine residue at the active site of the enzyme (8). In contrast, the XL-15-encoded enzyme was resistant to inhibition by these agents; as compared with the G21-expressed activity, 100-fold or greater concentrations of the two compounds were required to inhibit both 5D and 5'D activities in membranes prepared from XL-15-injected oocytes.

Table 1. G21- and XL-15-encoded expression of deiodinase activity in *X. laevis* oocyte membranes

| cDNA | Activity | $K_m$, $\mu$M | $V_{max}$,* units | $V_{max}/K_m$ |
|---|---|---|---|---|
| G21 | 5' | 0.18 | 12.1 | 66.3 |
| G21 | 5 | | No activity detected | |
| XL-15 | 5' | 0.44 | 0.5 | 1.2 |
| XL-15 | 5 | 0.002 | 1.3 | 727.0 |

In two additional experiments, similar kinetic data were obtained.
*Units = pmol/min·mg of protein.

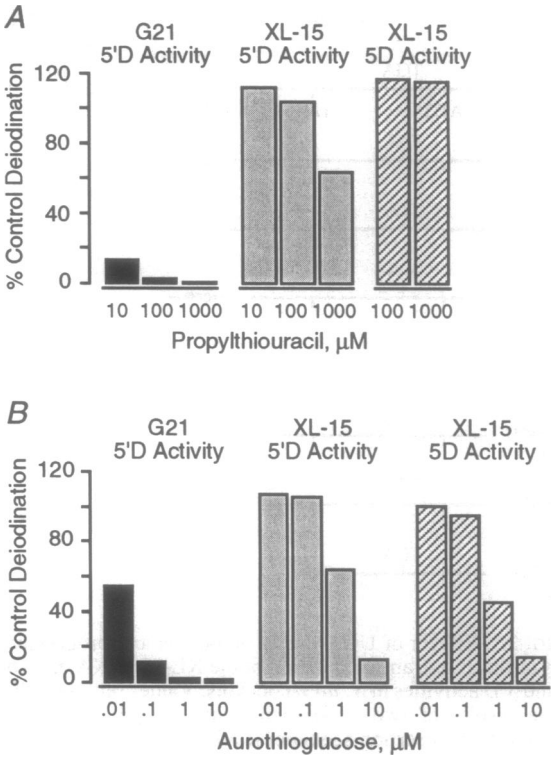


FIG. 2. Effects of deiodinase inhibitors on expressed 5D and 5'D activities in *X. laevis* oocytes. (*A*) Effect of PTU on XL-15- and G21-encoded activities. (*B*) Effect of AThG on XL-15- and G21-encoded activities. Single assays were done for each data point. Control incubations in the absence of inhibitors were done in duplicate with a coefficient of variation of <5%. Essentially identical results with both compounds were obtained in a second experiment.

This resistance of the XL-15 deiodinase to inhibition by PTU and AThG raised the possibility that the encoded protein is not a selenoprotein or that selenocysteine is not involved in the catalytic activity. To investigate this further, site-directed mutagenesis of the XL-15 cDNA was done to alter the presumed selenocysteine-encoding TGA codon to another stop codon (TAA) or one encoding leucine (TTA) or cysteine (TGT). Fig. 3 shows complete loss of both 5D and 5'D activity with the stop and leucine mutant constructs. The cysteine mutant retained ≈24% of the wild-type 5D activity, but no 5'D activity could be demonstrated, further emphasizing that the XL-15-encoded deiodinase is primarily an inner ring deiodinase. Deletion analysis of sequences in the 3'-untranslated region demonstrated that nt 1187–1382, which include the putative selenocysteine insertion element, are necessary for the expression of a functional deiodinase (Fig. 3).

Confirmation of the identity and physiological significance of the XL-15 deiodinase was sought by comparing the levels of 5D and 5'D activity with the patterns of expression of the XL-15 mRNA in the tail from control and $T_3$-treated *X. laevis* tadpoles and in several *X. laevis* cell lines, cultured with and without $T_3$. As shown in Fig. 4, 5D activity in the tail from tadpoles not exposed to $T_3$ increased as tadpoles progressed

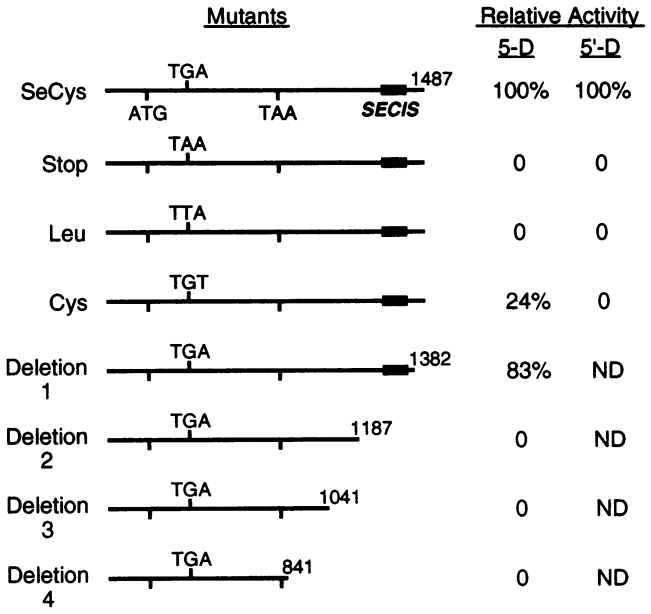


FIG. 3. Effects of UGA codon mutations and progressive deletions of the 3'-untranslated region of the XL-15 cDNA on expressed 5D and 5'D activities in *X. laevis* oocytes. Values represent the mean of the results of two experiments that agreed closely. SECIS, selenocysteine insertion sequences; ND, not determined.

from stage 54 (premetamorphosis) to stage 60 (climax) and then declined at stage 63. This pattern is identical to that previously noted for the expression of XL-15 mRNA in the tail (13). The marked stimulation of 5D activity by $T_3$ in tail from stage 54 tadpoles is also consistent with previous observations showing rapid up-regulation of the mRNA by $T_3$ (13). In contrast, 5'D activity differed little in tail tissue from stage 54 and 60 tadpoles and was not stimulated by $T_3$ at stage 54 (Fig. 4), suggesting that this activity was catalyzed by a different deiodinase. Consistent with this thesis, the $K_m$ value for 5'D activity in stage 60 tadpole tail homogenates using $rT_3$ as substrate was 35 nM, a value markedly lower than that induced in oocytes by the injection of XL-15 transcripts (Table 1). The inclusion of PTU (100 $\mu$M) in tail homogenates did not inhibit either 5- or 5'-deiodination (data not shown), indicating that activity typical of the 5'DI was not present.

The expression of XL-15 mRNA differed considerably in three *X. laevis* cultured cell lines (Fig. 5*A*). Basal expression of mRNA is exceedingly low in XTC cells, but it was induced markedly after exposure for 48 hr to 5 nM $T_3$. This induction is resistant to inhibitors of protein synthesis, indicating that up-regulation of the XL-15 gene in XTC cells is a direct response to TH (A.K. and D.D.B., unpublished observation). XLA cells contain a relatively high basal level of XL-15

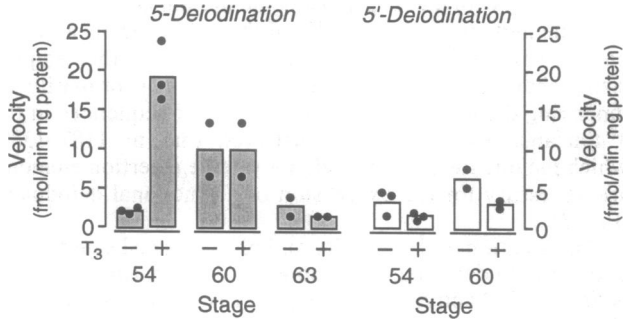


FIG. 4. 5D and 5'D activity in stage 54, 60, and 63 *X. laevis* tadpoles tail tissue untreated (−) or pretreated (+) with $T_3$. Points represent separate homogenate preparations.

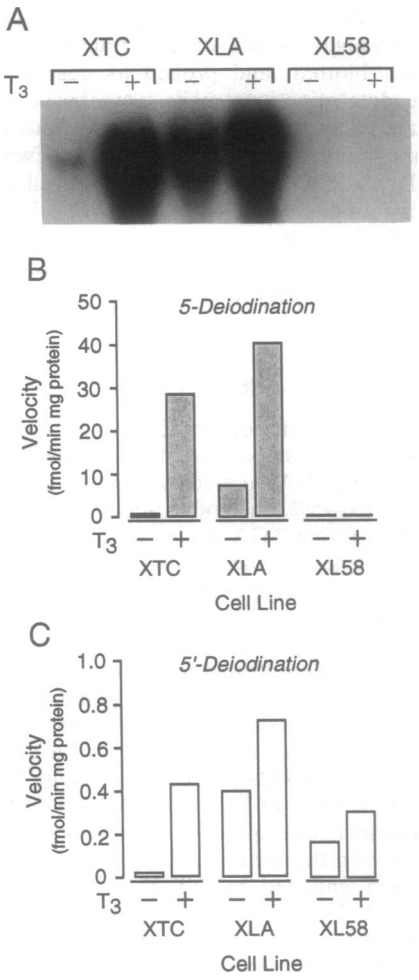


FIG. 5. Relative levels of XL-15 mRNA and deiodinase activities in *X. laevis* cultured cell lines. (*A*) Northern analysis of XL-15 mRNA in cultured cell lines treated with (+) and without (−) $T_3$. (*B*) 5D activity in cultured cells treated with (+) and without (−) $T_3$. (*C*) 5'D activity in cultured cells treated with (+) and without (−) $T_3$. Each bar represents the result of a single determination. Essentially identical results were obtained in a second experiment.

mRNA, which increases further after $T_3$ treatment. XL58 cells exhibit yet a third pattern of XL-15 expression; the level of mRNA is undetectable in the basal state and is unaffected by $T_3$. The pattern of 5D activity in these cell lines is identical to that of XL-15 mRNA expression, whereas the pattern of 5'D activity is not (Fig. 5 *B* and *C*). Furthermore, the $K_m$ value for $T_3$ of the 5D activity in the XTC and XLA cell lines is 3 nM, providing further evidence that it is encoded by the XL-15 gene. It is notable that the XL58 cells, which exhibit no detectable XL-15 mRNA, contain significant amounts of a 5'D activity (Fig. 5*C*), which manifested a relatively low $K_m$ when $rT_3$ was used as substrate (24 nM), suggesting, as in the case of the tadpole tail, that this outer ring activity is encoded by a gene other than XL-15. PTU (100 $\mu$M) did not inhibit either 5D and 5'D activity in any of the cultured cell extracts (data not shown).

## DISCUSSION

These studies show that the XL-15 cDNA encodes a deiodinase that differs markedly in properties from the G21-encoded rat 5'DI, despite the similarities in their sequences—including the presence of selenocysteine. Indeed, the XL-15 enzyme possesses all the characteristics of a 5DIII, including a $K_m$ value in the nanomolar range for $T_3$, requirements for

relatively high dithiothreitol concentrations, and insensitivity to inhibition by PTU and AThG (11, 24). It thus represents the only cDNA isolated for this class of deiodinases and the only nonmammalian deiodinase cDNA to be characterized. The finding that the XL-15-encoded deiodinase also catalyzes 5'-deiodination, although at a considerably lower catalytic efficiency, is unexpected. The properties of this 5'D activity (high $K_m$ for $rT_3$ and resistance to inhibition by PTU and AThG) are remarkably similar to those recently described for an unusual 5'D activity found in the kidney of a teleost fish (tilapia) (25). Unfortunately, the presence or absence of 5D activity in the tilapia kidney was not reported.

The close correlation in the patterns of expression of XL-15 mRNA and 5D activity in *X. laevis* tail and cultured cells lends further support to the conclusion that this cDNA encodes the 5DIII. In this regard, a notable property of the XL-15 gene is that it is markedly and rapidly induced by $T_3$ before metamorphic climax (13), a feature consistent with the postulated protective role of the 5DIII during development. The finding that exogenous $T_3$ does not stimulate XL-15 expression during metamorphic climax in *X. laevis* (stages 60 and 63) is likely due to the fact that this and other $T_3$-responsive genes are already fully induced by the high levels of endogenous hormone present at these stages. This has been shown to be the case in *Rana catesbeiana* tadpoles (26). Both *X. laevis* tadpole tail and cultured cells were noted in these studies to express a 5'D activity the characteristics of which (insensitivity to PTU and $K_m$ value in the nanomolar range using $rT_3$ and dithiothreitol as substrate and cofactor, respectively) match most closely those of the mammalian type II 5'D (24). These findings are analogous to those reported (1) in *R. catesbeiana* tadpoles, where only types II and III deiodinase activities have been described.

These studies demonstrate that, like the G21-encoded 5'DI (7), selenocysteine is critical for the catalytic activity of the XL-15-encoded enzyme, a finding consistent with the significant nucleotide and amino acid conservation surrounding the selenocysteine codons in these proteins. However, given that the sensitivity of the mammalian 5'DI to PTU and AThG has been attributed to the presence of selenocysteine at its active site (8), it is surprising that the XL-15-encoded 5DIII contains this uncommon amino acid. This result indicates that factors in addition to the presence of selenocysteine can influence sensitivity of the enzyme to inhibition by these compounds. As a corollary, resistance to PTU and AThG cannot be used as a criterion to exclude the presence of selenocysteine in deiodinase enzymes, raising the possibility that as-yet-uncharacterized deiodinases (such as the type II or mammalian type III) may also be selenoproteins.

These studies were supported by grants from the National Institutes of Health (D.L.S.G., D.D.B., and V.A.G.) and the Mathers Foundation (D.D.B.). R.A.S. is a fellow of the American Cancer Society.

1. Galton, V. A. (1992) *Trends Endocrinol. Metab.* **3**, 96–100.
2. St. Germain, D. L. (1994) *Trends Endocrinol. Metab.* **5**, 36–42.
3. Kaplan, M. M. (1986) in *Thyroid Hormone Metabolism*, ed. Hennemann, G. (Dekker, New York), pp. 231–253.
4. Galton, V. A. & Hiebert, A. (1987) *Endocrinology* **120**, 2604–2610.
5. Galton, V. A., McCarthy, P. T. & St. Germain, D. L. (1991) *Endocrinology* **128**, 1717–1722.
6. Visser, T. J. & Schoenmakers, C. H. H. (1992) *Acta Med. Austriaca* **19**, Suppl. 1, 18–21.
7. Berry, M. J., Banu, L. & Larsen, P. R. (1991) *Nature (London)* **349**, 438–440.
8. Berry, M. J., Kieffer, J. D., Harney, J. W. & Larsen, P. R. (1991) *J. Biol. Chem.* **266**, 14155–14158.
9. Berry, M. J., Kieffer, J. D. & Larsen, P. R. (1991) *Endocrinology* **129**, 550–552.
10. Safran, M., Farwell, A. P. & Leonard, J. L. (1991) *J. Biol. Chem.* **266**, 13477–13480.
11. Santini, F., Chopra, I. J., Hurd, R. E., Solomon, D. H. & Teco, G. N. (1992) *Endocrinology* **130**, 2325–2332.
12. Buckbinder, L. & Brown, D. B. (1992) *J. Biol. Chem.* **267**, 25786–25791.
13. Wang, Z. & Brown, D. D. (1993) *J. Biol. Chem.* **268**, 16270–16278.
14. Wang, Z. & Brown, D. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11505–11509.
15. Sharifi, J. & St. Germain, D. L. (1992) *J. Biol. Chem.* **267**, 12539–12544.
16. St. Germain, D. L. & Croteau, W. (1989) *Mol. Endocrinol.* **3**, 2049–2053.
17. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
18. Nieuwkoop, P. D. & Faber, J. (1956) *Normal Table of Xenopus laevis Daudin* (North-Holland, Amsterdam).
19. Samuels, H. H., Stanley, F. & Casanova, J. (1979) *Endocrinology* **105**, 80–85.
20. Kanamori, A. & Brown, D. D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6013–6017.
21. St. Germain, D. L. (1988) *Endocrinology* **122**, 1860–1868.
22. Berry, M. J., Banu, L., Harney, J. W. & Larsen, P. R. (1993) *EMBO J.* **12**, 3315–3322.
23. Berry, M. (1992) *J. Biol. Chem.* **267**, 18055–18059.
24. Leonard, J. L. (1991) in *Thyroid Hormone Metabolism: Regulation and Clinical Implications*, ed. Wu, S. (Blackwell, Boston), pp. 1–28.
25. Mol, K., Kaptein, E., Darras, V. M., de Greef, W. J., Kühn, E. R. & Visser, T. J. (1993) *FEBS Lett.* **321**, 140–144.
26. Galton, V. A. & St. Germain, D. L. (1985) *Endocrinology* **117**, 912–916.