The thyroid hormone receptor (TR) β genes in Xenopus laevis are regulated by thyroid hormone in all organs of an animal during metamorphosis. This autoregulation appears to be critical for systematic transformations of different organs as a tadpole is transformed into a frog. To understand this autoregulation, we have previously identified a thyroid hormone response element in the hormone-dependent promoter of the X. laevis TRβA gene. We report here the detailed characterization of the promoter. We have now mapped the transcription start site and demonstrated the existence of an initiator element at the start site critical for promoter function. More important, our deletion and mutational experiments revealed a novel upstream DNA element that is located 125 base pairs upstream of the start site and that is essential for active transcription from the promoter. Promoter reconstitution experiments showed that this novel element does not function as an enhancer, but acts as a core promoter element, which, together with the initiator, directs accurate transcription from the promoter. Finally, we provide evidence for the existence of a protein(s) that specifically recognizes this element. Our studies thus demonstrate that the TRβA promoter has a unique organization consisting of an initiator and a novel upstream promoter element. Such an organization may be important for the ubiquitous but tissue-dependent temporal regulation of the gene by thyroid hormone during amphibian metamorphosis.

Thyroid hormone (T3) is the causative agent of amphibian metamorphosis, a process that systematically changes most, if not all, organs of a tadpole to prepare the animal for adult terrestrial life (1–3). The hormone is known to regulate the transcription of target genes through their nuclear receptors or thyroid hormone receptors (TRs) (4–11). Thus, it is believed that T3 induces a cascade of gene regulation in each tissue or organ to effect the metamorphic transition (12). Many T3 response genes have been isolated from various metamorphosing tadpole tissues, and their developmental expression profiles have implicated potential roles during metamorphosis (12–14).

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¶ The abbreviations used are: T3, thyroid hormone; TR, thyroid hormone receptor; TRE, thyroid hormone response element; bp, base pairs; PCR, polymerase chain reaction; UPE, upstream promoter element; Inr, initiator; RXR, retinoid X or 9-cis-retinoic acid receptor.

Among the T3 response genes are the TR genes themselves. Two TRα and two TRβ genes have been isolated from Xenopus laevis (15, 16), whereas only one TRα gene and one TRβ gene have been cloned from Rana catesbeiana (17, 18). Consistent with their roles in mediating T3 effects, all TR genes are expressed during metamorphosis and can be up-regulated by T3 treatment of premetamorphic tadpoles (17–20). In particular, the Xenopus TRβA genes have been shown to be directly regulated by T3 at the transcriptional level (21–24). This T3 regulation appears to be mediated mostly by a thyroid hormone response element (TRE), consisting of two near-perfect repeats of AGGTCA separated by 4 bp. Interestingly, promoter studies using transient transfection assays in frog tissue culture cells failed to identify any other elements necessary for the TRβA promoter due to the lack of information on the transcription start site (22, 23).

Promoters recognized by RNA polymerase II generally contain a TATA box and/or an initiator that directs specific transcription initiation. In many genes, the TATA element is the primary core element responsible for positioning the basal transcription machinery on the promoter (25–27). However, many other genes lack a TATA element and, instead, contain an initiator. The initiator encompasses the transcription start site and is sufficient to position the basal transcription complex. This specific positioning of the basal transcription machinery at a promoter by a TATA and/or initiator element allows basal transcription, which can be enhanced by transcription activators. To determine the nature of the TRβA promoter, we have now mapped its start site by introducing the TRβA promoter into Xenopus oocytes and analyzing the resulting transcript by primer extension and by PCR cloning of the 5’-end of TRβA mRNA from tadpoles. Deletion and mutational studies demonstrated a unique nature of the TRβA promoter, consisting of a novel promoter element and an initiator, thus different from the two major classes of RNA polymerase II promoters mentioned above. We further show that specific proteins exist to recognize the novel promoter element, thus likely allowing specific transcription initiation and activation by TRs.

MATERIALS AND METHODS

Plasmid Constructs—The wild-type pTRβA promoter construct was generated by cloning a 1.9-kilobase EcoRI fragment containing 1.6 kilobases of TRβA promoter sequence and ~0.3 kilobase of chloramphenicol acetyltransferase gene sequence from plasmid pCAT-WT (22) into pBlueScript II KS(−) (Stratagene). To make a construct of the promoter with a 5’- and/or a 3’-deletion (bearing a HindIII restriction site and located at an appropriate position for the desired deletion) and a 3’-primer (bearing a BglII restriction site and located at a downstream position for the desired deletion) were used to PCR-amplify a promoter fragment from the wild-type template. The amplified fragment was then cloned into the wild-type plasmid after removing the promoter sequence by HindIII and BglII digestions.
A Novel Element for TRβ mRNA Transcription

To make a specific mutation in the UPE or Inr region, a specific primer bearing the desired mutation was used to prime second strand DNA synthesis on a single-stranded wild-type construct using a transfection kit (Amersham Pharmacia Biotech). The resulting DNA was transformed into *Escherichia coli* to obtain the mutant promoter plasmid. A single-stranded DNA was prepared using a QIAGEN kit as described by the manufacturer. The RNA antisense primer (5'-GTTACATCTTGACTATTATGCA-3') was included in the primer extension reaction to quantify the endogenous H4 mRNA level.

**PCR Cloning of the 5' End of the TRβ mRNA**—The anchor PCR cloning procedure was performed according to Frohman et al. (32) with slight modifications. Ten micrograms of stage 64 tadpole RNA were reverse transcribed as described (22). 5'-AAAAGCTTGAAGTGAGATGATTATAATAA-3' and 5'-AGGTTAATATAATATCTCCTCAATGAAGTTGAGATGATTATAATAA-3' were used as the forward and reverse primers, respectively. The cDNAs were enriched by phenol extraction, phenol/chloroform extraction, and ethanol precipitation and were resuspended in 1× Tris/EDTA. One-fifth of the cDNAs were precipitated again with ethanol in an ammonium acetate buffer and resuspended into 20.6 μl of 1× Tris/EDTA. For tailing, 2.4 μl of 2.5 mM dATP, 6 μl of 5× tailing buffer (Life Technologies, Inc.), and 1× of terminal deoxynucleotidyltransferase (Life Technologies, Inc.) were added, and the mixture was incubated for 12 min at 37 °C and heated for 15 min at 65 °C. The reaction mixture was diluted to 500 μl in 1× Tris/EDTA, and 5-μl aliquots were used for amplification in 50 μl of PCR mixture (5 μl of 10× Taq polymerase buffer with MgCl₂ (Promega), 0.8 μl of 25 mM dNTP, 0.65 μl of 0.1 μg/ml of Taq DNA polymerase, 0.8 μl of 0.1 μg/ml primer I, and 2.5 units of Taq DNA polymerase (Promega)) for adapter primer sequence, ref. 32). Using a DNA thermal cycler (Perkin-Elmer), the mixture was denatured at 94 °C for 4 min, annealed at 42 °C for 2 min, and extended at 72 °C for 30 min before 40 cycles of amplification using a step program (94 °C, 40 s; 55 °C, 2 min; and 72 °C, 3 min), followed by a 30-min final extension at 72 °C. PCR products were cloned with the Original TA Cloning kit (Invitrogen). Individual clones were isolated and sequenced with the T7 Sequenase Version 2.0 DNA sequencing kit (Amersham Pharmacia Biotech). Seven independent TRβ cDNA clones were obtained that had only a few base changes (different among different clones) compared with the TRβ genomic clone. These changes were likely derived from PCR errors due to the use of Taq polymerase and/or sequence polymorphisms. However, all had their 5'-end at position +1 or +2.

**Gel Mobility Shift Assay**—Two nanograms of 32P-labeled double-stranded oligonucleotides were mixed with 9 μg of cell extract, made from *X. laevis* tissue culture cell line XL58 as described (22), in 20 μl of 1× binding buffer (20 mM Tris-HCl (pH 7.5), 40 mM NaCl, 5 mM MgCl₂, 1 mM dithiothreitol, 0.1% Triton X-100, and 10% glycerol) containing 1 μg of poly(dI-dC). After a 20-min incubation at room temperature, the reaction mixture was separated directly on a 5% polyacrylamide gel (runner and stacking; Tris borate/EDTA) at 4 °C for 4 h. The gel was dried and exposed to film to visualize TRβ.

**RESULTS**

**Promoter Analysis in Frog Oocytes Identifies a Minimal Region Essential for TRβ mRNA Transcription**—The two *X. laevis* TRβ genes (TRβA and TRβB) are regulated identically during development and by thyroid hormone (16, 33). Genomic structure analyses have revealed that each TRβ gene produces mRNAs with two alternative 5'-ends, i.e., having two different 5'-exons (exons a and b, respectively) (33). Although the relative locations of the two exons, i.e., whether exon b is located upstream of exon a or vice versa, have yet to be determined, both exons are independently transcribed (21, 33). The expression from the promoter upstream of exon a is maintained at low but constitutive levels. In contrast, the promoter upstream of exon b is repressed in the absence of T3, but is activated to high levels when T3 is present. We (22) and others (23) have previously analyzed this T3 inducible promoter (upstream of exon b) of the TRβA gene and identified a strong TRE that mediates the strong autoduction of the receptor gene (Fig. 1A). However, the start site of the TRβA promoter had not been determined, possibly due to the low abundance of the mRNA in vivo or generated from transcription (22). Thus, it has not been possible to identify any elements other than the TRE that are necessary for reporter gene expression in transient transfection assays (22).

To determine the transcription start site, we microinjected a single-stranded reporter construct, which contained 46 bp of
the previously reported exon b (16) and another 1.6 kilobases of upstream sequence, into *Xenopus* oocytes in the presence or absence of overexpressed TR/RXR heterodimers and/or T3. The single-stranded DNA is known to be replicated into the double-stranded form and assembled into chromatin through a replication-coupled assembly process, mimicking that in somatic cells (24, 29). Primer extension analysis showed that there was a very low level of transcription from the promoter in the absence of T3 and TR/RXR (Fig. 1B). Overexpression of TR/RXR in the absence of T3 caused a further reduction of transcription. In the presence of both T3 and TR/RXR, the transcription was strongly activated, in agreement with the early transfection studies. Both the basal and T3-induced transcripts initiated from position +1 (Fig. 1, A and B), 264 bp upstream of the 5‘-end of the published exon b sequence (16). Thus, the previously published exon b sequence was derived from a partial mRNA clone truncated at the 5‘-end, and the TRE is located 262 bp downstream of the transcription start site (Fig. 1A).

To confirm that the start site identified in the oocyte is also used in tadpoles, the anchor PCR or rapid amplification of cDNA ends PCR method (32) was used to clone the 5‘-end of TRβA mRNA. For this purpose, stage 64 tadpole RNA was reverse-transcribed with a TRβA-specific primer and PCR-amplified with another specific primer and the adapter primer. The resulting cDNA was cloned, and individual clones were isolated and sequenced. Seven independent TRβA cDNA clones were obtained. Three clones had their 5‘-ends at position +1, and four clones at position +3 (Fig. 1A). These results indicate that position +1 is also the start site in metamorphosing tadpoles when TRβ genes are highly expressed (20, 31).

As earlier transfection studies showed that TRβA plasmid constructs with 5‘-deletions passing site +1 still gave strong reporter signal (Ref. 22, where position +1 is equivalent to
position +270 in this paper), we were interested to determine whether these constructs were able to direct accurate transcription in frog oocytes. For this purpose, we microinjected the same double-stranded plasmid constructs used in the earlier studies into frog oocytes. Since double-stranded plasmids do not undergo replication and consequently are assembled through a different pathway into a chromatin form that yields high levels of basal transcription (24, 29), it was not necessary to overexpress TR/RXR and/or to add T$_3$ to the oocyte culturing medium. Consistently, primer extension analysis of the RNA

**Fig. 3.** Deletion analyses identify a minimal promoter region located at -154 to +7. 5'-Deletion (A) and 3'-deletion (B) constructs were microinjected into oocytes in double-stranded form, which allowed efficient transcription of the wild-type (WT) promoter in the absence of both T$_3$ and TR/RXR. After overnight incubation, the RNA was isolated and analyzed by primer extension with primer I for the TR$\beta$ transcript and with the H4 primer for the endogenous histone H4 mRNA, which served as an internal control (indicated by arrowheads).
isolated from oocytes injected with the full-length TRbA promoter (wild-type, −1336 to +316) showed that the RNA with the expected size was efficiently transcribed in the oocytes (Fig. 2). However, many of the short constructs failed to produce specific transcripts (data not shown). To define the promoter sequence, several additional deletion constructs were made around the start site and analyzed in oocytes. Constructs with 5'-deletions up to −276 or −172 (pTR6 and pTRp11, respectively) were still able to direct accurate transcription from position +1, as were the constructs with 3'-deletions up to +266, +244, or +8 (pTRp9, pTRp6, and pTRp10, respectively) (Fig. 2). On the other hand, those constructs with 5'-deletions passing −172 (pTRp4, pTRp5, pTRp7, and pTRp8) failed to produce any transcripts from the start site (+1). Thus, these data suggest that the reporter activity detected by chloramphenicol acetyltransferase assay reported for the transient transfection experiments with some of the constructs (22) was most likely due to non specific transcription from the reporter plasmid, which was enhanced by the TRbA DNA sequence inserted in front of the chloramphenicol acetyltransferase coding region through a yet unknown mechanism. Furthermore, these results suggest that the region from −172 to +8 is sufficient for accurate basal transcription from the promoter.

**Deletion and Mutational Analyses Reveal the Existence of a Novel DNA Element and an Initiator in the TRbA Promoter.** To further characterize the DNA sequences necessary for the basal promoter activity, additional 5'- and 3'-deletion constructs were made and injected into oocytes in the double-stranded form. Primer extension analysis showed that 5'-deletion up to −154 (pTRp5'-7) still yielded an active promoter, whereas a further deletion of 13 bp (pTRp5'-6) or more resulted in an inactive promoter (Fig. 3A). Thus, the region from −154 to −141 is an essential element for the activity.

Transcription studies of promoter constructs with additional 3'-deletions showed that the sequences downstream of +7 (pTRp3'-1) were not necessary for promoter activity, whereas deletion to +5 (pTRp3'-2) or −4 (pTRp3'-3) abolished accurate transcription (Fig. 3B). These results suggest that the region around the start site is critical for the promoter, i.e. the presence of an initiator.

Site-directed mutagenesis was then carried out to further define the UPE around −140 and the Inr. Three site-directed mutational constructs were made, each changing 6 residues to the recognition site of NdeI. Two of them were in the upstream region around −140, i.e. the UPE, and one was near the start site, i.e. the Inr. Consistent with the deletion analyses above, mutating residues −148 to −143 abolished the promoter function (Fig. 4, construct UPE/m2). On the other hand, changing residues −130 to −125 had no effect (construct UPE/m1). Thus, the UPE lies within −151 to −130. Similarly, the mutation of residues +6 to +11 to CATATG (construct Inr/m1) inactivated the promoter, confirming that sequence up to +7 is important for initiator function.

Inspection of the UPE and Inr sequences revealed that they are complementary to each other with the exception of a few residues. This prompted us to examine whether the sequences or the complementarity between them is critical for the promoter. We first mutated 5'-CCCGCC-3' in the UPE to 5'-GGGGG-3' (UPE/m3) or 5'-GGGGG-3' in the Inr to 5'-CCCGCC-3' (Inr/m2) and analyzed the promoter activities of the resulting constructs. Both were found to be inactive, although slight activity was detected for UPE/m3 (Fig. 4). This is consistent with the deletion and mutational studies for UPE above and with the fact that initiators require the sequences both upstream and downstream of the start site (26, 27). We then introduced both the UPE and Inr mutations into a single promoter construct. The rationale was that these mutations would maintain the GC content in the mutant promoter compared with the wild-type promoter. In addition, we reestablished the complementarity between the UPE and Inr, and the potential duplex formed between the UPE and Inr would have similar stability. However, the resulting promoter still failed to direct
accurate transcription (Fig. 4, construct UPE/Inr/m). Thus, the sequences of the UPE and Inr are important for promoter function, which is further supported by the detection of proteins that specifically recognize UPE and Inr sequences (see below).

**The Novel DNA Element (UPE) Is Not an Enhancer, but Constitutes an Essential Part of the Basal Promoter**—The inability of the UPE deletion and mutational constructs to support transcription suggests that the UPE may function as either a basal promoter element or an enhancer. In the latter case, the activity of the basal promoter may be too weak to be detected by the primer extension assay (once the UPE is mutated) and should be rescued by adding a different enhancer. To distinguish between these two possibilities, we reintroduced the UPE or a truncated UPE in both orientations into a deletion construct that had no UPE and tested the transcriptional activity of the resulting constructs (Fig. 5). The results showed that the truncated UPE failed to rescue the promoter function (Fig. 5, constructs 5 and 6), as expected. Interestingly, the UPE was able to rescue the promoter when placed in the same orientation as in the wild-type promoter (Fig. 5, construct 4), but it failed to do so when placed in the opposite orientation (construct 3). Thus, the UPE functions in an orientation-dependent manner, thus most likely as a promoter element, not as an enhancer. Consistently, when we placed the UPE in either orientation into a construct containing the enhancerless SV40 early promoter, we found that it failed to alter the promoter activity (data not shown).

To provide further evidence that the UPE is a basal promoter element, we replaced the UPE with other known enhancer elements and analyzed the activity of the resulting promoter constructs. For this purpose, we placed one or three copies of the Gal4 DNA-binding sites were inserted upstream of an active promoter (pTRp5'-7, -154 to +316) or inactive promoter (pTRp5'-1, -63 to +316), both containing the TRE at +262. The original and new constructs were microinjected in the double-stranded form into oocytes with or without prior injection of TR/RXR in the presence of T₃ or Gal4-VP16 mRNA. After overnight incubation, the RNA was isolated and analyzed as in the legend to Fig. 3. The arrowhead and arrow indicate the internal control and TRβ products, respectively. The TRβ product migrated to a similar location as a product derived from non-specific priming of endogenous RNA. Due to the use of 32P-labeled primer I with a lower specific activity and lower levels of TRβ promoter DNA used in this experiment, the signal for the TRβ decreased relative to the nonspecific product. Thus, a signal derived from nonspecific endogenous RNA was observed for the inactive promoter (pTRp5'-1, lanes 3–8; compared with Fig. 3A, lane 8). The inactive promoter and its derivatives were not affected by either TR/RXR or Gal4-VP16 despite the presence of their binding sites. On the other hand, TR/RXR (lanes 1 and 2) and Gal4-VP16 (lanes 9 and 10) were able to activate the functional promoter containing their binding sites.

![Fig. 5. Promoter reconstitution suggests that the UPE functions as a basal promoter element. The truncated promoter (construct 1, -154 to +316) containing the minimal promoter sequence was transcriptionally active (lane 1), whereas a short construct (construct 2, -63 to +316) was inactive (lane 2). Placing the UPE back into construct 2 in the wild-type orientation (construct 4), but not in the opposite orientation (construct 3), restored the promoter activity (lanes 4 and 3, respectively). In contrast, placing a truncated UPE back into construct 2 in either orientation (constructs 5 and 6) did not rescue the promoter (lanes 5 and 6, respectively). Oocyte injection and transcript analyses were done as described in the legend to Fig. 3. The arrowhead indicates the internal control.](image1)

![Fig. 6. Promoter reconstitution experiments confirm that the UPE is essential for basal promoter function. One or three copies of the Gal4 DNA-binding sites were inserted upstream of an active promoter (pTRp5'-7, -154 to +316) or inactive promoter (pTRp5'-1, -63 to +316), both containing the TRE at +262. The original and new constructs were microinjected in the double-stranded form into oocytes with or without prior injection of TR/RXR in the presence of T₃ or Gal4-VP16 mRNA. After overnight incubation, the RNA was isolated and analyzed as in the legend to Fig. 3. The arrowhead and arrow indicate the internal control and TRβ products, respectively. The TRβ product migrated to a similar location as a product derived from non-specific priming of endogenous RNA. Due to the use of 32P-labeled primer I with a lower specific activity and lower levels of TRβ promoter DNA used in this experiment, the signal for the TRβ decreased relative to the nonspecific product. Thus, a signal derived from nonspecific endogenous RNA was observed for the inactive promoter (pTRp5'-1, lanes 3–8; compared with Fig. 3A, lane 8). The inactive promoter and its derivatives were not affected by either TR/RXR or Gal4-VP16 despite the presence of their binding sites. On the other hand, TR/RXR (lanes 1 and 2) and Gal4-VP16 (lanes 9 and 10) were able to activate the functional promoter containing their binding sites.](image2)
A Novel Element for TRβA Transcription

Two nanograms of 32P-labeled double-stranded oligonucleotide containing the UPE were mixed with protein extract from Xenopus tissue culture cells in the presence of 5, 10, 25, and 100 ng of the unlabeled UPE itself, a truncated UPE (mUPE), the Inr, or a mutated Inr (mInr). The resulting complexes were analyzed by gel mobility shift assay. The arrowhead points to the specific complex that was competed away by UPE, but not by mUPE or mInr. The Inr was able to compete due to its sequence similarity to UPE.

used gel mobility shift assay to determine whether proteins exist to recognize the UPE. We isolated protein extracts from Xenopus oocytes and from a Xenopus tissue culture cell line that is known to be able to regulate endogenous TRβ gene expression in a thyroid hormone-dependent manner, just like in tadpoles. When a 32P-labeled UPE oligonucleotide was mixed with the extract, several complexes were formed (Fig. 7). The same results were obtained when oocyte extract was used (data not shown). All of them could be competed away by the unlabeled UPE itself, but the major one could not be competed by the truncated UPE (mUPE) (Fig. 7) or by a mutant UPE bearing the mutations that inactivate its promoter activity (data not shown).

Consistent with the sequence similarity between the UPE and Inr, the Inr oligonucleotide could compete away the major complex. On the other hand, a mutated Inr failed to compete away this complex (Fig. 7). Thus, this complex represents the binding of the UPE by a sequence-specific DNA-binding protein, which also recognizes the Inr, and an intimate correlation exists between the binding of the sequence-specific DNA-binding protein to the UPE and Inr and the TRβA promoter activity.

DISCUSSION

Thyroid hormone receptors mediate the biological effects of thyroid hormone. Perhaps the most dramatic T3-dependent process is amphibian metamorphosis, during which an aquatic tadpole is transformed into a terrestrial frog. As expected, the TR genes are highly expressed during amphibian metamorphosis (17–20, 31, 35–38). Interestingly, the expression of TRβ genes has been shown to be regulated by T3 temporally in a tissue-dependent manner that correlates with tissue-specific changes during development, even though they are regulated by T3 ubiquitously in all organs (31, 37, 39–42), and this regulation is directly mediated by TRs themselves (22, 23). We have shown here that this spatial and temporal autoregulation appears to involve, in addition to a TRE, an initiator and a novel promoter element located ~140 bp upstream of the start site.

Each of the TRβ genes (TRβA and TRβB) in X. laevis is transcribed from two promoters based on transcript analysis (21, 33). One of the promoters is constitutively expressed at low levels, and the other is thyroid hormone-inducible. We (22, 33) and others (23) have shown previously by transient transfection that this T3-inducible promoter of the TRβA gene contains a strong TRE (Fig. 1A), which mediates transcriptional repression by unliganded TR/RXR and activation by T3-bound TR/RXR. However, these earlier studies failed to reveal any other DNA elements critical for promoter function due to the use of an indirect reporter assay and a lack of information on the transcription start site. By directly analyzing the transcripts derived from the promoter both in the absence and presence of TR/RXR and/or T3, we have shown here that the basal and T3-induced transcription starts at 262 bp upstream of the TRE. We have further defined a minimal promoter for accurate transcription that includes the sequence from −154 to +7 relative to the start site. Within this promoter region, no TATA box is present, suggesting that the promoter belongs to the class of TATA-less promoters. Consistently, we have found that the promoter contains an essential initiator, a key feature of TATA-less promoters (26, 27).

Sequence comparison reveals no obvious resemblance of the initiator to any groups of initiators identified so far (25, 43, 44). While transcription from TATA-less promoters usually starts from an A residue, primer extension analysis clearly indicate that a G residue is used. Thus, the TRβ initiator is a novel element.

In addition to the initiator, our deletion and mutational analysis also revealed the existence of an important, novel UPE. The activity of the promoter depends on the presence of both the initiator and the UPE since it is abolished when either one is mutated or deleted. Several lines of evidence argue that the UPE functions as a novel core promoter element, but not as an enhancer. First, the UPE cannot be substituted by single or multiple TRE- or Gal4-binding sites, which, however, can mediate the strong transcriptional activation by liganded TR/RXR or Gal4-VP16, respectively, when the UPE is also present. Second, the function of UPE is orientation-dependent. In general, transcription enhancers function in an orientation-independent manner. The absolute requirement for both the initiator and UPE is exceptional since, in other TATA-less promoters, the initiator is sufficient to direct low levels of basal transcription, although the presence of binding sites for transcription factors like SP-1 can augment promoter activity.

The Xenopus TRβA promoter bears some similarities to the human TRβ promoter (45). Both promoters are autoregulated by TRs themselves, and TREs have been identified in the promoters. In addition, binding sites for transcription factor SP-1 or related factors are present in both genes and are important for human promoter function (46, 47). Although the SP-1 sites are dispensable for Xenopus promoter function in tissue culture cells or oocytes (Fig. 2) (22), it cannot be ruled out that they may play a role in development.

Distinct differences, however, exist between the human and Xenopus TRβ promoters. The human promoter has a TATA-like motif and Oct-1 elements (45–47). The Xenopus promoter lacks such elements. Instead, it contains an initiator element and an upstream promoter element.

A surprising feature of the Xenopus TRβ promoter comes from the sequence similarity between the UPE and initiator (Fig. 1) (22). The UPE and initiator are oriented in opposite directions in the TRβ promoters and thus could potentially form a heteroduplex. Interestingly, no transcription initiated

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2 J. Wong, V. C.-T. Liang, L. M. Sachs, and Y.-B. Shi, unpublished observations.
from the UPE region, but in an opposite direction could be detected using a primer located upstream (5' to 3' direction is toward the UPE) of the UPE (data not shown), indicating that the sequence differences between the UPE and initiator and/or other sequences in the minimal promoter are important for the directionality of the promoter.

We have also tested whether the potential secondary structure formed due to the complementarity of the initiator and UPE is involved in promoter function. Reciprocal mutations that inactivate the UPE or initiator, the identity and nature of this protein(s) will be of particular interest for future studies on TR promoter regulation.

The mutations/deletions that inactivate the UPE or initiator also abolish the ability to compete for binding. Thus, the binding by this protein(s) correlates with the transcription function of the UPE and initiator. The identity and nature of this protein(s) will be of particular interest for future studies on TRβA promoter regulation.

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