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Expression of the Two Maize TATA Binding Protein Genes and Function of the Encoded TBP Proteins by Complementation in Yeast

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A single gene encodes the TATA binding protein (TBP) in yeasts and animals. Although two TBP-encoding genes (*Tbp*) previously were isolated from both Arabidopsis and maize, the expression and in vivo function of the encoded plant TBPs were not investigated. Here, we report that the two highly conserved maize *Tbp* genes are unlinked and reside within larger, ancestrally duplicated segments in the genome. We find quantitative differences in *Tbp1* versus *Tbp2* transcript accumulation in some maize tissues. These nonidentical expression patterns may indicate differences in the tissue-specific regulation of these genes, which might allow the two encoded maize TBP isoforms to perform nonoverlapping functions in the plant. In addition, we show that the maize TBP products, unlike animal TBPs, are functionally interchangeable with yeast TBP for conferring yeast cell viability. This is a conclusive demonstration of in vivo activity for a nonyeast TBP protein, and these complementation results point to particular amino acids in TBP that are likely to influence species-specific protein interactions.

INTRODUCTION

Largely characterized in animals and yeast, the TATA binding protein (TBP) subunit of the TFIID transcription initiation factor interacts with the TATA box sequence in the promoter of most eukaryotic nuclear protein–encoding genes. In addition to performing this early DNA recognition function during basal and activated transcription by RNA polymerase II, TBP also serves as a critical subunit in the initiation complexes for RNA polymerases I and III (reviewed in Green, 1992; Rigby, 1992; Sharp, 1992). In all three polymerase systems, TBP appears to function via precise interactions with other proteins in the transcription complex (Dynlacht et al., 1991; Timmers and Sharp, 1991; Pugh and Tjian, 1992). Despite early suggestions that multiple TFIID factors exhibiting different TATA target site specificities might exist within an individual organisms (Chen and Struhl, 1988; Simon et al., 1988), only a single TBP-encoding gene (herein denoted *Tbp*), whose product performs all known TBP activities, has been identified in yeasts, animals, and insects (see Peterson et al., 1990). In contrast, two *Tbp* genes encoding distinct TBP isoforms have been isolated from plants such as Arabidopsis and maize (Gasch et al., 1990; Haass and Feix, 1992). The two encoded TBPs conceivably could differ in their DNA binding specificity, in specific interactions with other transcription proteins, or in their regulated expression in the plant.

TBP proteins from evolutionarily diverse species all share a strikingly conserved ~180–amino acid C-terminal domain, which is necessary and sufficient as a complete unit for DNA binding and basal transcription (Horikoshi et al., 1990; Peterson et al., 1990; Poon et al., 1991). This conserved domain, which contains two 60– to 66–amino acid imperfect direct repeats separated by a basic linker region, was shown by x-ray crystallographic analysis of Arabidopsis TBP2 (Nikolov et al., 1992) to carry twofold symmetry and adopt a saddlelike conformation. β-strands in the direct repeats are thought to straddle the DNA, and α-helices in the basic segment and extreme C terminus are accessible on the surface of the molecule for possible interaction with other transcription proteins. The TBP N-terminal domain, extremely variable in both length and sequence, may also contribute outward-facing surfaces; however, the precise function of this domain remains largely unknown.

The *Tbp* genes from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* can complement an otherwise TBP-deficient *S. cerevisiae* strain to support all of the diverse TBP activities necessary for cell viability (Cormack et al., 1991; Gill and Tjian, 1991; Poon et al., 1991). Because human and Drosophila TBP were not able to complement, TBP proteins have been concluded to carry species-specific functional differences, and such specificity determinants were shown to map to multiple and diverse parts of the C-terminal domain

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(Cormack et al., 1991; Gill and Tjian, 1991). It is not known whether this functional noninterchangeability between yeast and animal TBPs derives from individual amino acid residue differences that influence direct contacts with other yeast transcription factors or from overall differences in protein conformation.

Here, we report several findings involving the expression of the two maize *Tbp* genes and the in vivo activity of the encoded TBP proteins. We demonstrate that a single, inbred maize genome carries two *Tbp* genes, whose loci are unlinked and map within larger, duplicated chromosomal segments. We identify quantitative differences in the tissue-specific transcript expression patterns of these genes, indicating that the two encoded TBPs might carry nonoverlapping functions in some tissues via their differential abundance. We show that maize TBP proteins are functionally interchangeable with yeast TBP for supporting yeast cell viability and do not exhibit the species specificity differences observed for animal TBPs. This is an effective demonstration of in vivo function for an intact, nonyeast TBP.

RESULTS

Inbred Maize Expresses Two Distinct *Tbp* Genes

A fragment of a maize TBP-encoding gene (*Tbp*) was polymerase chain reaction (PCR) amplified from the maize genome using partially degenerate oligonucleotide primers corresponding to regions of amino acid identity within the second direct repeat among fungal (Horikoshi et al., 1989; Hoffmann et al., 1990), human (Peterson et al., 1990), and Drosophila (Hoey et al., 1990) TATA binding proteins. Predominating among the PCR reaction products was a discrete 205-bp fragment, which contained 110 bp of unique coding sequence, 66% similar to the *S. cerevisiae* sequence, interrupted by a 95-bp intron that is positioned identically to the third intron in *S. pombe Tbp* (Hoffmann et al., 1990). Using this PCR fragment to probe a maize B73 inbred seedling cDNA library, we isolated two cDNA classes, denoted mzTbp1 and mzTbp2, encoding products with TBP homology, as shown in Figure 1.

Several of the eight independent mzTbp2 cDNAs (the longest, 1.35 kb) carried a complete protein-encoding region; however, all four mzTbp1 cDNAs were incomplete. We reconstructed a complete, in-frame *Tbp1* coding region by joining the longest partial cDNA to a *Tbp1* 5′ region fragment generated by 5′ rapid amplification of cDNA ends PCR. Unlike previous reports (Gasch et al., 1990; Haass and Feix, 1992), in which cloned plant *Tbp* sequences were obtained from DNA libraries derived from unstated plant cultivars with unknown levels of allelic diversity, we isolated *Tbp* cDNAs from a single, well-defined inbred line whose level of heterozygosity is negligible. Therefore, we can exclude allelic polymorphism at a single locus as the source for the two maize cDNA classes. The coding region sequences of our *Tbp1* and *Tbp2* cDNA

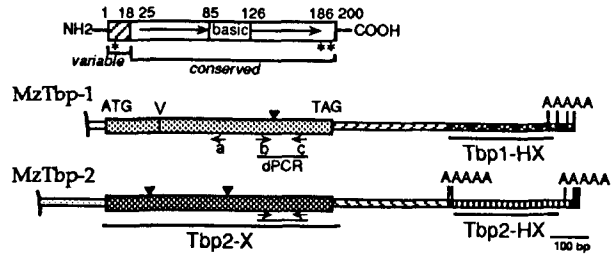

Figure 1. Structure of Maize *Tbp1* and *Tbp2* Genes and Encoded TBP Proteins.

Each gene structure represents a compilation of multiple, independent MzTbp1 and MzTbp2 cDNA clones. The highly conserved coding regions (shaded boxes) are delimited by ATG initiation and TAG stop codons. The 3′ untranslated regions are bipartite; the 82% conserved proximal region (diagonal striping) is adjacent to the highly diverged distal segment (different, darkly patterned boxes). Two major polyadenylation regions (denoted by AAAAA) are each composed of multiple, minor termination sites (vertical lines) found in individual cDNA clones. Filled inverted triangles indicate introns trapped within cDNA clones. A "V" shows the BspHI site at which the MzTbp1 5′ PCR and 3′ cDNA fragments were joined in frame to generate an intact coding region. Small arrows indicate PCR primers: a, the *Tbp1* gene–specific 28-mer; b and c, degenerate 20-mers used to isolate the initial genomic *Tbp* probe fragment (dPCR). Restriction fragments (Tbp2-X, Tbp1-HX, and Tbp2-HX) used as hybridization probes are shown. The structure of the encoded 200–amino acid products, including the short N-terminal variable domain (striped box), the 182–amino acid conserved C-terminal domain (open box), and the locations of the amino acid direct repeats (arrows) and the intervening basic region are shown. Amino acid polymorphisms between these products are indicated by asterisks. Numbers indicate the amino acid positions bordering these features.

clones are identical to those previously reported by Haas and Feix (1992); however, the noncoding regions are slightly polymorphic (data not shown).

The two maize *Tbp* transcripts are extremely similar. The 600-bp coding regions share 96.3% nucleotide similarity, whereas the 5′ and 3′ noncoding regions are more diverged (86 and 68% identity, respectively). Furthermore, the 3′ noncoding regions are bipartite, containing adjacent proximal and distal stretches that share medium (82%) and low (<50%) identity, respectively (Figure 1). The more diverged, distal segments, denoted Tbp1-HX and Tbp2-HX, constitute gene-specific probes. Flanking the distal segment are two major regions of polyadenylation, defined by the termination sites found among independent cDNA clones. Whereas *Tbp2* contains two widely spaced putative polyadenylation signals (data not shown), which appear to direct transcript termination within each of the two major termination regions, DNA polymorphisms in *Tbp1* eliminate the upstream signal, resulting in termination of all *Tbp1* cDNAs downstream. Thus, individual maize *Tbp* transcripts are predicted to vary by up to 290 nucleotides, a size heterogeneity consistent with the smeared transcript doublet bands spanning ~1.1 to 1.4 kb observed on RNA gel blots (see Figure 5).

The Two *tbp* Loci Are Linked in the Maize Genome

Genomic DNA gel blot and molecular mapping analysis provided additional information about the genomic relationship of the two cloned *Tbp* sequences. As shown in Figure 2A, blots containing B73 inbred genomic DNA probed with the complete, conserved *Tbp* coding region (Tbp2-X) reveal two bands for each digest at low stringency, with one band preferentially washing off at high stringency. Reprobing the same membrane with the Tbp1-HX gene-specific probe resulted in a single hybridizing species per lane, corresponding to the band that bound more weakly to the *Tbp2*-derived coding probe. The general presence of two nonidentical *Tbp* sequences in the genome was demonstrated further by a similar analysis of several other diverse inbred maize lines and by showing that all F_1 progeny of a self-crossed B73 inbred plant gave 100% cosegregation of the two Tbp2-X-hybridizing bands (data not shown). These results are consistent with two nonidentical *Tbp* genes as the source for the two cDNA classes.

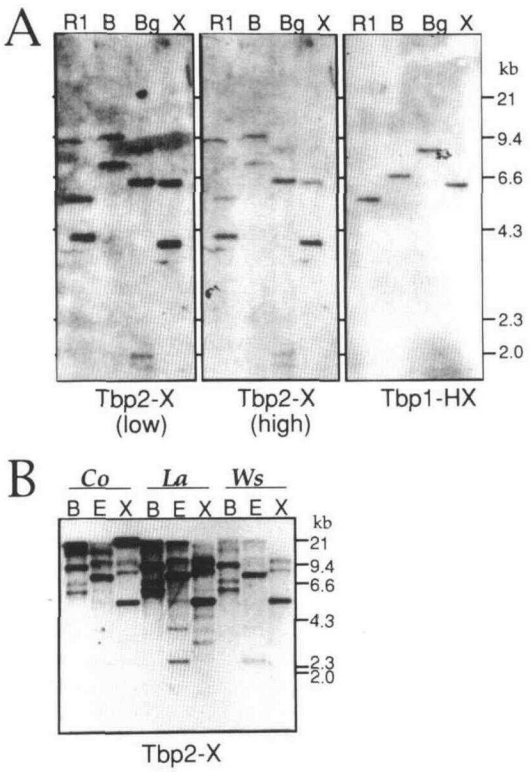

Figure 2. Genomic DNA Gel Blot Analysis of Maize and Arabidopsis.

(A) Maize B73 inbred genomic DNA digested with EcoRI (R1), BamHI (B), BglII (Bg), and XhoI (X) was hybridized with the Tbp2-X coding region probe (see Figure 1) and washed first at low stringency (low) and then at high stringency (high). The same membrane was next hybridized with the Tbp1-HX gene–specific probe, as labeled, and washed at high stringency.

(B) Arabidopsis genomic DNA from the Columbia (*Co*), Landsberg *erecta* (*La*), and Wassilewskija (*Ws*) ecotypes was digested with BamHI (B), EcoRV (E), and XbaI (X) enzymes with sites not represented in the published Arabidopsis *Tbp* coding sequences. Hybridization was at 40°C below melting temperature with the maize Tbp2-X coding region probe, which shares 75 and 78% identity with the published Arabidopsis *Tbp*1 and *Tbp*2 coding regions, respectively.

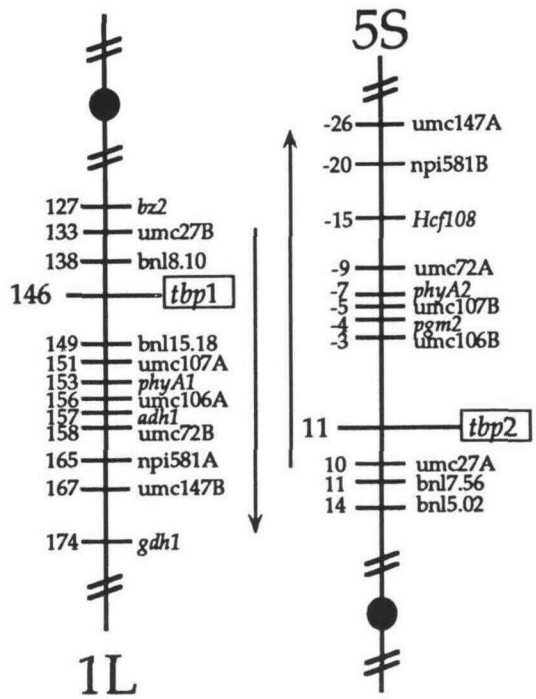

Figure 3. Genomic Map Positions of Unlinked Maize *tbp*1 and *tbp*2 Loci.

Portions of maize chromosome arms 1L and 5S are shown, with some of the molecularly mapped loci on each chromosome indicated. Loci with known products are italicized, and RFLP loci are in unitalicized letters. Numbers refer to relative map positions along each chromosome; a unit of 1 represents 1 centimorgan. The mapped *tbp* loci are boxed. These maps were derived from the recombinant inbred populations of B. Burr and coworkers, and analogous positions were identified within other mapping populations. Vertical arrows delineate the extent of, and show the relative directions of, the imperfect duplicated segments shared by these chromosome arms. Dark circles represent centromeres. This figure is not to scale. Double slashes indicate chromosomal regions left out of this schematic.

To determine the genomic linkage relationship of the two maize *Tbp* genes, the Tbp2-X coding fragment was used as a restriction fragment length polymorphism (RFLP) probe under low-stringency conditions against genomic DNAs from three different populations of recombinant inbred maize lines. In all populations, the *Tbp*1 gene mapped near the *alcohol dehydrogenase-1* (*Adh1*) gene on chromosome arm 1L, and *Tbp*2 sequences localized to chromosome arm 5S near *phosphoglucomutase-2* (*Pgm2*), as shown in Figure 3. Thus, the two maize *Tbp* genes, although highly conserved and

closely related, are not clustered. However, these *tbp* loci are contained within larger chromosomal segments that appear to have been imperfectly duplicated and inverted between chromosome arms 1L and 5S during maize genome evolution (Helentjaris et al., 1988). The *tbp* loci are situated at analogous positions within these regions, roughly delimiting one end point of each duplication (see Figure 3). Neither *tbp* locus correlates with the position of any genetically mapped, phenotypically defined maize gene.

Two TBP-encoding genes have been isolated from Arabidopsis (Gasch et al., 1990). Because the chromosomal locus positions of these genes have not been determined and the cultivar origins of these sequences were not stated, it remained a possibility that these cloned sequences might represent alleles rather than separate genes (loci). To find additional evidence for distinct loci, we examined three diverse Arabidopsis ecotypes (inbred lines) by low-stringency genomic DNA gel blot analysis using the maize Tbp2-X coding probe, as shown in Figure 2B. At least two hybridizing bands are visible in every DNA digest of each ecotype, consistent with the presence of two *Tbp* genes in a single Arabidopsis genome. Low-stringency hybridization of the maize coding region probe to genomic DNA from several other plants, including barley, rice, sorghum, and tobacco (data not shown), similarly indicates multiple *Tbp* genomic sequences present among diverse plant species.

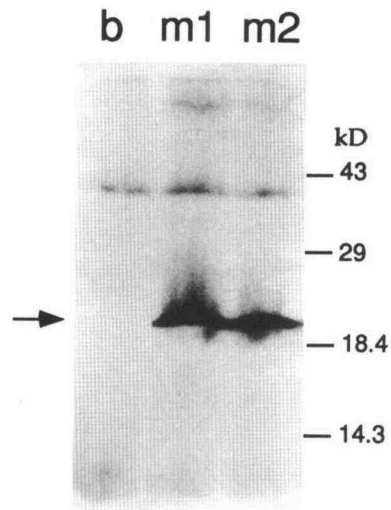

Figure 4. In Vitro Translation Products of Maize *Tbp* Genes.

In vitro–transcribed RNA from linearized maize *Tbp*1 and *Tbp*2 cDNA templates was translated in vitro using rabbit reticulocyte lysates and ^{35}S-methionine. Labeled products are resolved on an SDS–polyacrylamide gel. The arrow indicates identically sized ~22-kD TBP proteins specific for the maize *Tbp*1 (m1) and *Tbp*2 (m2) genes. This product is not present in blank lysates (b) programmed without RNA.

Maize TBP1 and TBP2 Proteins Are Highly Conserved

The *Tbp*1 and *Tbp*2 open reading frames each encode 200–amino acid polypeptides sharing a predicted molecular mass of 22.3 kD. Consistent with this prediction, identically sized, ~20-kD in vitro translation products were generated from synthetic transcripts of each gene, as shown in Figure 4. Therefore, it is unlikely that either maize *Tbp* cDNA represents an untranslatable pseudogene. In agreement with the results of Haass and Feix (1992), the encoded maize TBP1 and TBP2 proteins share 98.5% overall amino acid sequence identity, differing at only three nonconsecutive amino acids, one in the variable N terminus and two at the extreme C terminus (see Figure 1). The overall length and domain structures of maize TBP1 and TBP2 are extremely similar to that of the TBP proteins of two dicots, Arabidopsis (Gash et al., 1990) and potato (Holdsworth et al., 1992) and somewhat less similar to one from a more closely related monocot, wheat (Kawata et al., 1992).

Maize *Tbp*1 and *Tbp*2 Transcripts Exhibit Quantitative Expression Pattern Differences

Total accumulated *Tbp* message in poly(A)$^+$-selected mRNA from diverse tissues of the maize plant was examined by RNA gel blot analysis using several *Tbp* gene fragment probes. First, as shown in Figure 5, the Tbp2-X conserved coding region probe, which cross-hybridizes to both *Tbp*1 and *Tbp*2 transcripts, reveals a diffuse transcript doublet spanning ~1.1 to 1.4 kb in every tissue examined. This heterogeneous *Tbp* transcript size range is in full agreement with the lengths of the longest complete cDNA clones obtained (1.15 and 1.35 kb). Although the relative level of total, accumulated *Tbp*2-X–hybridizable transcript varies greatly among the different vegetative, floral, and gametophytic tissues, all lanes were loaded with approximately equal amounts of RNA. (The ethidium bromide–stained gel is shown; however, staining of the residual rRNA bands does not necessarily indicate uniform poly[A]$^+$ RNA levels.) Control hybridizations using maize actin and ubiquitin probes (Figure 5) confirmed that every sample contained intact, hybridizable RNA (even pollen, which exhibits extremely low *Tbp* message levels). However, neither of these control genes expresses its message uniformly among these many tissues (J.M. Vogel and B. Kloeckener-Gruissem, unpublished observations), and we know of no other gene that expresses its transcript at equal levels in all these diverse tissues. Therefore, without a reliable reference, an accurate, quantitative comparison of *Tbp* message levels between tissues is not possible. Nevertheless, it is possible to compare *Tbp*1 versus *Tbp*2 message levels within a single tissue. Hybridization of duplicate membranes with the Tbp1-HX and Tbp2-HX gene–specific probes, followed by linear quantitation of the hybridization signals, revealed transcript size and distribution patterns qualitatively similar and, for most tissues, quantitatively similar to the pattern identified with the conserved coding probe (Figure 5). In a few lanes, however, the two gene-specific

hybridization signals differ quantitatively, indicating that *Tbp*1 and *Tbp*2 transcripts accumulate to different relative levels in these tissues. For example, whole tassel (but not isolated pollen) appears to contain much more *Tbp*2 than *Tbp*1 message, whereas both primary root and developing embryo accumulate relatively higher levels of *Tbp*1 full-length transcript.

Although both gene-specific probes identified a full-length transcript pattern similar to that for the coding probe, the *Tbp*1-specific probe also detected a prominent, second hybridizing species of ~600 bp in some tissues. This shorter transcript is most abundant in, but not exclusive to, the leaflike tissues of the plant and is not detectable with the *Tbp*2-specific probe (Figure 5). Furthermore, it is not likely to represent an alternatively spliced form of *Tbp*1 mRNA, because it hybridizes only weakly with the more proximal 3′ noncoding fragment (data not shown) and is undetectable using probes representing the entire coding region (Figure 5), subfragments of the *Tbp*1 coding region, or the 5′ untranslated region (data not shown). To determine the relative orientation of the short transcript, we used each strand of the Tbp1-HX fragment separately as a probe against blots carrying poly(A)$^+$ RNA from a subset of the tissues illustrated in Figure 5. As shown in Figure 6, the (+) strand probe, representing the DNA strand with an orientation equivalent to the *Tbp* mRNA, did not hybridize to either the full-length *Tbp*1 message or the shorter transcript. The

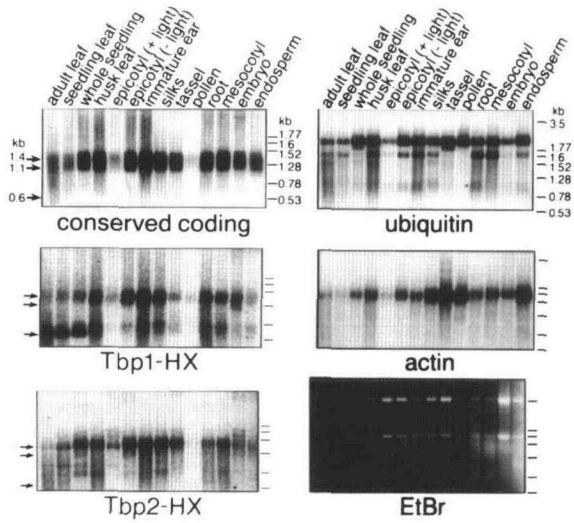

Figure 5. RNA Gel Blot Analysis of Maize *Tbp* Transcripts.

Identical membranes containing equal amounts of poly(A)$^+$–selected RNA from the indicated maize tissues were hybridized with probes representing either the highly conserved maize *Tbp* coding region or the indicated maize *Tbp* gene–specific fragments. Control hybridizations of these membranes used maize actin and ubiquitin cDNA inserts, and the ethidium bromide (EtBr) stain of the gel prior to blotting is also shown. All hybridizations and washes were performed at high stringency. Two arrows indicate the 1.1- to 1.4-kb full-length *Tbp* transcript doublet, and a single arrow shows the position of the shorter 600-bp transcript, which was detected only by Tbp1-HX.

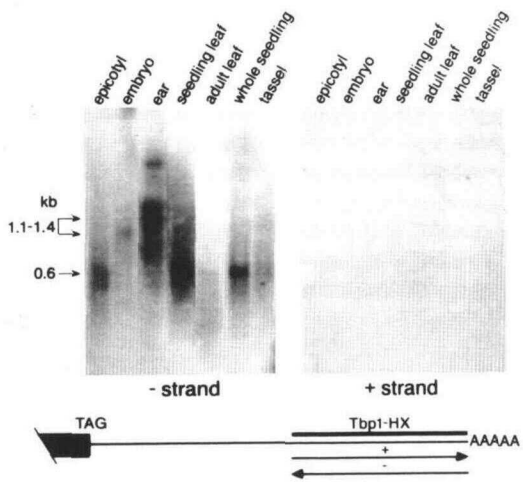

Figure 6. Directionality of the Short *tbp*1 Locus Transcript.

Duplicate membranes containing poly(A)$^+$–selected RNA were hybridized with single-stranded versions of the Tbp1-HX probe, originating from the *Tbp*1 distal 3′ untranslated region (see Figure 1). The tissue origin of each RNA sample is shown. The (+) strand probe is in the same orientation as the full-length *Tbp*1 message; the (−) strand is in the opposite orientation. The integrity of both probes was verified by subsequent hybridization against cloned DNA (data not shown).

(−) strand probe, complementary to *Tbp*1 mRNA, hybridized to both full-length *Tbp*1 message, most prominent in embryo and ear, and to the short transcript, visible primarily in the leaf tissues. Thus, the short RNA and the full-length *Tbp*1 message must be transcribed in the same orientation. Altogether, these results are most consistent with the shorter transcript representing a second, independent polyadenylated RNA originating from the *tbp*1 chromosomal locus. The Tbp1-HX region contains no open reading frames in the direction of the short transcript, and we could find no homology to any sequence in the GenBank or EMBL data bases. However, we cannot yet exclude the possibility that this short RNA species contains *Tbp*1 intron sequences or extremely short stretches of coding region that would prevent efficient hybridization or that it is some other processed form of *Tbp*1 message.

Both Maize *Tbp* Genes Functionally Complement Yeast *Tbp* in Vivo

Translation in vitro confirmed that the maize *Tbp*1 and *Tbp*2 genes encode intact, identically sized polypeptides (Figure 4). In addition to showing structural integrity, however, it was important to demonstrate that each encoded TBP is a functional protein. As a stringent test of the multiple and diverse TBP activities essential for sustaining cell viability, we investigated the function of each maize TBP protein using a plasmid shuffle in vivo complementation assay. Briefly, a Leu$^-$ *S. cerevisiae*

strain, carrying a *TRP*1 insertion/deletion of the chromosomal TBP-encoding gene (designated y*Tbp*) and harboring the y*Tbp* gene on a low-copy–number *URA*3 vector, was transformed with high-copy–number *LEU*2 vectors, carrying either the y*Tbp* or maize *Tbp* genes under transcriptional control of the strong *ADH*1 promoter. The resulting transformants (Trp^+, Leu^+, Ura^+) were replica plated to medium containing the toxic uracil analog 5-fluoroorotic acid (5-FOA) to select for loss of the *URA*3 plasmid carrying the y*Tbp* gene. Growth of these strains on 5-FOA indicates that the *Tbp* gene carried on the *LEU*2 plasmid alone is able to functionally complement the chromosomal deletion.

As shown in Figure 7A, transformants containing the *LEU*2 vector that lacks any insert grow on minimal medium but not on minimal medium containing 5-FOA, confirming the efficacy of the 5-FOA selection and demonstrating that, in the absence of any other *Tbp* gene, y*Tbp* maintained on the *URA* plasmid is essential for cell viability. Strains transformed with either the yeast or the maize *Tbp* genes carried on the *LEU* vector had indistinguishable growth rates when these cells were grown on minimal medium, indicating that overexpression of either yeast or maize *Tbp* genes does not inhibit cell growth. Resistant colonies arose after replica plating these isogenic strains, carrying either the yeast or maize *Tbp* genes on *LEU* plasmids, to 5-FOA (Figure 7A), although colonies expressing the maize *Tbp* genes arose and grew more slowly, even when streaked onto rich medium (Figure 7B). We isolated and sequenced the maize *Tbp*/*LEU*2 plasmids from the 5-FOA–resistant transformants to determine whether cell viability resulted from recombination, occurring before 5-FOA selection, between the y*Tbp* gene on the *URA* plasmid and maize *Tbp* on the *LEU* plasmid. Both of the introduced maize *Tbp* coding regions in these 5-FOA–resistant strains were intact and unaltered (data not shown).

Expression of the heterologous maize TBP proteins as the sole TBP isoform in these 5-FOA–resistant transformants was confirmed using immunoblot analysis with a monoclonal antibody that was generated against the C-terminal domain of Drosophila TBP but cross-reacts with the evolutionarily conserved yeast, human (R. Weinzierl, personal communication), and maize (this report) TBP isoforms. As shown in Figure 7C, a protein with an apparent molecular mass of 27,000 D, the predicted size of *S. cerevisiae* TBP (Horikoshi et al., 1989), is specifically recognized in crude extracts from cells carrying the intact yeast *Tbp* gene. This 27-kD protein is more abundant when the y*Tbp* gene is carried on high-copy–number plasmids (transformants Y-1 and Y-2) compared to its normal chromosomal position (wild-type strain H1511). Independent 5-FOA–resistant transformants expressing either the maize *Tbp*1 or *Tbp*2 gene (lanes labeled mzTBP1 and mzTBP2, Figure 7C) contain a protein migrating at ~20,000 D, consistent with the predicted size of maize TBP. The apparently low levels of maize TBP proteins detected in these extracts may not reflect their true abundance; the maize TBPs may contain amino acid polymorphisms within the undefined epitope region, possibly resulting in reduced antibody affinity.

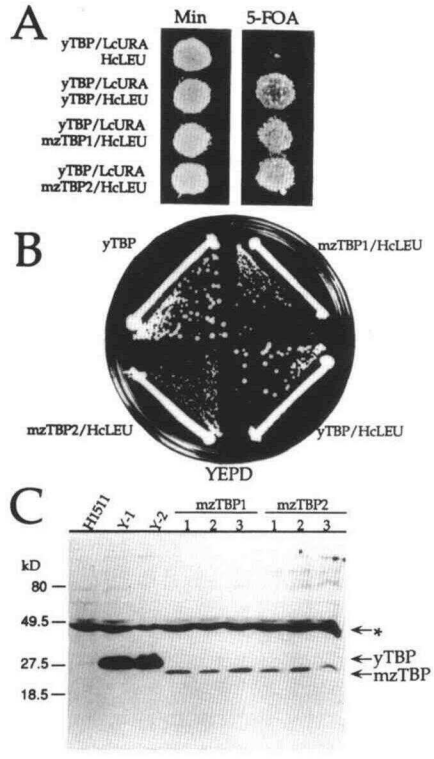

Figure 7. Complementation Analysis of Yeast TBP-Deficient *S. cerevisiae* by Maize *Tbp*.

(A) Plasmids (described in Methods) containing the indicated *Tbp* gene on the high-copy–number *LEU*2 plasmid (HcLEU) were introduced into the YMC14 yeast strain, which harbors a disrupted chromosomal y*Tbp* gene but carries a functional gene on a low-copy–number plasmid (yTBP/LcURA). Single-colony transformants containing either vector alone (yTBP/LcURA, HcLEU), yeast *Tbp* (yTBP/LcURA, yTBP/HcLEU), or maize *Tbp*1 (yTBP/LcURA, MzTBP1/HcLEU) or *Tbp*2 (yTBP/LcURA, MzTBP2/HcLEU), carried on the high-copy–number *LEU*2 vector, were patched onto minimal (Min) medium and grown to confluence and then replica plated to minimal medium supplemented with 5-FOA. Plates were incubated for 2 days (Min) or 4 days (5-FOA).

(B) The wild-type yeast strain H1511 (shown as yTBP) and the 5-FOA–resistant colonies described in **(A)**, carrying and expressing the indicated *Tbp* gene on the high-copy–number *LEU*2 vector, were streaked onto YEPD rich medium and incubated for 5 days.

(C) TBP proteins expressed in wild-type yeast strain H1511 and individual Trp^+, Ura^-, Leu^+ transformants following 5-FOA selection were detected using immunoblot analysis. Equal amounts of each yeast extract (1.0 OD_{595} unit) were loaded, and the anti-TBP monoclonal antibody used cross-reacted with the 27-kD yeast (yTBP) in both wild-type and yeast gene transformants (Y-1 and Y-2) and with the ~20-kD maize TBP (mzTBP) in the mzTBP1 and mzTBP2 maize gene transformants. A cross-reacting, unidentified, ~45-kD protein (asterisk) is ubiquitous in all extracts.

Alternatively, the maize TBP products may be unstable or could be translated poorly in yeast cells. In any case, the 27-kD yeast TBP isoform is completely undetectable in these extracts, and the maize TBP proteins are present at approximately equal levels among independent transformants. These results demonstrate that both maize TBP proteins can complement yeast TBP for supporting cell viability.

DISCUSSION

Relatively little is known about TFIID factors and general transcription initiation mechanisms in the plant nucleus. *Tbp* cDNA clones encoding the TATA binding protein subunit of the TFIID transcription initiation factor have been isolated from a few plant species (Gasch et al., 1990; Haass and Feix, 1992; Holdsworth et al., 1992; Kawata et al., 1992), and all encode proteins similar in size and structure. Although animals and yeasts contain only a single *Tbp* gene, two distinct *Tbp* gene sequences were isolated previously from Arabidopsis (Gasch et al., 1990) and maize (Haass and Feix, 1992). It remained possible that the Arabidopsis genes might represent alleles of a single locus, because their chromosomal positions are not mapped and their strain origin(s) is unstated. Nevertheless, our own analysis of three diverse inbred Arabidopsis ecotypes and our examination of a well-defined maize inbred provide confirmation for the suggestion that the two sequences in each plant represent distinct genes (loci). Only one *Tbp* gene has been isolated from each of potato and wheat, although it is possible that additional genes may yet be identified. In any case, nothing was known regarding the location and organization of *Tbp* genes in any plant genome.

We have isolated two classes of functional *Tbp* cDNA from a single inbred maize stock and demonstrate that inbred maize genomes carry both sequences as separate, unlinked loci. Although possible, neither locus corresponds to the map position of any genetically defined maize mutant. These highly conserved *Tbp* genes are contained within large, unlinked, ancestrally duplicated segments identified previously in the maize genome (Helentjaris et al., 1988). This duplicated region on chromosome arms 1L and 5S is imperfect and inverted in relation to the centromeres, encompasses at least 30 centimorgans, and shares at least 14 homologous RFLP loci and one other mapped gene pair (*phy*A1 and *phy*A2) (Helentjaris et al., 1988; Burr and Burr, 1991). It is not known whether this and other segmental duplications in the maize genome (Helentjaris et al., 1988) also are carried by closely related grass species whose 10 chromosomes appear generally to be colinear with those of maize (Doebley and Stec, 1991; Whitkus et al., 1992). It is also not known whether any of these duplications exist in the genomes of more distantly related plants, such as *Arabidopsis*, whose genomes are not colinear with maize. Given the greater intraspecies divergence of the two *Tbp* genes in Arabidopsis (Gasch et al., 1990) than in maize, it is tempting to speculate that maize may have lost one of a pair of ancestral plant *Tbp* genes, which both remain in some other species (i.e., Arabidopsis) but that maize carries a more recent duplication of the remaining ancestral gene. In any case, the evolutionary origin of multiple *Tbp* genes among plants remains obscure, and multiple TBP-encoding genes in at least some plant genomes are unique compared to other eukaryotes.

Conceivably, the two nonidentical maize *Tbp* genes might encode products with nonoverlapping functions in the plant. Based upon the three-dimensional structure deduced for Arabidopsis TBP2 (Nikolov et al., 1992), the predicted DNA binding, β-strand regions of the two maize proteins are identical, as shown in Figure 8. The three amino acids that are polymorphic between maize TBPs fall into the apparently outward-facing regions of the protein, indicating their potential for mediating protein–protein contacts with other transcriptional proteins (Nikolov et al., 1992). Potential phosphorylation of the accessible serine in TBP2 at polymorphic position 193, for example, may provide a functional distinction between the two nearly identical maize TBP isoforms. As shown in Figure 8, the sole amino acid position that defines protein pairs between maize and Arabidopsis is at the extreme C terminus, at position 198 (a valine in maize TBP1 and At1 but isoleucine in maize TBP2 and At2). Therefore, if any functional distinction between the two TBP proteins has been conserved between Arabidopsis and maize, then extremely subtle TBP structural differences must be involved.

Nonoverlapping TBP functions for the maize TBPs could result from the coexpression of distinct protein isoforms with dissimilar transcriptional activities or from differential spatial or temporal expression of functionally equivalent protein products. We have identified differences in the transcript expression patterns for the maize *Tbp* genes. Although *Tbp*1 and *Tbp*2 transcripts each accumulate in all maize tissues examined, the relative levels of these two transcripts differ in some parts of the plant. These quantitative differences in transcript expression, most notable in the tassel (but not the pollen), primary root, and developing embryo, indicate differences in the tissue-specific regulation of the *Tbp* genes. The biological significance of these quantitatively different expression patterns remains unclear. Nevertheless, if these differences are reflected at the protein level, then the resulting differential availability of the two TBP isoforms may provide additional flexibility for transcript initiation in some tissues of the plant.

We have demonstrated that both maize *Tbp* cDNAs encode proteins that can support transcription in vivo, as evidenced by their ability to complement an otherwise lethal TBP-deficient yeast strain to support cell viability. This complementation assay is a stringent test of the diverse functions required of TBP, including its binding to the TATA element of RNA polymerase II promoters, its interaction with other transcription factors involved in RNA polymerase II transcription, and its various roles in DNA binding and protein–protein interaction required for RNA polymerase I and polymerase III transcription (see reviews by Green, 1991; Rigby, 1992; Sharp, 1992). Only the conserved C-terminal domain of the TBP protein, from either *S. cerevisiae* or *S. pombe*, is required for yeast cell viability (Horikoshi et

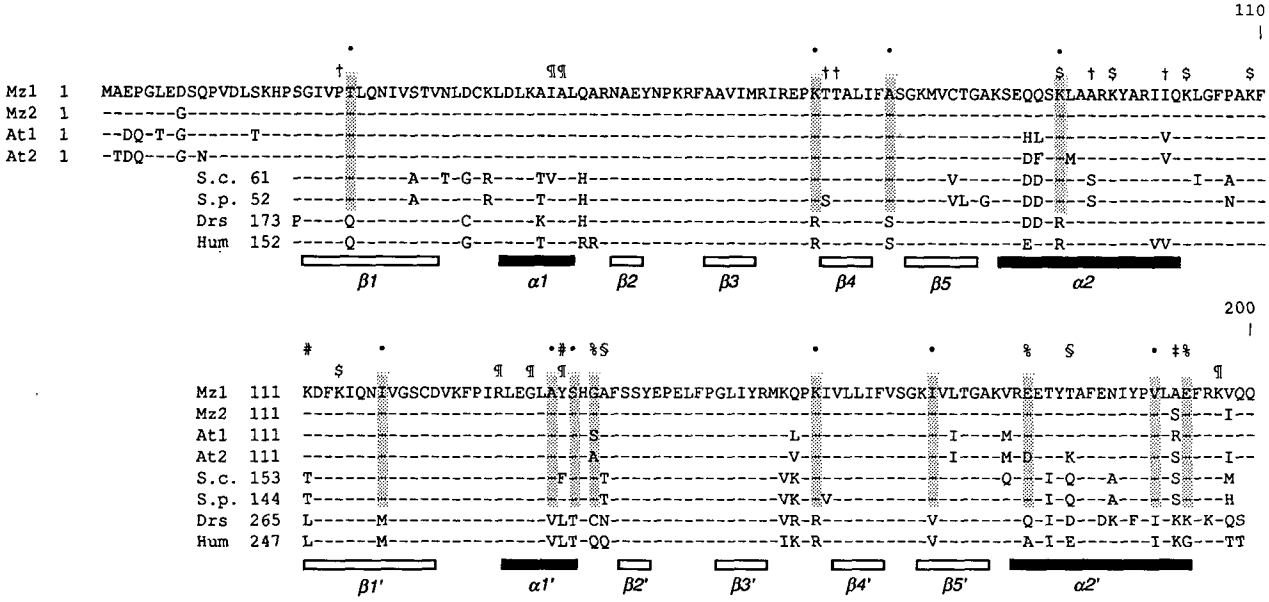

Figure 8. Amino Acid Sequence and Structural Comparison of TBP Proteins from Diverse Species.

The complete amino acid sequence of maize TBP1 (Mz1) is shown, and other TBP protein sequences are depicted underneath. Amino acid identity with Mz1 is indicated with a dash. The 18-residue N-terminal domain sequences are shown for the maize and Arabidopsis (At1 and At2) TBPs, but only the C-terminal 180 to 182 amino acids are shown for the *S. cerevisae* (S.c.), *S. pombe* (S.p.), Drosophila (Drs), and human (Hum) isoforms. Horizontal open and filled boxes show the locations of β-strands and α-helices, respectively, as determined by x-ray crystallographic analysis of Arabidopsis TBP2 (Nikolov et al., 1992). Amino acid positions in the conserved C-terminal domain, in which maize proteins differ from those of both Drosophila and human, are indicated above the Mz1 sequence: •, [Mz1=Mz2=S.c.=S.p.] ≠ [Drs=Hum]; %, [Mz1=Mz2=S.c.=S.p.] ≠ [Drs] ≠ [Hum]; #, [Mz1=Mz2] ≠ [S.c.] ≠ [Drs=Hum]; §, [Mz1=Mz2] ≠ [S.c.=S.p.] ≠ [Drs] ≠ [Hum]; ‡, [Mz1] ≠ [Mz2=S.c.=S.p.] ≠ [Drs=Hum]. The subset of 13 of these positions at which maize and yeast TBPs share the identical amino acid are shaded. Individual amino acid residues in *S. cerevisiae* TBP involved in protein–protein interactions with yeast SPT3 (Eisenmann et al., 1992) (¶), TFIIA (Buratowski and Zhou, 1992) ($), or RNA polymerases (see Nikolov et al., 1992) (†) also are shown above the Mz1 sequence. The entire α2 helix region is encompassed within a segment shown to be involved in the interaction between TBP and E1A (see also Nikolov et al., 1992).

al., 1990; Cormack et al., 1991; Gill and Tjian, 1991; Poon et al., 1991). In contrast, the intact human and Drosophila TBP proteins and their C-terminal domains cannot function in yeast (Cormack et al., 1991; Gill and Tjian, 1991; Poon et al., 1991). This failure to complement has been proposed to result from species-specific differences that prevent the animal isoforms from fully supporting RNA polymerase II transcription or from sustaining non-RNA polymerase II activities in the yeast cell. Because single amino acid alterations in yeast TBP can prevent its functional interaction with other transcription proteins (see Nikolov et al., 1992), it is possible that some subset of the 24 amino acid positions that differ between the yeast and animal C-terminal domains (see Figure 8) might prevent animal TBP function in yeast.

Thirteen of these 24 polymorphic amino acids are conserved between yeast and maize TBP proteins (shaded in Figure 8). Given that both maize TBPs can function in yeast, some of these 13 shared amino acids could be required for yeast cell activity. Furthermore, because the TATA box binding functions of animal and fungal TBPs are interchangeable (see Kelleher et al., 1992), only residues involved in interactions with other transcriptional proteins, and not in DNA binding, are likely to be involved. Based upon the structure of Arabidopsis TBP2 (Nikolov et al., 1992), many of these shared 13 amino acids appear to be contained within the four α-helices that lie across the outer surface of the molecule. (All α-helices as well as β-strands, likely to contact the DNA, are indicated in Figure 8.) A few of these 13 residues correspond to amino acid positions shown by mutational analyses to be involved in mediating specific protein–protein interactions in the yeast cell and therefore are the best candidates for species specificity determinants. For example, the presence of a lysine at position 91 (maize TBP numbers) may be required for yeast cell function because both human and Drosophila TBPs each carry arginine, and a mutation at this position disrupts yeast TBP–TFIIA interactions (Buratowski and Zhou, 1992). Alternatively, amino acids 134, 135, and 136, which overlap residues important in yeast TBP for functional interaction with the yeast SPT3 factor (Eisenmann et al., 1992), may constitute a specificity determinant. Perhaps functional interaction of TBP with some subset of proteins in the yeast cell may require an aromatic amino acid (Tyr or Phe) at position 135.

These results demonstrate that each of the two TBP proteins, encoded by unlinked, highly conserved genes in the

maize genome, can function in yeast. We demonstrate that an intact, nonfungal TBP protein can complement yeast TBP in vivo. Detailed amino acid structure–function analysis of the maize *Tbp* genes should allow the identification of precise TBP protein residues that serve as species-specific determinants, thereby providing valuable insight into understanding protein–protein contacts essential for TBP function during transcription.

METHODS

Maize and Arabidopsis Stocks

All analyses used seeds and tissues from the maize (*Zea mays*) inbred line B73 obtained from Pioneer Hi-Bred International, Inc. (Johnston, IA). Genomic DNAs from *Arabidopsis thaliana* of the Columbia, Landsberg *erecta*, and Wassilewskija ecotypes were a generous gift from M. Williams (University of California, Berkeley). This maize inbred line and each of the Arabidopsis ecotypes have been inbred extensively, and each genome should carry a single allele at every locus. Maize recombinant inbred (RI) individuals of two independent families, CM37 × T232 (48 RI individuals maintained by at least nine selfed generations) and CO159 × TX303 (41 individuals) (Burr and Burr, 1991), were kindly provided by B. Burr (Brookhaven National Laboratory, Upton, NY). Maize actin and ubiquitin cDNA clones were kindly provided by R. Meagher (University of Georgia, Athens) and P. Quail (U.S. Department of Agriculture Plant Gene Expression Center, Albany, CA), respectively.

Degenerate Primer Polymerase Chain Reaction and cDNA Clone Isolation

A fragment of a maize TATA binding protein (*Tbp*) gene was amplified from the B73 genome using degenerate polymerase chain reaction (PCR) primers corresponding to regions shown to be conserved among yeast, Drosophila, and human TBP proteins. The 12-fold degenerate 20-mers used as PCR primers (see Figure 1) were 5′-TGTGATGTGAA-(A/G)TT(T/C)CC(C/T/A)AT-3′ (forward) and 5′-GGCTTCACCAT(C/T)C-(G/T)(G/A)TA(G/A)AT-3′ (reverse), corresponding to *Saccharomyces cerevisiae* TBP amino acids 164 to 170 (CDVKFPI) and 194 to 200 (IYRMVKP), respectively. Degenerate PCR primer amplifications, using 1 μg of CsCl-purified B73 genomic DNA, were performed for three cycles using a 35°C annealing temperature, and for 35 additional cycles at 50°C. PCR fragments were gel purified, treated with T4 DNA polymerase (New England Biolabs), and then individually subcloned into pBluescript KS− (Stratagene).

Clones containing *Tbp* sequence were verified by DNA sequencing (Sequenase v2.0; U.S. Biochemicals Corp.), and a 205-bp PCR fragment carrying maize *Tbp* sequences was used to probe an amplified maize cDNA library (Barkan and Martienssen, 1991) constructed in λ UniZAP-XR (Stratagene) using poly(A)$^+$-selected RNA isolated from light-grown, 2-week-old maize B73 seedlings. Approximately 3 × 10^6 recombinant clones were screened under low-stringency conditions (hybridization in 6.6 × SSC [1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate], 10 mM EDTA, 50 mM sodium phosphate, 0.1% SDS, 5 × Denhardt's solution [1 × Denhardt's solution is 0.02% Ficoll, 0.02% PVP, 0.02% BSA] at 45°C [40°C below melting temperature]). Filters were washed in 3 × SSC, 0.2% SDS at 45°C. Four independent cDNA clones of one sequence class (denoted mzTbp1) and eight of a second class (mzTbp2) were isolated. No mzTbp1 clone contained a complete coding region. The 5′ end of the mzTbp1 transcript was amplified from poly(A)$^+$ RNA of immature embryos, an abundant source of *Tbp*1 message, using 5′ rapid amplification of cDNA ends PCR, essentially as described by Frohman et al. (1988). The reverse orientation *Tbp*1 gene–specific 28-mer, 5′-GCAAGCTTAGATTGCTGCTCGCTCTTAG-3′, corresponds to maize TBP amino acid positions 141 to 150 (see Figure 1) and is the most polymorphic coding region segment between the truncated mzTbp1 and full-length mzTbp2 clones. The resulting 375-bp PCR fragment was sequenced to verify its distinctiveness from *Tbp*2 and was then subcloned into the longest of the truncated *Tbp*1 cDNA clones at the unique BspHI site to generate a complete in-frame *Tbp*1 coding sequence. The ability of this reconstructed cDNA to encode a full-length TBP protein was verified by in vitro translation (see below). Complete DNA and deduced protein sequences for these cDNAs are registered in the GenBank sequence data base as accession numbers L13301 and L13302, respectively.

Nucleic Acid Isolation and Gel Blot Analysis

Total genomic DNA was isolated from maize B73 seedlings and from adult leaf or immature ear tissue from all recombinant inbred and parental maize stocks (Burr and Burr, 1991) using the phenol-detergent mini-prep method (Chomet et al., 1991). Isolation of total RNA from tissues of B73 plants was as previously described (Kloeckener-Gruissem et al., 1992). Polyadenylated RNA was isolated from these total RNAs by double selection on oligo(dT) columns (Type VII; Calbiochem).

For each lane of a gel, 10 μg of maize or 2.5 μg of Arabidopsis genomic DNA was digested with a fourfold excess of restriction endonuclease (New England Biolabs) for 3 to 16 hr. Digestion reaction products were fractionated on 0.8 to 1.0% agarose gels and blotted in 10 × SSC onto Duralon-UV nylon membranes (Stratagene). RNA gels contained 2 μg of poly(A)$^+$-selected RNA per lane, and analyses were performed as previously described (Kloeckener-Gruissem et al., 1992).

All hybridizations were performed with ^{32}P-labeled probes (≥1 × 10^8 cpm/μg) generated by random nanomer priming (Stratagene). Hybridizations for maize DNA and RNA gel blots were conducted at 65°C under aqueous conditions (Chomet et al., 1991). Blots were washed at either low (2 × SSC, 0.5% SDS; 65°C) or high (0.1 × SSC, 0.1% SDS; 65°C) stringency, as indicated. Gel blots containing Arabidopsis DNA were probed with the heterologous Tbp2-X (see below) maize *Tbp* coding region fragment (75 and 78% similar to the two cloned Arabidopsis genes; Gasch et al., 1990) at very low stringency (hybridization at 9 × SSPE, 5 × Denhardt's solution, 10% sarcosine at 50°C; washes in 1 × SSPE [0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4], 0.1% SDS at 50°C. Membranes were exposed to Kodak X-AR film at −80°C using Lightening Plus intensifying screens or directly to a phosphorimaging screen (Molecular Dynamics, Sunnyvale, CA) for linear quantitation of hybridization signals.

Fragments of the maize *Tbp* cDNAs used as probes are as follows: the conserved coding region, designated Tbp2-X (the 631-bp XhoI-XhoI fragment containing the complete *Tbp* coding region from the longest mzTbp2 cDNA; this fragment shares 95, 75, and 78% identity with the corresponding maize *Tbp*1 and Arabidopsis *Tbp*1 and *Tbp*2 coding regions, respectively [Gasch et al., 1990]); the *Tbp*1 gene–specific fragment, TBP1-HX (a 240-bp fragment from the distal-most 3′

noncoding region of *Tbp1*); and the *Tbp2* gene–specific fragment, Tbp2-HX (the analogous 250-bp region of *Tbp2*). The two gene-specific fragments are less than 50% similar to one another. Single-stranded versions of Tbp1-HX were generated from linearized, denatured plasmid templates by run-off DNA synthesis (Sequenase v2.0) using primers specific to either the T3 or T7 phage promoter flanking the insert.

Locus Mapping with Recombinant Inbreds

Molecular mapping with maize RI lines was performed as described previously (Burr and Burr, 1991). Specifically, Tbp2-X was used as a restriction fragment length polymorphism (RFLP) probe at low stringency against gel blots containing RI and parental DNAs digested with BglII. Under these conditions (final wash at 2 × SSC at 65°C), both strong and medium intensity hybridization to *Tbp2* and *Tbp1* bands, respectively, can be viewed simultaneously. Alleles for each of these two genes were typed for all RI individuals in comparison to the parental line alleles. The resulting numerical arrays were sent to B. Burr (Brookhaven National Laboratories, Upton, NY) for computer-aided determination of chromosomal map positions for these maize *tbp1* and *tbp2* loci. Concurrently, these genes were also kindly mapped by D. Blair and M. Katz in the Pioneer Hi-Bred mapping populations.

Protein Identification

cDNAs carrying the complete maize *Tbp1* and *Tbp2* coding regions, subcloned into pBluescript SK+, were linearized at a common HincII site in their 3′ noncoding regions. The linearized, gel-purified plasmids served as templates for in vitro transcription reactions using phage T3 RNA polymerase (Promega). One-hundred nanograms of these in vitro–synthesized RNAs were used to program ^{35}S-methionine–labeled TBP protein synthesis using nuclease-depleted rabbit reticulocyte lysates (Promega). In vitro translation products were fractionated on 12% SDS–polyacrylamide discontinuous gels, and ^{35}S-labeled protein products were identified by enhanced fluorography on Kodak X-AR film.

Yeast Strains and Complementation Assays

Oligonucleotide site-directed mutagenesis (Su and El-Gewely, 1988) was performed to construct maize *Tbp* genes containing an NcoI site at the AUG start codon (underlined) using the forward oligonucleotide primer 5′-CGTGCGTGATTCCCATGGCGGAGC-3′ and a HpaI site at the termination codon (underlined) of *Tbp1* and *Tbp2* using 5′-CAGCAATGATGTTAACTGATGTGAAGG-3′ and 5′-CAGCAATGATGTTAACTTGTGAAGG-3′, respectively, as primers. The single-stranded DNA templates used for mutagenesis were derived from pBluescript SK– vectors carrying either a 0.92-kb maize *Tbp1* cDNA fragment (from the StuI site in the 5′ noncoding region to the PstI site in the 3′ untranslated region) or a 1.25-kb maize *Tbp2* cDNA (EcoRI and XhoI sites upstream and downstream, respectively), with each insert containing complete, intronless protein-encoding regions. These two cDNA fragments were cloned into the vector in the same orientation at the HincII and PstI sites or at the EcoRI and XbaI polylinker sites, respectively.

To generate the yeast expression plasmid C18-2, the annealed oligonucleotides 5′-AGCTTAAGATCTAAACCATGGAAGCGGCCGCA-3′ and 5′-AGCTTGCGGCCGCTTCCATGGTTTAGATCTTA-3′ were inserted into the single HindIII site between the *alcohol dehydrogenase-1* (*ADH1*) promoter and terminator in the *LEU2*-marked 2μ plasmid vector, pAAH5 (Ammerer, 1983). The NcoI site within the linker is oriented such that it is adjacent to the *ADH1* promoter. Following digestion of the C18-2 vector with NotI, filling in these ends with the Klenow fragment of DNA polymerase I, and then digesting with NcoI, fragments of the maize *Tbp* genes (NcoI-HpaI) were inserted.

The wild-type yeast strain H1511 (MATα, *ura3-52*, *trp1-D63*, *leu2-3*, *GAL2*$^+$), an ascospore derivative of S288C, was transformed (Ito et al., 1983) with the low-copy–number *URA3*-containing yeast plasmid pRS316ADHyTBP (Gill and Tjian, 1991), which contains the *Saccharomyces cerevisiae* *yTbp* gene under transcriptional control of the *ADH1* promoter. A *tbp*Δ chromosomal allele was constructed in this strain by transforming it from Ura$^+$, Trp$^-$ to Ura$^+$, Trp$^+$, replacing the chromosomal *yTbp* sequences with a 2.0-kb EcoRI-BamHI fragment containing the 1.1-kb *TRP1* gene. To distinguish between homologous integration of this fragment at the *yTbp* chromosomal locus rather than into the *URA* plasmid–borne gene copy, the selected Ura$^+$, Trp$^+$ transformants were replica plated to 5-fluoroorotic acid (5-FOA) and screened for 5-FOA sensitivity (as described by Gill and Tjian, 1991), which would indicate the presence of a functional *yTbp* gene only on the *URA3* plasmid. Cells grown from a Ura$^+$, Trp$^+$, 5-FOA–sensitive colony, denoted YMC14, were then transformed with the modified high-copy–number *LEU2* plasmid derivative, C18-2, or with the same *LEU2* plasmid containing the yeast *Tbp*, maize *Tbp1*, or maize *Tbp2* gene (Gill and Tjian, 1991), under transcriptional control of the strong *ADH1* promoter. Trp$^+$, Ura$^+$, Leu$^+$ colonies were then replica plated to 5-FOA plates to select for 5-FOA resistance, indicative of *URA* plasmid loss. The only *Tbp* gene copy in these final Trp$^+$, Ura$^-$, Leu$^+$ colonies is contained on the *LEU2* plasmid.

Immunoblot Analysis

Following 5-FOA selection, individual Trp$^+$, Ura$^-$, Leu$^+$ yeast transformants were grown in 5 mL of YEPD rich medium (Ammerer et al., 1983) to densities of 6.5 to 9.0 OD_{600}. Whole-cell extracts were prepared by glass bead disruption using a vortexer (Jazwinski, 1990). Total protein concentrations were determined at A_{595} by the method of Bradford (see Jazwinski, 1990). Extract volumes corresponding to 1.0 A_{595} unit were loaded and run on a 12% SDS–polyacrylamide discontinuous gel (0.75 mm), and the unstained gel was electroblotted (semidry) to an Immobilon-P (Millipore Corp., Bedford, MA) membrane in 49 mM Tris base, 39 mM glycine, .04% SDS, 20% methanol. Immunoblot analysis using chemiluminescence detection was performed as described by the manufacturer (ECL; Amersham Corp.). The monoclonal antibody, 58C9, was used as primary antibody at a 1:500 dilution. This antibody was generated using the C-terminal domain of Drosophilia TBP as immunogen and has also been demonstrated to bind specifically to yeast and human TBP isoforms (ascites generously provided by R. Weinzierl and R. Tjian, University of California, Berkeley). The secondary antibody, horseradish peroxidase–conjugated goat anti–mouse IgG (Amersham Corp.), was used at a 1:10,000 dilution.

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