The *Xenopus* YB3 protein binds the B box element of the class III promoter

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Received April 1, 1991; Revised and Accepted June 28, 1991

EMBL accession no. X60217

ABSTRACT

We have isolated a *Xenopus* cDNA encoding the YB3 protein which binds specifically to the B box promoter element of class III genes. Northern analysis shows YB3 is expressed in a variety of adult tissues. Fractionation of oocyte S150 extracts demonstrates YB3 is present in phosphocellulose fraction IIIC, as well as in the fraction isolated by B box DNA affinity chromatography. Silver staining indicates that YB3, or a protein of the same mobility in SDS gels, is the most abundant component in either fraction.

INTRODUCTION

RNA polymerase III transcribes a variety of genes including those encoding 5S RNA and tRNAs (for reviews, see 1 and 2). Transcription of tRNA-type genes requires at least two factors known as TFIIB and TFIIC, whereas an additional factor, TFIIA, is required for 5S RNA transcription. The core promoter of tRNA-type genes consists of two separable elements of approximately 10 bp each, known as A and B boxes. TFIIC binds primarily to the B box, but also protects the A box in DNase I footprint assays (3). TFIIC promotes binding by TFIIB (4), which in the yeast system has been shown to bind a region preceding the initiation site (5). The core promoter of 5S RNA genes is a 50 bp internal control region (ICR) (6). TFIIA binds the ICR (7) and facilitates binding by TFIIC (4). Interaction of TFIIB with the 5S gene requires prior binding by both TFIIA and TFIIC (4,8). These factors form a stable complex with the promoter (4,8–12) which remains associated throughout multiple rounds of transcription initiation *in vitro* (10).

The gene encoding *Xenopus* TFIIA has been isolated and the 39K protein is well characterized (13,14). There is evidence that yeast and human TFIIB is a single component (15,16). TFIIC has been partially characterized from several systems including yeast (17–20), human (3,21,22), *Drosophila* (23), and *Bombyx* (24). Most evidence suggests that factor TFIIC is an aggregate of polypeptides with molecular mass of 300–600K. Studies utilizing crosslinking reagents or antibodies indicate yeast TFIIC is comprised of several components ranging in size from 55K to 145K (17–20). Estimates vary as to the subunit composition of human TFIIC. Specific DNA binding activity has been reported for polypeptides ranging in size from 55K to 230K (21,22,25,26).

We have used the expression cloning method (27) in an attempt to identify proteins which bind the B box promoter element, and might therefore be involved in class III gene expression. We have isolated a *Xenopus* cDNA encoding a protein, designated YB3, which binds specifically to the B box.

METHODS

Expression library screening

A *Xenopus* ovary λ gt11 expression library, kindly provided by Richard Harland, was screened by the filter binding protocol (27). The radiolabeled DNA probe contained 10 tandem copies of a synthetic oligonucleotide (gatcAAAGGTTGTGGGTTCCGATTCCCA) containing the B box (underlined) of the OAX gene (satellite I) (28,29). The library was plated at a density of 40,000 plaque forming units (pfu) per 143 cm² dish. The plates were incubated at 42°C for 4 hours until plaques were visible, and then overlaid with nitrocellulose filters impregnated with isopropyl thiogalactoside (IPTG) to induce cDNA expression. After 6 hours at 37°C, the filters were lifted and dried. The bound protein was denatured in 6M guanidine hydrochloride in binding buffer (25 mM NaCl, 5 mM MgCl₂, 7 mM DTT, and 25 mM HEPES, pH 7.9). The bound proteins were renatured by stepwise reduction in the concentration of guanidine hydrochloride. Filters were then blocked by incubating in binding buffer containing 5% non-fat dry milk. The double stranded probe (10⁷ cpm/ml) was incubated with the filters for 1 hour at 4°C in binding buffer containing 0.25% non-fat dry milk and 1 μ g/ml denatured salmon sperm DNA. The filters were then washed briefly in binding buffer and exposed to film.

DNA Sequencing

For sequencing, the cDNA inserts were subcloned into Bluescript vector (Stratagene). Double stranded DNA sequencing was performed using modified T7 polymerase according to the Sequenase II protocol (Stratagene). Sequencing products were resolved on 6% polyacrylamide gels containing 8M urea.

Northern analysis

Total RNA was isolated from *Xenopus* adult tissues and embryos by homogenization in guanidine isothiocyanate, followed by phenol extraction and ethanol precipitation using reagents and procedures supplied by Stratagene. The RNA (10 μ g) was electro-

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phoresed in 1.2% agarose gels containing 1% formaldehyde in 40 mM MOPS, pH 7, 1 mM EDTA, and 10 mM sodium acetate buffer, and then transferred to nitrocellulose filters and baked at 80° for 2 hours under vacuum. The filters were stained briefly in methylene blue to visualize ribosomal RNA, and then hybridized overnight at 42°C with random primed probes (500,000 cpm/ml). Hybridization and prehybridization solutions contained 6×SSPE, 2×Denhardt's reagent, 50% formamide, 0.1% SDS, and 50 µg/ml salmon sperm DNA. Following hybridization, the filters were washed in 1×SSPE, 0.1% SDS at room temperature for 20 minutes, followed by two 20 minute washes at 68°C in 0.2×SSPE.

Fractionation of oocyte S150 extracts

Oocyte S150 extracts were prepared from stage 6 oocytes as previously described (30). Mature oocytes were separated from immature stages by collagenase treatment of whole ovaries. The intact stage 6 oocytes were centrifuged in two additional volumes of buffer A (20 mM TRIS-HCl, pH 7.6, 70 mM KCl, 0.1 mM EDTA, 2 mM DTT, 20% glycerol) in an SW41 rotor for 30 minutes at 30K RPM. The S150 supernatant was then fractionated according to established procedures (31) using phosphocellulose ion exchange resin (Whatman P11). The S150 extract was passed over P11 in buffer A, and washed with ten column volumes of the same buffer. Fraction IIIB was eluted with buffer A containing 350 mM KCl and contained TFIIB activity and the bulk of the RNA polymerase III activity. Fraction IIIC was eluted with 550 mM KCl and contained TFIIC activity. These fractions were dialysed against buffer A and used directly in transcription assays or were electrophoresed in SDS polyacrylamide gels for Western analysis.

B Box DNA affinity chromatography

Multimerized oligos containing either the OAX or Adenovirus VA 1 B box sequences were coupled to CNBr activated Sepharose according to directions provided by supplier (Pharmacia). The VA 1 oligo used was gatcGACGACCGGGTTCGAACCCG-GG, with B box underlined. Oocyte S150 extracts (15 ml) were passed over a 0.5 ml DNA affinity column in the presence of 1 µg/ml poly (dI-dC). The column was then washed with 10 mls of buffer B (20 mM HEPES, pH 7.5, 70 mM KCl, 7 mM DTT, 0.1 mM EDTA), and bound material eluted in 1 ml of buffer B containing 550 mM KCl. This fraction was dialysed and used directly in transcription assays or electrophoresed in SDS-polyacrylamide gels.

In Vitro Transcription Assays

Transcription assays were performed in a 20 µl volume contained 100 ng of pXls11 (32) containing one repeat unit of the Xenopus somatic-type 5S RNA gene in pUC18. Reactions contained 7 µl of fractions TFIIB and TFIIC which was saturating concentration for 100 ng of pXls11. TFIIC was isolated from immature ovaries as 7S particles complexed with 5S RNA, and the protein purified as previously described (33). Transcription reactions contained 1 µl of TFIIC which was saturating concentration for the amount of DNA present. Reaction mixtures also contained 5mM MgCl₂, 2 mM DTT, 0.6 mM ATP, CTP, and UTP, 20 µM GTP and 10 µCi of α³²P GTP. Reaction were incubated at room temperature for 2 hours and then stopped with 5 volumes of 20 mM EDTA, 0.5% SDS. The transcription products were purified by phenol extraction, ethanol precipitation,

and then electrophoresed in sequencing gels. The gels were dried and exposed to film for 2–4 hours.

Generation of antibodies to YB3 fusion protein

An overnight culture of E.coli strain Y1089 was infected with phage λgt11-YB3 and grown at 37°C for 1 hour in the presence of 10 mM IPTG to induce expression of the fusion protein. The bacteria were lysed in SDS gel loading buffer containing 2.5% SDS and electrophoresed in 5% polyacrylamide-SDS gels. The gel was briefly stained in 0.05% Coomassie blue and a gel slice

A

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1      TCGGCCACCGCGGCTCAGTTACCATCAACACCGCGGGGAAGTTAAAGCGAGC
58     CAACGCGCGCGCGAGTCACACCTCCGAAGTCAACCAACTAACCATCAAGACACA
118    ATGAGCAGCGAGGTTGAACACAGAGCAGCAGCAGCAGTTCAGGAGCAAGCGCGG
      M S S E V E T Q E Q Q P D A L E G K A G      20
178    CAGGAACACCGCGCGCGGAGGAGGAGGTCATCCGCAACAGGTTTGGGACACA
      Q E P A A T V G E K K V I A T K V L G T      40
238    GTCAATGGTTTAAATGTGCGCAATGTTACGGCTTTTAAACAGGAATGACACCAAG
      V K W F N V R N G Y G F T N R N D T K E      60
298    GATGTGTTGTACACCAATGCCATCAAGAAGTAACCTAGGAAGTACCTTCGGAGT
      D V F V H O T A I K N N P R K V Y L R E      80
358    GTGGGGATGGTGAACGTGTGGAGTTGATGTAGTTGAAGGTGAAGGAGTCCGAGG
      V G D G E T V E F D V V E G E K G A E A      100
418    GCTAATGTACAGGTCCAGGTCTGTTCAGTCCAGGCGAGCAATATGCCGAGACG
      A N V T G P G P V P V Q G S K Y A A D R      120
478    AATAATACAGCGCTATCCAGTCCGAGGTCCTCCAGTAATACAGCAAACTAC
      N N Y R R Y P R R G P P R N Y Q Q N Y      140
538    CAAAACAGTGAGAGTGGAGAAAGGAGGAGGAGTGCACCAAGAGAGAGG
      Q N S E S G E K A E G N E S A P E G E G      160
598    ACAATCAACACGTCCATGCCGAGAGGGGTTATCCACCACTTCTACTCAGGAG
      T N Q Q R P C P R R R Y P P P F Y S R R      180
658    CCATATGGGCGCAGACCACTACTCCAATGTTCTGTTCCAGGAGAAAGTGTGAGG
      P Y G R R P Q Y S N V P V Q G E S A E G      200
718    GCAGAGAGCCAGGTGCGAGGCAACGAGCAGCTGTGAGGCGAATATGTATAGAG
      A E S Q G A G E Q P R V R Q N M Y R G      220
778    TTCAGCCGCAATTCGCGAGGGGACCAACCGTCAGAGACACCCAGAGAGATGGA
      F R P Q F R R G P P R Q R Q P R E D G N      240
838    GAAGAGGACAGGAAATCAGGGGATGAACCCAGAGTCATGATGCCACCTCATG
      E D K E N Q G D E T S A C I I L M R      260
898    CGGTACCGCGTAACCTTAACACAGCAGCAGCCGCAAGAAACCTTAACACCA
      R Y R R N F N Y R R R R P E N P K P Q D      280
958    GGTAAAGAGACCAAGGCGCGCAACATCAGCTGAGAACACGTCGCTCCGAGGCT
      G K E T K A A E T S A E N T S A P E A E      300
1018   CAGGCGGGGCTGAGTAATACCGGCTTACCAACTCTACCACTATCCGCTTTTGT
      Q G G A E      305
1078   CAAAATGTCAAGACAGAAATTCAGCAATAGAAATGAACAAAGAAATGGAAGT
1138   CCTAAGTGTGCTTTTGTCTTTGACGAGTAACGTCATCTGCATAGATACTG
1198   AGATGGTTTTATTTTATCTAAAGCTCTTTTGGGAATGAGAAATTTTAAACAT
1258   TTCTCAATGTACCTTCAAGGTTTAAATGTTCTCTATCTGTCACCACTAAGAT
1318   TAAGAACTAATTTTAAATTTGTAATAGCGTACACCTAATTTCTTTCAAAAGT
1378   AAACGCAAGCATCTGTTAATAAGGTTCTTCATCAAAAAA

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B

Figure 1A. Nucleotide sequence of YB3 cDNA. The deduced amino acid sequence is shown below. The region which is most highly conserved in related Y box binding proteins (35–39) is underlined. **B.** Charge distribution in the YB3 protein. Computer analysis (GenePro) shows alternating regions of net positive and negative charge along the 305 amino acid sequence of YB3.

containing 10 μ g of the fusion protein was emulsified with Freund's complete adjuvant and injected into a rabbit to elicit antibody production. Two additional boosting injections of the same amount of protein with Freund's incomplete adjuvant followed at 30 day intervals.

Western analysis

Protein fractions were resolved in SDS polyacrylamide gels and transferred electrophoretically to nitrocellulose filters. The filters were blocked for one hour with TBST (10 mM Tris-HCl, pH 7.9, 150 mM NaCl, 0.05% Tween-20) containing 1% BSA, and then incubated with a 1:1000 dilution of anti-YB3 fusion protein antisera in TBST. Bound rabbit antibodies were detected by standard procedures using biotinylated goat anti-rabbit antisera, followed by streptavidin-alkaline phosphatase conjugate, and BCIP, NBT, according to the protocol for the BRL Immunoselect system (Bethesda Research Laboratories).

In vitro translation of YB3 protein

YB3 RNA was transcribed from a truncated form of the YB3 cDNA insert in Bluescript vector. The YB3 cDNA was truncated at the 5' end by cleavage with Hinc II, leaving 27 bp preceding the ATG at position 118. T3 RNA polymerase was used to transcribe RNA from the T3 promoter upstream of the YB3 cDNA insert. This RNA was translated in rabbit reticulocyte lysates (Promega) in the presence of 35 S labeled methionine.

Gel mobility shift assays

Expression of the YB3 fusion protein was induced in E.coli lysogens with IPTG as described above. The bacteria were pelleted, resuspended in buffer A containing 200 μ g/ml PMSF, 2 mM EDTA, and lysed by repeated freeze thaw cycles using dry ice- ethanol baths. The lysate was centrifuged at 30KG for

30 minutes. The supernatant was passed over P11 in buffer A and the bound material eluted with buffer A containing 550 mM KCl. Following dialysis against 100 mM KCl, 20 mM HEPES, pH 7.9, 7 mM DTT, this fraction (1 μ l) was incubated for 30 minutes with 10 ng of a radiolabeled 251 bp DNA fragment containing the OAX gene and 1 μ g of poly (dI-dC). The protein DNA mixtures were electrophoresed for 3 hours at 4°C in 6% polyacrylamide gels containing 0.25 $\times$ TBE. Competitor DNAs were multimerized double stranded oligos containing the OAX B box sequence (gatcAAAGGTTGTGGGTTCGATTCCCA) or a mutant B box sequence (gatcAAAGGTTGTGGTTATCGGCCCA).

Nucleotide sequence accession number

The sequence reported here has been deposited in the EMBL library under the accession number X60217.

RESULTS

Expression cloning of the gene encoding YB3

A Xenopus ovary λ gt11 expression library was screened by the filter binding protocol (27). The probe was a multimerized oligo containing the OAX B box region (gatcAAAGGTTGTGGGTTCGATTCCCA). OAX (satellite I) refers to a multicopy gene family which is expressed specifically in oocytes (28,29). The OAX B box is a perfect match for the consensus B box sequence, GGTTCGANTCC (1). The primary screening yielded four clones encoding fusion proteins which bound the OAX promoter. These were purified and subcloned into Bluescript vector (Stratagene) for sequencing. Partial sequence analysis indicated these four represented the same gene product. The longest cDNA insert of 1426 bp was fully sequenced and found to contain an open reading frame encoding a protein of 305 amino acids and predicted molecular weight of 35K, which we have designated YB3 (fig. 1). The ATG at position 118 is likely to be the translation start site or close to the start site; Northern analysis indicates the YB3 transcript in Xenopus tissues is only 200 bp longer than this cDNA (fig.2). Further, *in vitro* transcription/translation of a truncated form of this cDNA having only 27 bp preceding the ATG at position 118 resulted in a protein product of identical

Figure 2. Expression of YB3 during embryonic development. **A.** Northern analysis was performed using total RNA (10 μ g) isolated from ovary and various stages of embryos. The RNA was electrophoresed in formaldehyde containing agarose gels, transferred to nitrocellulose and hybridized with a radiolabeled YB3 cDNA probe. The autoradiograph was exposed to film for one hour. The positions of 18S and 28S RNAs are indicated. **B.** The same RNA samples were hybridized with a probe containing the OAX gene. **C.** Methylene blue staining of the nitrocellulose filter after transfer shows the relative amounts of ribosomal RNA.

Figure 3. Expression of YB3 in adult tissues. **A.** Northern analysis was performed with total RNA (10 μ g) isolated from various adult tissues, hybridized with a labeled YB3 cDNA probe. **B.** Methylene blue staining of the nitrocellulose filter shows the relative amounts of ribosomal RNA.

size to the native protein as shown by Western analysis (fig. 5A). The YB3 protein does not contain any of the motifs previously associated with DNA binding proteins such as helix-turn-helix, helix-loop-helix, zinc fingers, or leucine zippers (34). One unusual aspect of the amino acid sequence is an alternating pattern of basic and acidic regions (fig. 1B). A computer homology search showed YB3 to be closely related to a family of Y box binding proteins (35–39).

YB3 is ubiquitously expressed

YB3 was cloned from an oocyte cDNA expression library on the basis of binding to the oocyte-specific OAX gene. To determine whether YB3 was specifically expressed in ovary, Northern blot analysis was performed. Total RNA was isolated from ovary and various stages of embryos and electrophoresed in formaldehyde containing agarose gels. The RNA was then transferred to nitrocellulose, and hybridized to the radiolabeled YB3 cDNA insert. Autoradiographs revealed a 1.6kb YB3 transcript which is somewhat larger than that of the cloned YB3 cDNA (1426 bp), and is similar to sizes reported for related Y box transcripts (35–39). YB3 is expressed at high levels in ovary, as well as gastrula and later stages of embryos (fig. 2A). In early stages of embryogenesis, the level of YB3 expression is low. This may reflect the general delay in onset of embryonic transcription until the mid-blastula transition (40).

Since the YB3 gene was isolated on the basis of binding to the OAX gene, this raised the possibility that it encodes a specific regulatory factor for OAX. Northern analysis showed the OAX gene was expressed in ovary and in gastrula stage embryos (fig. 2B), but was thereafter inactive. The observation that YB3 is expressed in later stage embryos, following shutoff of OAX expression, argues against YB3 being a specific positive regulator of OAX. Expression of other class III genes, such as somatic 5S RNA, begins at gastrula stage, coincident with the onset of YB3 expression, and continues in later stage embryos (40). To assay for expression of YB3 in adult tissues of *Xenopus*, total RNA was isolated from a variety of tissues. Hybridization with the YB3 cDNA probe showed this gene is expressed in all adult tissues tested (fig. 3).

Figure 4. In vitro transcription assays using P11 fraction IIIIC and the fraction isolated by B box DNA affinity. *In vitro* transcription assays contained TFIIIA, TFIIIB, and no TFIIIC (lane 1), P11 fraction IIIIC from immature ovaries (lane 2) or mature oocytes (lane 3), or the fraction isolated by Adenovirus VA I or OAX B box DNA affinity chromatography (lanes 4 and 5).

Figure 5. YB3 is present in P11 fraction IIIIC. **A.** Western analysis was performed with P11 fractions IIIIC or fractions isolated by binding to OAX or VA B box DNA affinity columns. The protein samples (~2 µg/lane) were electrophoresed in SDS polyacrylamide gels, transferred to nitrocellulose, and incubated with antisera generated against the YB3 fusion protein. An adjacent lane contained radiolabeled translation products of the YB3 cDNA obtained with rabbit reticulocyte lysates. An autoradiograph is shown in lane 4. The molecular weights of the prestained markers (lane M) were determined by comparison with unstained proteins. **B.** Western analysis was performed on P11 fractions IIIIC (550 mM KCl eluate), IIIB (350 mM KCl eluate), and the flowthrough (fraction IIIA) (lanes 1–3) isolated from mature oocyte S150 extracts. The IIIIC fraction in lane 4 was isolated from immature ovary extracts. The Western blot in lane 5 shows mature oocyte fraction IIIIC reacted with preimmune serum. **C.** Lanes 1 and 2 show Western analysis of P11 fractions IIIIC and the fraction isolated by OAX B box DNA affinity chromatography (1–2 µg/lane). The arrow denotes the 55K band recognized by antisera to the YB3 fusion protein. Lanes 3 and 4 show silver staining patterns of the same samples in SDS polyacrylamide gels. The arrow indicates the position of 55K proteins. In lane 3, BSA refers to bovine serum albumin added exogenously to stabilize TFIIIC activity.

YB3 is present in phosphocellulose fraction IIIC

The chromatographic behavior of YB3 was analyzed using phosphocellulose (P11) ion exchange resin. Oocyte S150 extracts were fractionated according to standard procedures by P11 chromatography (31). Fraction IIIB, containing TFIIB activity and RNA polymerase III, was eluted with 350 mM KCl. Fraction IIIC, containing TFIIC activity, was eluted with 550 mM KCl. These fractions, along with purified TFIIA, were sufficient to reconstitute transcription *in vitro* of a 5S RNA gene (fig. 4). Fraction IIIC could be substituted in transcription reactions by fractions which bound to B box DNA affinity columns (lanes 4 and 5). The B box DNA affinity columns were prepared by coupling multimerized oligos containing either the OAX or VA I B box sequences to Sepharose. The phosphocellulose fractions and fractions isolated by B box DNA affinity chromatography were assayed for the presence of YB3 by Western analysis. Protein samples were resolved in SDS polyacrylamide gels, transferred electrophoretically to nitrocellulose filters, and incubated with antibodies to the YB3 fusion protein. The antibodies recognized a 55K protein present in P11 fraction IIIC and in the fraction isolated by B box affinity chromatography (fig. 5A). An adjacent lane contained radiolabeled YB3 protein synthesized *in vitro* in rabbit reticulocyte lysates. An autoradiograph (lane 4) showed that the labeled YB3 protein had the same mobility as the 55K protein recognized by antibodies to the YB3 fusion protein. The apparent molecular weight of 55K for YB3 in SDS gels is larger than the 35K size predicted from the cDNA sequence, and may reflect postsynthetic modification. The Western blot in figure 5B shows more clearly that YB3 is

present in P11 fraction IIIC (lane 1), and not in fraction IIIA (flowthrough) (lane 3) or fraction IIIB (lane 2). The 55K protein was also detected in fraction IIIC isolated from immature oocytes (lane 4). The preimmune serum did not recognize the 55K protein in fraction IIIC (lane 5).

A protein which comigrates with YB3 is the most abundant component in phosphocellulose fraction IIIC or in fractions bound to B box affinity columns

Silver staining of material bound to B box affinity columns revealed a predominant protein (fig. 5C, lane 4) which comigrated with the 55 K protein detected by anti-YB3 antibodies (lane 2). Silver staining revealed two major components in P11 fraction IIIC (lane 3), one of which corresponds to BSA added exogenously to stabilize factor TFIIC activity. The second band, or group of bands, comigrated with the protein detected by anti-YB3 antibodies (lane 1). Thus, a protein(s) which comigrates with YB3 is the most abundant component of P11 fraction IIIC or B box affinity purified material.

To assay for possible involvement of YB3 in transcription of class III genes, we attempted to immunodeplete this protein from fractions containing TFIIC activity using the antisera directed against the YB3-fusion protein. The antisera inhibited transcription at concentrations below that required to deplete YB3, suggesting nonspecific effects on transcriptional activity (data not shown). This approach may require antisera generated against the native, nonfusion YB3 protein, rather than the denatured fusion protein.

Mutations within the B box abolish binding by YB3

Gel mobility shifts were performed with a labeled DNA fragment containing the OAX gene and the YB3 fusion protein. The fusion

Figure 6. Mutations within the B box abolish binding by YB3. **A.** A radiolabeled DNA fragment containing the OAX gene was electrophoresed in 6% polyacrylamide/0.25×TBE gels in the absence (lane 1) or presence (lane 2) of partially purified YB3 fusion protein. In lanes 3 and 4, multimerized oligonucleotides containing the OAX B box were present in 50 or 500 fold higher concentrations than the labeled DNA fragment. Lanes 5 and 6 contained 50 or 500 fold higher concentrations of a mutant B box sequence, shown below. Lanes 9–12 contained zero, 10, 50, or 500 fold greater concentration of an irrelevant plasmid DNA. Lanes 7 and 8 contained proteins extracted from bacteria which had not been induced to express the fusion protein. The arrow at left indicates the position of the specific complex competed with B box oligos. **B.** The mutant oligo used in these gel shift competitions contained five base changes within the B box as shown.

Figure 7. Sequence homologies between the B box and Y box promoter elements. **A.** The Y box of the HLA-DRA gene is compared to the B box sequence of the OAX gene. The OAX B box (underlined) matches the consensus GGTTCGANTCC. The B and Y boxes have in common a GATT motif, as well as TTC and CCA motifs which are out of alignment by one base. **B.** The sequences between –5 and +20 of the TFIIC2 binding site within the 5S RNA gene (44) are shown in the inverted orientation. Sequence homologies to the B box and Y box are indicated. **C.** The sequences downstream of the B box elements of the oocyte-specific tMET and OAX genes have in common a A/TCCT motif. This motif is also present at the same relative position downstream of a core 8 bp Y box in a number of major histocompatibility genes, such as HLA DQβ (45–47).

protein was partially purified from bacterial extracts by P11 chromatography. This P11 fraction was incubated with the labeled DNA fragment and then electrophoresed in 6% polyacrylamide gels. Several retarded bands were observed (fig. 6A, lane 2), one of which was specifically competed with the OAX B box oligo (arrow, lanes 3 and 4). This complex was not competed with an irrelevant plasmid (lanes 9–12) which preferentially competed the nonspecific bands. This specific complex was not produced by a equivalent P11 fraction obtained from bacteria which were not induced to express the YB3 fusion protein (lanes 7 and 8). To determine if the B box was required for binding, competitions were performed with a mutant OAX oligo having five base substitutions within the B box which eliminated the GAAT motif (GGTTCGATTCC to GGTATCGGCC). This mutant oligo was unable to compete the specific retarded complex (lanes 5 and 6).

DISCUSSION

We report the isolation of a gene encoding YB3 which binds to the B box promoter elements of the *Xenopus* OAX and Adenovirus VA1 genes. Mutations within the B box abolished binding by YB3 in competition gel shift assays. Northern analysis revealed YB3 is expressed in a variety of adult tissues. Western analysis of fractionated oocyte S150 extracts showed that YB3 was present in phosphocellulose fraction IIIC, and was also present in the fraction isolated by B box DNA affinity chromatography. Silver staining showed YB3, or a protein of the same mobility, was the most abundant component in P11 fraction IIIC or the fraction isolated by B box DNA affinity chromatography.

A computer homology search showed YB3 to be closely related to the Y box family of DNA binding proteins which includes YB1 (35), EF1 (36,37), dbpB (38), FRG-Y1 (39), FRG-Y2 (39) and dbpA (38). The amino acid sequence of YB3 is 84–89% homologous to FRG-Y1, EF1, or YB-1, but has considerably less homology with FRG-Y2 or dbpA, except for a conserved region of 70 amino acids near the N terminus. Curiously, this domain exhibits 50% homology to the entire coding region of a bacterial protein of unknown function known as the *E. coli* cold shock protein (41,42). YB1 and other members of this gene family have been found to bind to a class II promoter element known as the Y box which is found upstream of a variety of genes (35,36,39). The Y box (CTGATTGGCCA) contains an inverted CCAAT motif. There is sequence homology between the OAX B box and the Y box, notably a common GATT motif, as well as flanking TTC and CCA motifs (fig. 7A). However, homology to the Y box is not required for binding by YB3; this protein also binds to the VA1 B box (fig. 5A) which lacks significant Y box homology; the GATT motif is replaced by GAAC. This indicates that YB3 is able to recognize the B box, independently of Y box homology. The B box is the primary binding site for factor TFIIC. The 50 bp core promoter (+50–97) of the 5S gene lacks a B box sequence (43), yet a component of HeLa factor TFIIC, termed C2, has been shown to bind a region (–8 to +18) around the initiation site (44). Within this binding site, in the opposite orientation, we have noted limited homology to the B box (fig. 7B), as well as a superimposed homology to the Y box, such that the region from +7 to +19 is homologous to either the B box or Y box at 9 out of 13 positions. Thus, two distinct TFIIC binding sites exhibit homology to the Y box, suggesting that these sequence similarities may not be coincidental. The class

III B box may be evolutionarily related to the class II Y box, a type of CCAAT element. In support of this notion, some B box and Y box sequences exhibit limited sequence homology even outside the core elements; an A/TCCT motif present downstream of the OAX and tMET B boxes is similarly situated downstream of the Y box of a number of MHC genes, including A β , DO4 β 1, and DQ β 2 (45–47) (fig. 7C).

The finding that YB3 binds the B box promoter element raises the possibility that this protein might be involved in class III gene regulation. YB3 appears to be an abundant B box binding protein in *Xenopus* oocyte S150 extracts. This protein, or a protein which comigrates in SDS gels, is the predominant component in the fraction isolated by B box DNA affinity chromatography. A protein comigrating with YB3 was also the major component in phosphocellulose fraction IIIC isolated from oocyte S150 extracts. Since these two fractions are routinely used as the source of TFIIC activity in *in vitro* transcription reactions, an abundant B box binding protein, other than TFIIC, could affect transcriptional activity by competing for binding by TFIIC. Such competition could also occur *in vivo*. An abundant B box binding protein might preferentially compete with TFIIC for binding to templates with relatively low affinity for this factor. As an example of such competition, the oocyte 5S gene, which forms relatively unstable complexes with TFIIC, is selectively inactivated by association with histone H1 (48).

Another possible role for YB3 is as a subunit of factor TFIIC. By Southwestern analysis, Seifart and coworkers (25) identified a 55K B box binding protein in a highly purified fraction containing HeLa TFIIC. Since the YB3 protein was similarly identified as a B box binding protein by a Southwestern procedure (27), this suggests YB3 could represent the *Xenopus* homolog of the HeLa 55K B box binding protein. However, the observation that a protein which comigrates with YB3 is the single most abundant component in our *Xenopus* fractions containing TFIIC activity argues against its being a subunit of a multicomponent TFIIC, since other components should be present in equimolar amounts. Future experiments will be aimed at assessing the role of YB3 in class III gene expression, as a B box binding protein which might compete for binding by TFIIC, as a possible component of factor TFIIC, or as an unrelated class III regulatory protein.

ACKNOWLEDGEMENTS

We thank Richard Maki for a critical reading of the manuscript, Craig Hauser, Charles Van Beveren, Antonio Celada, and Robert Oshima for many helpful discussions, and Ann Bookser for assistance in preparing the manuscript. This research was supported by a grant from the National Institutes of Health (GM34888).

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