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Genes encoding transcription factor IIIA and the RNA polymerase common subunit RPB6 are divergently transcribed in *Saccharomyces cerevisiae*

(zinc finger/RNA polymerase III/5S rRNA)

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ABSTRACT **The gene encoding *Saccharomyces cerevisiae* transcription factor TFIIIA has been found adjacent to *RPB6*, a gene that specifies a subunit shared by nuclear RNA polymerases. Analysis of DNA upstream of the *RPB6* gene revealed an open reading frame that predicts a protein, designated PZF1, with nine C_2H_2 zinc fingers. The presence of nine C_2H_2 zinc fingers in PZF1 protein, a hallmark of amphibian TFIIIA proteins, suggested that PZF1 might be a TFIIIA homologue. We found that purified recombinant PZF1 specifically binds the internal control region (ICR) of the 5S rRNA gene in *S. cerevisiae*. The presence of nine C_2H_2 zinc fingers, the specific binding to ICR DNA, and the similarity of the predicted molecular mass of PZF1 with that determined for purified yeast TFIIIA, together indicate that PZF1 is TFIIIA. The yeast and amphibian TFIIIA proteins share only a limited number of residues outside of those normally conserved in C_2H_2 zinc fingers; these conserved residues may provide clues to the sequence specificity of these proteins. The *PZF1* gene was found to be single copy, transcribed into a 1.5-kilobase mRNA, and essential for yeast cell viability. Interestingly, the yeast RPB6 and TFIIIA coding sequences are divergently transcribed and are separated by only 233 base pairs, providing the potential for coregulated expression of components of RNA polymerases and the 5S rRNA component of ribosomes.**

The transcriptional activator transcription factor IIIA (TFIIIA) is required for initiation of 5S rRNA transcription by RNA polymerase III (refs. 1–4; reviewed in ref. 5). Synthesis of 5S rRNA begins with the binding of TFIIIA to the internal control region (ICR) of the 5S rRNA gene (6, 7), followed by sequential binding of factors TFIIIC and TFIIIB and RNA polymerase III (1, 3, 4, 8). Unlike TFIIIC and TFIIIB, which are required for synthesis of both tRNA and 5S rRNA, TFIIIA appears to be exclusively required for 5S rRNA synthesis. In addition to its ability to specifically bind the 5S rRNA-encoding DNA ICR, TFIIIA is able to specifically bind 5S rRNA (9, 10).

Cloning and analysis of the TFIIIA gene product from *Xenopus laevis* (11) lead to recognition of a tandemly repeated pattern of cysteine and histidine residues that came to be known as zinc fingers (12, 13). Structural and mutational analyses of zinc finger peptides (14–19) have provided evidence that the zinc finger is a structural domain that is responsible for sequence-specific recognition of DNA. TFIIIA in *X. laevis* has nine C_2H_2 zinc fingers, which bind to the ICR of the 5S gene (20–22). Nonfinger residues of the C terminus appear to be important for transcriptional activation (20). Thus far, TFIIIA genes have been isolated only from the amphibians *X. laevis* (11), *Xenopus borealis* (23), and *Rana catesbeiana* (23). All three of the amphibian TFIIIA proteins have nine contiguous C_2H_2 zinc fingers.

Since TFIIIA plays a key role in the synthesis of 5S rRNA, and because the molecular interactions between zinc fingers and specific DNA sequences are being studied intensively, the isolation of TFIIIA genes from genetically tractable organisms has been of great interest. However, attempts to isolate the TFIIIA genes from other eukaryotes have been unsuccessful thus far, apparently because of the lack of sequence similarity between amphibian TFIIIAs and those of other eukaryotes. Here we describe the isolation and characterization of a gene for a nonamphibian TFIIIA from the yeast *Saccharomyces cerevisiae*.§ The yeast TFIIIA protein possesses the nine zinc fingers found in amphibian TFIIIA proteins but is otherwise substantially different in sequence.

MATERIALS AND METHODS

Plasmids and Yeast Strains. Plasmid pPZF1 was constructed by inserting a 3-kilobase (kb) *PZF1*-containing *Nsi* I/*Sac* I DNA fragment into the *Nsi* I/*Sac* I sites of pGem9Zf(+). pPZF4 was constructed from pPZF1 by replacement of the coding region of *PZF1* with *Bam*HI/*Sma* I sites (24) followed by insertion of a 1.75-kb *HIS3*-containing DNA fragment. pPZF5, the plasmid used to obtain the complete DNA sequence of *PZF1*, has a 4-kb *Bgl* II DNA fragment inserted into the *Bam*HI site of pBluescript KS+. *PZF1* was sequenced from both strands of pPZF5 plasmid DNA. pPZF7 contains the coding region of *PZF1*, minus the first 30 base pairs (bp), flanked with PCR-synthesized restriction sites (a *Bam*HI site on the 5′ end and an *Eco*RI site on the 3′ end) to permit insertion into the *Bam*HI/*Eco*RI sites of the glutathione *S*-transferase (GST; RX:glutathione R-transferase, EC 2.5.1.18) expression vector, pGEX-2T (Pharmacia LKB). The first 30 bp encoding the N-terminal 10 amino acids were deleted to allow for induced expression of GST–PZF1 after it was noted that isopropyl β-D-thiogalactoside (IPTG)-mediated induction of the full-length *PZF* gene in pGEX-2T killed the bacterial cells shortly after addition of IPTG. pNW9 contains an ≈310-bp *Bam*HI/*Eco*RI *S. cerevisiae* ICR-containing DNA fragment [approximately positions +180 through −120 of the 5S rRNA gene, obtained by PCR of the plasmid pDPL13-5S (kindly provided by J. Segall, University of Toronto)] ligated to *Bam*HI/*Eco*RI digested pGem7Zf(+). All DNA fragments generated by the PCR were determined to be error-free by DNA sequence analysis. Yeast strains were as follows: Z321, *MAT***a**/*MATα ura3-52*/

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Abbreviations: GST, glutathione *S*-transferase; ICR, internal control region; IPTG, isopropyl β-D-thiogalactoside; TFIIIA, transcription factor IIIA.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M90638).

ura3-52 his3Δ200/his3Δ200 leu2-3/leu2-3 leu2-112/leu2-112 lys2Δ201/lys2Δ201 ade2/ade2; WY20, *MATa/MATα ura3-52/ura3-52 his3Δ200/his3Δ200 leu2-3/leu2-3 leu2-112/leu2-112 lys3Δ201/lys2Δ201 ade2/ade2 pzf/pzf1Δ1::HIS3*.

Deletion of *PZF1*. To create the *pzfΔ1::HIS3* allele, a 3.5-kb *Spe* I/*Xmn* I *HIS3*-containing fragment cut from pPZF4 was used to transform the yeast diploid Z321. Genomic DNA was prepared from the His^+ transformant WY20 and subjected to Southern analysis to verify substitution of the chromosomal copy with *HIS3*. WY20 cells were sporulated and subjected to tetrad analysis.

Bacterial Expression and Purification of PZF1. Overnight cultures of pPZF7 cells were diluted 1:50 and grown for 2–3 hr at 37°C followed by addition of IPTG to a final concentration of 1 mM. Three hours after IPTG induction, 50-ml aliquots were pelleted and stored at −70°C. Pellets were sonicated in 1 ml of TKZD (50 mM Tris·HCl, pH 8.0/200 mM KCl/10 mM $ZnCl_2$/1 mM dithiothreitol) followed by addition of Triton X-100 to 1%. GST–PZF1 fusion protein was adsorbed from the supernatant with 200 μl of glutathione Sepharose 4B (Pharmacia LKB) for 1 hr at 4°C. The affinity gel was pelleted and washed four times with 0.5 ml of TKZD, and the fusion protein was eluted with 200 μl of TKZD plus 5 mM reduced glutathione for 5 min at room temperature.

Mobility-Shift DNA Binding Assay. A DNA fragment containing the yeast ICR region was used to demonstrate sequence-specific DNA binding with the GST–PZF1 fusion protein. Plasmid pNW9 was digested with *Bam*HI/*Eco*RI, purified, and end-labeled using the Klenow fragment of DNA polymerase I. Binding reaction mixtures contained 20 μl of TKZD containing 5% glycerol, 5 μg of bovine serum albumin, 2 μg of dI-dC, 5 μl of purified GST–PZF1, plus 1 μg of competitor DNA when specified. The sequences of the wild-type and mutant duplex 51-mer DNA oligonucleotides are represented in Fig. 2*B*. Linearized pUC19 DNA was used as a nonspecific competitor. All reaction mixtures were incubated for 10 min at room temperature before addition of the radioactively labeled ICR-containing DNA, followed by an additional incubation for 10 min at room temperature. Gel electrophoresis conditions were as described by Challice and Segall (25).

RESULTS

A Gene Adjacent to *RPB6* Encodes a Zinc Finger Protein. The *RPB6* gene encodes a subunit shared by RNA polymerases I, II, and III in *S. cerevisiae*, and it is located on chromosome XVI (26). Sequence analysis of DNA upstream of *RPB6* revealed a 1.3-kb open reading frame that is oriented opposite that encoding RPB6 (Fig. 1*A*). The 1.3-kb open reading frame and the RPB6 coding sequences are separated by only 233 bp.

The 1.3-kb open reading frame predicts a 429-amino acid protein (50 kDa) containing nine C_2H_2 zinc fingers, and we designated this putative protein PZF1 (Fig. 1*B*). The nine C_2H_2 zinc fingers belong to a class of proteins that have two invariant cysteine and histidine residues spaced within the general pattern $F/YXCX_{2-4}CX_3FX_5LX_2HX_{3-4}HX_5$, where X is any amino acid (27). Proteins that contain this class of zinc fingers include the *Xenopus* transcription factor TFIIIA, the human factor Sp1, and the yeast factor ADR1, among others (28, 29). The TFIIIA proteins distinguish themselves by containing nine C_2H_2 zinc fingers, but alignment of PZF1 amino acid residues with those of TFIIIA revealed only ≈20% sequence identity, with most of the shared residues being in the general pattern exhibited by the C_2H_2 zinc finger proteins. Although PZF1 appeared likely to be a DNA binding factor, its identity was unclear.

***PZF1* Is an Essential Single-Copy Gene.** We further investigated *PZF1* by determining its copy number, identifying a specific transcript, and ascertaining whether the gene is essential for yeast cell viability. To determine *PZF1* copy number, Southern blots containing immobilized restriction digests of *S. cerevisiae* genomic DNA were probed with *PZF1* DNA fragments at moderate stringency. The pattern of hybridization, in which only a single band producing a strong signal was observed (data not shown), indicated that *PZF1* was a single copy gene in haploid yeast. *PZF1* DNA hybridized to a 1.5-kb mRNA on a Northern blot containing immobilized yeast mRNA (data not shown).

To construct a yeast strain lacking the *PZF1* gene, the entire protein coding region of the plasmid-borne *PZF1* gene was first deleted and replaced with a unique restriction site. A *HIS3* DNA fragment was inserted into the newly created restriction site to produce the *pzfΔ1::HIS3* allele. One chromosomal copy of the gene in diploid yeast cells was replaced by the method of Rothstein (30), producing strain WY20. Tetrad analysis of the sporulation products of WY20 revealed that deletion of *PZF1* produces nonviable haploid cells (Table 1). Thus, *PZF1* is essential for cell viability.

PZF1 Binds to the ICR of the 5S rRNA Gene. Three pieces of evidence led us to suspect that PZF1 might be the *S. cerevisiae* transcription factor TFIIIA, despite limited sequence similarity between yeast PZF1 and amphibian TFIIIA. First, PZF1 has nine C_2H_2 zinc fingers, a hallmark of TFIIIA. Second, Wang and Weil (31) recently purified an ≈48-kDa protein doublet from *S. cerevisiae* possessing TFIIIA activity, closely corresponding to the predicted size of

B

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  1 GATCACTTGTAGCCTTAATCTTTACACTGTTACCTGCAAAATGTGTCTTTTTAGCCTTTC
 61 TTCAAGGTTGTGATTGATGCCCTTTGAATGATATTCTTAAAAAAAAAAAAATTTTTTTTC
121 AATTAAATATCATACTATACGAATAATCAATATACAATGATTATTTATTATAAGAAGAAC
181 TAACTATCAAAATAAGGGCATTAAGCACAGTAGTAACTGTTGAAATCGCTGTCAAATGGGA
                                                          M  G    2
241 GGAGAGGTTCTAAATAATGAAGGAATGCCACTGGCCGAACTTAAACAGGAGACCATTCCC
    G  E  V  L  N  N  E  G  M  P  L  A  E  L  K  Q  E  T  I  P   22
301 ATATCACGATCAGAGAGTTCAGAATCATTGAACAGTTTAACATCAACTAGAAGCTCATCG
    I  S  R  S  E  S  S  E  S  L  N  S  L  T  S  T  R  S  S  S   42
361 AGCAATAGGCCAAAGACATATTTCTGTGACTATGATGGCTGTGATAAGGCATTTACAAGA
    S  N  R  P  K  T  Y  F  C  D  Y  D  G  C  D  K  A  F  T  R   62
421 CCTTCAATTTTGACTGAACACCAATTAAGCGTACATCAGGGTTTAAGAGCATTTCAGTGT
    P  S  I  L  T  E  H  Q  L  S  V  H  Q  G  L  R  A  F  Q  C   82
481 GATAAGTGTGCAAAATCCTTCGTTAAAAAGAGTCACTTAGAGAGACACTTGTATACGCAT
    D  K  C  A  K  S  F  V  K  K  S  H  L  E  R  H  L  Y  T  H  102
541 TCTGATACGAAACCATTCCAATGTTCTTATTGTGGAAAAGGAGTGACGACTCGCCAGCAA
    S  D  T  K  P  F  Q  C  S  Y  C  G  K  G  V  T  T  R  Q  Q  122
601 CTGAAGCGACACGAAGTAACGCATACCAAATCTTTCATTTGTCCAGAGGAAGGATGCAAC
    L  K  R  H  E  V  T  H  T  K  S  F  I  C  P  E  E  G  C  N  142
661 CTCCGATTCTACAAGCATCCACAATTAAGGGCACATATTTTATCTGTTCATTTACATAAA
    L  R  F  Y  K  H  P  Q  L  R  A  H  I  L  S  V  H  L  H  K  162
721 CTAACCTGTCCACACTGCAATAAAGCTTTCAGAGCCATATAGCTAAGAAATCACATT
    L  T  C  P  H  C  N  K  S  F  Q  R  P  Y  R  L  R  N  H  I  182
781 TCTAAACATCACGATCCTGAGGTAGAAAATCCTTACCAATGTACTTTTGCTGGTTGTTGC
    S  K  H  D  P  E  V  E  N  P  Y  Q  C  T  F  A  G  C  C  202
841 AAAGAGTTTCGGATATGGTCACAATTGCAATCGCATATAAAAATGATCATCCTAAATTA
    K  E  F  R  I  W  S  Q  L  Q  S  H  I  K  N  D  H  P  K  L  222
901 AAATGTCCTATTTGTAGCAAACCATGTGTGGGGGAAAATGGTTTACAAATGCACATGATT
    K  C  P  I  C  S  K  P  C  V  G  E  N  G  L  Q  M  H  M  I  242
961 ATTCATGACGACTCACTAGTAACCAAAAATTGGAAGTGTCATATATGTCCTGATATGTCT
    I  H  D  D  S  L  V  T  K  N  W  K  C  H  I  C  P  D  M  S  262
1021 TTTTCTAGAAAACATGATCTTCTCACGCATTATGGGTCTATTCATACGGAAGAAGATATC
    F  S  R  K  H  D  L  L  T  H  Y  G  S  I  H  T  E  E  D  I  282
1081 CCACTGGAACTAAAATATAAAATTTCTGATATTCAACAACTTGTCCAAGACCACGGAGTT
    P  L  E  L  K  Y  K  I  S  D  I  Q  Q  L  V  Q  D  H  G  V  302
1141 CAACTGGGGAATAGCAAACACTCGAATGAGCAGGATGAAGAAGAAGATTTCCAATAGGTTG
    Q  L  G  N  S  K  H  S  N  E  Q  D  E  E  K  I  S  N  R  L  322
1201 AGGAAAAGGAGAAAACTTACCGAAAACAATAATGTAGAGTTTTACAGAATGAAGTGGAT
    R  K  R  R  K  L  T  E  N  N  N  V  E  F  L  Q  N  E  V  D  342
1261 TTAGAAAAGCGACTTGAGTCTGGTGAAAATGGGCTAAACCTTTTATTGAATACCGTTGGA
    L  E  K  R  L  E  S  G  E  N  G  L  N  L  L  L  N  T  V  G  362
1321 AGAAAATACAGATGCTTTTACAATAATTGTTCTAGGACTTTCAAGACAAAGGAAAAATAC
    R  K  Y  R  C  F  Y  N  N  C  S  R  T  F  K  T  K  E  K  Y  382
1381 GAGAAACATATAGACAAGCACAAAGTACATGAGCTAAAGTTAAAAATATTACAGGAAAAG
    E  K  H  I  D  K  H  K  V  H  E  L  K  L  K  I  L  Q  E  K  402
1441 GAAGAAAATAAAACGCTAGTGGACCAGAACCATAAGGAACCTTTTATAATTCAAAAGGAA
    E  E  N  K  T  L  V  D  Q  N  H  K  E  P  F  I  I  Q  K  E  422
1501 ACGCAGTCAGCGGGTGACAAATAAGCATTTTTTTTTTTTATTTTATATGCTAATTATATT
    T  Q  S  A  G  D  K  *                                      429
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FIG. 1. The *PZF1* gene. (*A*) Orientation of the *PZF1* and *RPB6* genes. Arrows represent gene orientation; lengths of shaded and solid rectangles reflect relative gene sizes. (*B*) DNA sequence of the *PZF1* gene. The nine zinc fingers of the predicted amino acid sequence are underlined. The 233-bp 5′ untranslated region shown corresponds to the promoter region shared by PZF1 and RPB6 as diagramed in *A*.

Table 1. Tetrad analysis of diploid cells with PZF gene replacements

Genotype at target locus	Viable spores per tetrad			
	1	2	3	4
pzf1/pzf1Δ1::HIS3	2	17	0	0

All viable spores were His^-.

the PZF1 gene product (50 kDa). Third, attempts to isolate the yeast gene encoding TFIIIA by using either low-stringency hybridization with the *Xenopus* gene or antibodies against the *Xenopus* TFIIIA have failed, apparently because of significant differences in the TFIIIA gene and protein in these two species (31).

To determine whether PZF1 was yeast TFIIIA, we investigated whether PZF1 would bind specifically to the ICR of the *S. cerevisiae* 5S rRNA gene. Since purified yeast TFIIIA binds exclusively to DNA sequences contained in the ICR DNA *in vitro* (31), we used a mobility-shift binding assay to assess whether PZF1 has this TFIIIA function. Recombinant PZF1 protein fusions to either maltose binding protein or GST were generated with two different *Escherichia coli* expression systems and purified to homogeneity. The purified PZF1 protein fusions were tested for their ability to bind a radioactively labeled DNA fragment containing the *S. cerevisiae* 5S rRNA gene ICR by using a mobility-shift DNA binding assay. The behavior of maltose binding protein and GST–PZF1 fusions were comparable in this assay. Fig. 2 reveals a significant mobility shift of the labeled ICR-containing DNA fragment with the GST–PZF1 fusion protein (Fig. 2*A*, lane 1). Preincubation of PZF1 with unlabeled ICR DNA completely prevented the shift of the labeled ICR-containing DNA fragment (lane 2), while preincubation with nonspecific DNA did not (lane 4). The loss of PZF1 binding to the ICR-containing DNA fragment by competition with the oligonucleotide [representing the 51-bp region of DNA that contained the eight known yeast TFIIIA–ICR close contact points (25)] demonstrated that PZF1 binds specifically to the ICR of the 5S rRNA gene. When all eight of these contact points (25) were mutated, the mutant ICR fragments failed to compete with the binding of PZF to the wild-type ICR. This experiment further supported the competition experiment with the wild-type oligonucleotide, since it revealed that the residues shown to be TFIIIA–ICR contact points are critical for ICR binding as well. The specific binding of PZF1 to ICR DNA, the presence of nine C_2H_2 zinc fingers, and the similarity of the predicted molecular mass of PZF1 with that determined for yeast TFIIIA lead us to conclude that PZF1 is TFIIIA.

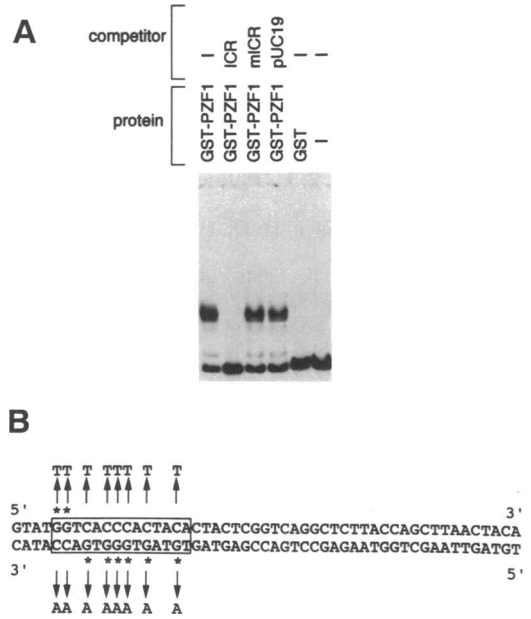


FIG. 2. Mobility-shift binding assay of the GST–PZF1 fusion protein. (*A*) Mobility-shift assay using a radiolabeled 310-bp fragment containing the *S. cerevisiae* ICR and purified GST–PZF1 fusion protein (GST–PZF1) or purified GST protein alone (GST). The following competitor DNAs were used: a duplex 51-mer DNA oligonucleotide containing the yeast ICR (ICR) (see *B*), a duplex 51-mer DNA oligonucleotide with mutations in bases involved in DNA–protein binding (mICR) (see *B*), and pUC19 as a nonspecific competitor. —, Absence of a component. The low intensity band above the unshifted labeled DNA represents a small percentage of proteolyzed PZF1 protein whose binding is blocked by competition with unlabeled ICR DNA. (*B*) Sequence of the duplex 51-mer containing the *S. cerevisiae* ICR. Residues +48 to +98 are on the lower (noncoding) strand, +48 is at the 5′ end, and +98 is at the 3′ end. The guanosines marked with an asterisk are TFIIIA–DNA contact points demonstrated by DNA methylation protection experiments with the TFIIIA–5S rRNA gene complex (25). Base changes (G to T, C to A) represented in the mutant ICR duplex DNA oligonucleotide are shown on both strands.

Comparison of Yeast and Amphibian TFIIIA Proteins. To date, only amphibian TFIIIA genes have been isolated and characterized. When aligned, the amino acid sequences of TFIIIA proteins from *X. laevis* and *X. borealis* are 84% identical (23). Those of TFIIIA from *X. laevis* and *R. catesbeiana* are 63% identical (23). Alignment of yeast TFIIIA with *X. laevis* reveals more substantial differences in sequence and size (Fig. 3). Only ≈20% of the amino acids are identical. Amphibian TFIIIAs have nine contiguous zinc finger domains, while the yeast protein has eight contiguous domains that are separated from a ninth by ≈80 amino acids. Finally, the molecular mass of yeast TFIIIA is ≈10 kDa greater than the amphibian TFIIIAs. These differences almost certainly account for the difficulties encountered in isolating the yeast TFIIIA gene by using amphibian DNA and

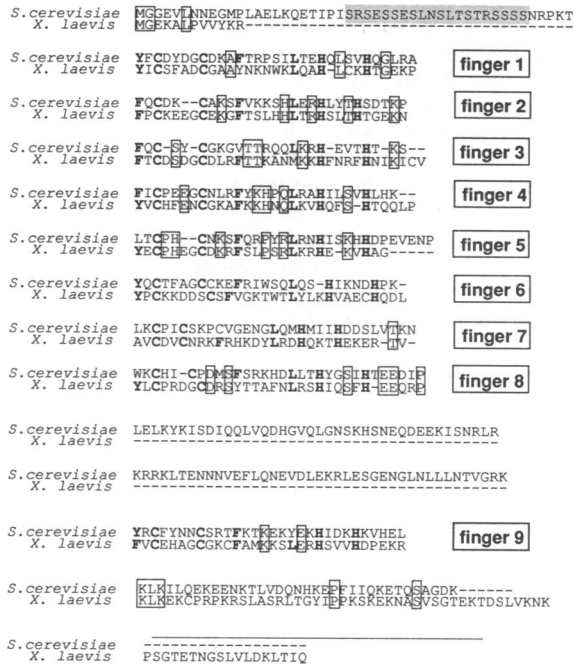
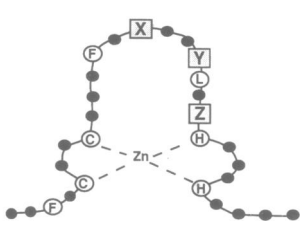


FIG. 3. Comparison of *S. cerevisiae* and *X. laevis* TFIIIA proteins. Amino acid sequences of the two proteins are aligned to show the nine zinc fingers. The invariant residues of C_2H_2 zinc fingers are in boldface. Other identical residues are boxed and shaded. A 20-amino acid serine-rich region near the TFIIIA N terminus is boxed.

A



B

finger	1	2	3	4	5	6	7	8	9
position	XYZ	XYZ	XYZ	XYZ	XYZ	XYZ	XYZ	XYZ	XYZ
Xenopus	KKA	SHR	TNK	KQV	LRR	KTK	HYD	TNS	MSR
Saccharomyces	RIE	KHR	TQR	KQA	RRN	IQS	GGM	RDT	TKK

FIG. 4. Comparison of *Xenopus* and *Saccharomyces* zinc finger residues that may be involved in DNA recognition. (*A*) Positioning of the X, Y, and Z residues within the C_2H_2 zinc finger modeled previously by Klevit (33). (*B*) Alignment of X, Y, and Z amino acids of the nine zinc fingers of *X. laevis* (*Xenopus*) and *S. cerevisiae* (*Saccharomyces*).

antibody probes. More importantly, they are likely to account for differences in 5S rRNA ICR interactions observed between *Xenopus* and *Saccharomyces* TFIIIA (32). For example, *S. cerevisiae* TFIIIA has a much smaller DNase I footprint (≈30 bp) with the 5S rRNA ICR compared to *Xenopus* (>50 bp) (32), and the *S. cerevisiae* TFIIIA has close contact with a different pattern of DNA residues (identified by methylation interference studies) compared to *Xenopus* TFIIIA (31).

DISCUSSION

The gene encoding *S. cerevisiae* transcription factor TFIIIA, *PZF1*, is located adjacent to the RNA polymerase subunit gene *RPB6* on chromosome XVI. The single copy *PZF1* gene is essential for yeast cell viability. The PZF1, or TFIIIA, protein contains the nine C_2H_2 zinc fingers characteristic of the amphibian TFIIIA proteins and binds specifically to DNA containing the yeast 5S rRNA ICR sequence.

Comparison of the yeast and amphibian TFIIIA proteins reveals that most of the sequence identity resides in amino acid residues within the zinc fingers (Fig. 3). Although the two proteins are only ≈20% identical overall, they are ≈30% identical within the zinc finger motifs. In addition, there are a substantial number of aligned residues that are conserved.

Recent studies of sequence specificity by two zinc finger domains, Zif268 and Krox-20 (18, 19), revealed that each zinc finger appears to bind 3 bp, with both Zif268 and Krox-20 zinc fingers making contacts exclusively with guanines of the DNA via arginine and histidine side chains. The main contacts were in the α-helix of the finger, which corresponds roughly to the region containing the histidine pairs in the C_2H_2 finger sequence (i.e., the $X_3LX_2HX_{3-4}HX_5$ portion of the entire $F/YXCX_{2-4}CX_3FX_5LX_2HX_{3-4}HX_5$ domain). By analysis of amino acid positions within the finger α-helix [designated X, Y, and Z in a recent review by Klevit (33)], Pavletich and Pabo (18) were able to correlate the presence of arginine or histidine residues at either position X, Y, or Z with specific binding to guanosines of the DNA (33).

Since the X, Y, and Z residues of the zinc finger appear to be involved in specific interactions with DNA, it may be instructive to compare these amino acid residues in each of the TFIIIA zinc fingers. This analysis indicates that the X, Y, and Z residues of fingers 1–5 are reasonably conserved in the *Saccharomyces* and *Xenopus* TFIIIA proteins, while those of fingers 6–9 differ substantially (Fig. 4). The differences in the two TFIIIA proteins probably reflect differences in the yeast and amphibian 5S rRNA ICR sequences but may also account for differences observed in the DNase I footprints produced by the yeast and amphibian factors (32).

Yeast TFIIIA differs from the amphibian proteins in two additional features—an 81-amino acid spacer region between the eighth and ninth fingers and a serine-rich stretch of amino acids near the N terminus of the protein. These domains may be required for interaction of TFIIIA with species-specific transcription factors or they may be necessary for distinct functions that vary among eukaryotic TFIIIA proteins.

The yeast RPB6 and TFIIIA coding sequences are divergently transcribed and are separated by only 233 bp. The proximity of the two genes strongly suggests that *RPB6* and *PZF1* share promoter regulatory elements. The divergently transcribed yeast genes *GAL1* and *GAL10* are coregulated; the coding sequences of these two genes are separated by ≈606 bp (34). Coordinated expression of RPB6 and TFIIIA might serve as a control point for synthesis of components required for both transcription (RNA polymerases) and translation (rRNA). It will be of interest to investigate the features of this small divergent promoter region that are involved in transcriptional regulation.

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