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The *Kluyveromyces* gene encoding the general transcription factor IIB: structural analysis and expression in *Saccharomyces cerevisiae*

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ABSTRACT

The *Kluyveromyces lactis* gene encoding the general transcription factor IIB (TFIIB) was isolated from a genomic library by complementation of the cold-sensitive phenotype conferred by a mutation in the *SUA7* gene, which encodes TFIIB in *Saccharomyces cerevisiae*. DNA sequence analysis of the *Kl-SUA7* gene revealed a 357 amino acid open reading frame that is 67% identical (81% overall similarity) to *S.cerevisiae* TFIIB. Comparison with other eukaryotic TFIIBs indicated that the most highly conserved sequence is located adjacent to the Zn-finger motif near the N-terminus. A plasmid shuffle system was used to replace the essential *Sc-SUA7* gene with *Kl-SUA7* in *S.cerevisiae*. The resulting strain was viable and phenotypically indistinguishable from the normal strain. However, transcription start site selection at the *ADH1* locus, shown previously to be affected by mutations in *Sc-SUA7*, was affected by *K.lactis* TFIIB. This result provides further evidence that TFIIB is a principal determinant of start site selection in yeast.

INTRODUCTION

TFIIB was initially identified from mammalian cell extracts as a general transcription factor essential for preinitiation complex formation *in vitro* (1,2). Comparable activities were subsequently identified and fractionated from fly (3) and yeast (factor e; ref. 4), establishing that TFIIB function is conserved among eukaryotic organisms. TFIIB associates with the transcription preinitiation complex subsequent to binding of TFIID to the TATA box, but immediately and necessarily preceding association of RNA polymerase II with the complex (5,6). TFIIB is a determinant of start site selection in yeast (7), and has also been implicated as the target of several transcriptional activator proteins *in vitro* (8–11). These data imply that TFIIB minimally contains contact sites for interaction with TFIID, RNA polymerase II, certain of the transcriptional activator proteins, and perhaps the initiator region of the DNA template.

Recently, DNA sequences encoding TFIIB from human (12,13), rat (14), frog (15), fly (16,17) and yeast (7) were isolated and sequenced. The metazoan TFIIB sequences are highly similar, with rat, frog and fly displaying, respectively, 99%, 94%, and 79% identity to the human sequence. The yeast sequence is less highly conserved, with 35% identity to human. Nonetheless, the sequence similarity between the yeast and human sequences spans the length of the two proteins, suggesting that TFIIB structural requirements encompass the entire protein. Inspection of the TFIIB sequences revealed several interesting structural features, including a Zn-finger motif near the N-terminus, two imperfect repeats and a potential amphipathic helix between the repeats (7,12). Functional analysis of human TFIIB demonstrated that the Zn-finger domain is important for recruitment of RNA polymerase II to the preinitiation complex and that the most conserved residues within the repeats are necessary for efficient interaction with TBP, the TATA-binding component of TFIID (18).

Comparative sequence analysis for homologous proteins from distantly related organisms can be an especially valuable method for identifying functionally important amino acid residues. Considering the extensive divergence between yeast and metazoan TFIIB sequences, we sought to determine the sequence of TFIIB from an organism more closely related to *S.cerevisiae*. Here we report the sequence of the gene (*Kl-SUA7*) encoding TFIIB from *K.lactis* and functional analysis of *Kl-SUA7* in *S.cerevisiae*.

MATERIALS AND METHODS

Strains, media and nomenclature

The *S.cerevisiae* strains used in this study are listed in Table 1. *K.lactis* strain Y-1140 was obtained from the American Type Culture Collection. Rich (YPD) and omission (−Ura and −His) media were prepared as described by Sherman (19). 5-fluoro-orotic acid (FOA) medium, used to select against *URA3*-containing plasmids, was prepared and used as described previously (20). The Csm$^-$ phenotype refers to distinctly impaired growth on YPD medium at 16° *C.S.cerevisiae* and *K.lactis SUA7* genes are distinguished by the prefixes *Sc-* and *Kl-*.

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Recombinant DNA and microbial techniques

Recombinant plasmids were constructed and screened according to standard techniques (21). Plasmids pDW11 (7) and pKL25 contain, respectively, the *Sc-SUA7* and *Kl-SUA7* genes in the *URA3 CEN* plasmid pRS316 (22). Plasmids pM299 and pKL15 are essentially identical to pDW11 and pKL25, respectively, except that the vector in both cases is the *HIS3 CEN* plasmid pRS313 (22). Plasmid DNA was isolated from *Escherichia coli* (23) and from yeast (24) as described previously. Transformations of *E.coli* and yeast were done by the calcium chloride (25) and lithium acetate (26) methods, respectively.

Gene replacement by plasmid shuffling

S.cerevisiae strains expressing either the *Sc-SUA7* or *Kl-SUA7* genes were constructed by a plasmid shuffle (27). The host strain was YIP91-13B (Table 1), which is disrupted at the *SUA7* chromosomal locus by the *LEU2* gene (the *sua7::LEU2* allele is described in ref. 7); viability of YIP91-13B is maintained by plasmid pDW11 (*Sc-SUA7 CEN URA3*). YIP91-13B was transformed with either pRS313 (*HIS3 CEN*) or its derivatives pM299 (*Sc-SUA7 CEN HIS3*) or pKL15 (*Kl-SUA7 CEN HIS3*), selecting in each case for His^+ transformants. The resulting strains were cured of pDW11 on FOA medium, which selects against the *URA3* marker. Accordingly, FOA^r colonies are indicative of functional TFIIB expression from the *HIS3* plasmid. This allows for phenotypes associated with expression of either *S.cerevisiae* or *K.lactis* TFIIB to be assessed in isogenic backgrounds.

DNA sequence analysis

The DNA sequence of the *Kl-SUA7* gene was determined by the dideoxy-terminator method (28), using the kit supplied by USB (Cleveland, OH). Both strands of a 1669 *Bam*HI/*Sau*3A−XhoI fragment were sequenced using the universal primer and single-stranded DNA derived from the indicated restriction fragments inserted into either M13mp18/19 (21) or pRS316 (22).

Determination of transcription start sites

Transcription start sites were determined by primer extension analysis. RNA was extracted from either *K.lactis* or *S.cerevisiae* strains grown in 50 ml YPD cultures to OD_{600} = 1.0, as described previously (7). Extension reactions were done using total RNA, AMV reverse transcriptase (Promega; Madison, WI), and [γ^{32}P]-ATP-labeled primers (7). *Kl-SUA7* start sites were determined using primer oJN-129, which corresponds to the template strand from position 47 → 30 (5′-CTAGATGAGAC-AGCTACA). *S.cerevisiae ADH1* transcription start sites were determined using primer oIP-87, which corresponds to the *ADH1* template strand from position 84 → 67 (CTTTGGAACTGG-AATATC). The cDNA products were analyzed in an 8% polyacrylamide DNA sequencing gel using sequenced DNA as size references.

RESULTS

Isolation of the *K.lactis SUA7* gene

The *K.lactis* homologue of the *S.cerevisiae SUA7* gene was cloned from a YEplac195 genomic library (W.Mulder and L.Grivell, unpublished) by complementation of the Csm^- phenotype conferred by the *sua7-1* allele of *S.cerevisiae* strain MH71-9C. From approximately 11,000 Ura^+ transformants, five Csm^+ colonies were isolated. When cured of plasmid DNA on FOA medium the Csm^- phenotype was restored for two of these strains. Plasmid DNA was recovered from both and reintroduced into MH71-9C. The resulting Ura^+ transformants were all Csm^+, confirming that complementation of the *sua7-1* mutation is conferred by plasmid DNA, rather than by strain reversion.

Restriction analysis revealed that the two plasmids contained overlapping DNA inserts, with the smaller of the two containing an insert of only 3 kb. Southern blot analysis showed that the insert was derived intact from the *K.lactis* genome and is present in single copy (data not shown). The entire 3 kb insert was transferred from the multicopy YEplac195 vector (29) to the low-copy-number pRS316 vector (22), creating plasmid pKL3. When introducted into MH71-9C, pKL3 complemented the Csm^- phenotype of *sua7-1*. Therefore the *K.lactis* DNA is able to complement the *sua7-1* allele when expressed from either a high-

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SCTFIIB     M----MTRESIDKRAGRRGPNLNIVLTCPECKVYPPKIVERFSEGDVVCA    46
KLTFIIB     MSLPAAASANVAVSSRRRGPNLNIVISCPECKVYPPKVVERFSEGDIVCA    50
HUTFIIB     MA----------STSRLDALPRV-TCPNHP--DAILVEDYRAGDMICP     35
RATFIIB     MA----------STSRLDALPRV-TCPNHP--DAILVEDYRAGDMICP     35
XLTFIIB     MA----------STSRIDALPKV-TCPNHP--DALLVEDYRAGDMICS     35
DMTFIIB     MA----------STSRLDN-NKV-CCYAHP--ESPLIEDYRAGDMICS     34
            *            . .*  .   *  * .     . ..* ...**..*.

SCTFIIB     LCGLVLSDKLVDTRSEWRTFSNDDHNGDDPSRVGEASNPLLDGNNLSTRI    96
KLTFIIB     LCGLVLSDRIVDTRSEWRTFSNDDQNGDDPSRVGEASNPLLDGNHLTTRI   100
HUTFIIB     ECGLVVGDRVIDVGSEWRTFSND-KATKDPSRVGDSQNPLLSDGDLSTMI    84
RATFIIB     ECGLVVGDRVIDVGSEWRTFSND-KATKDPSRVGDSQNPLLSDGDLSTMI    84
XLTFIIB     ECGLVVGDRVIDVGSEWRTFSND-KAAADPSRVGDAQNPLLSGGDLTTMI    84
DMTFIIB     ECGLVVGDRVIDVGSEWRTFSNE-KSGVDPSRVGGPENPLLSGGDLSTII    83
             ****..*...*. ********. ... ******.. ****....*.* *

SCTFIIB     GKGETTDMRFTKELNKAQGKNVMDKKDNEVQAAFAKITMLCDAAELPKIV   146
KLTFIIB     GQAQGSDARVTRDLNRAQSKSIVDKKDNELQAAYAKITMMCDAAELPKIV   150
HUTFIIB     GKGTGAASFDEFGNSKYQNRRTMSSSDRAMMNAFKEITTMADRINLPRNI   134
RATFIIB     GKGTGAASFDEFGNSKYQNRRTMSSSDRAMMNAFKEITTMADRINLPRNI   134
XLTFIIB     GKGTGSASFDEFGNSKYQNRRTMSSSDRAMMNAFKEITNMSDRINLPRNI   134
DMTFIIB     GPGTGSASFDAFGAPKYQNRRTMSSSDRSLISAFKEISSMADRINLPKTI   133
            * . ...   . .  *........*... .*. .*. . *  .**. .

SCTFIIB     KDCAKEAYKLCHDEKTLKGKSMESIMAASILIGCRRAEVARTFKEIQSLI   196
KLTFIIB     KDCAKEAYKLCFEERVLKGKSQESIMASVILVGCRRAEVGRSFKEILSLT   200
HUTFIIB     VDRTNNLFKQVYEQKSLKGRANDAIASACLYIACRQEGVPRTFKEICAVS   184
RATFIIB     VDRTNNLFKQVYEQKSLKGRANDAIASACLYIACRQEGVPRTFKEICAVS   184
XLTFIIB     IDRTNNLFKQVYEQKSLKGRSNDAIASACLYIACRQEGVPRTFKEICAVS   184
DMTFIIB     VDRANNLFKQVHDGKNLKGRSNDAKASACLYIACRQEGVPRTFKEICAVS   183
             * ... .*   . . ***.. ..  .. . ..**...* *.**** ..

SCTFIIB     HVKTKEFGKTLNIMKNILRGKSEDGFLKIDTDNMSGAQNL--TYIPRFCS   244
KLTFIIB     NVRKKEIGKTFTIIKNILREKNDRGFANIDTSNITTGQTSAETFIPRFCS   250
HUTFIIB     RISKKEIGRCFKLILKAL---------ETSVDLITTGD-----FMSRFCS   220
RATFIIB     RISKKEIGRCFKLILKAL---------ETSVDLITTGD-----FMSRFCS   220
XLTFIIB     RISKKEIGRCFKLILKAL---------ETNVDLITTGD-----FMSRFCS   220
DMTFIIB     KISKKEIGRCFKLTLKAL---------ETSVDLITTAD-----FMCRFCA   219
            ....**.*. ...  . *          ..... .....      .. ***.

SCTFIIB     HLGLPMQVTTSAEYTAKKCKEIKEIAGKSPITIAVVSIYLNILLFQIPIT   294
KLTFIIB     HLGLSVQVANAAEYIAKHSKDVNVLAGRSPITIAAAAIYMATLLFKLNIS   300
HUTFIIB     NLCLPKQVQMAATHIARKAVELDLVPGRSPISVAAAAIYMASQASAEKRT   270
RATFIIB     NLCLPKQVQMAATHIARKAVELDLVPGRSPISVAAAAIYMASQASAEKRT   270
XLTFIIB     NLGLTKQVQMAATHIARKAVELDLVPGRSPISVAAAAIYMASQASAEKRT   270
DMTFIIB     NLDLPNMVQRAATHIAKKAVEMDIVPGRSPISVAAAAIYMASQASEHKRS   269
            .* *.  *  .*...*..  ... ..*.***..*...**..          .

SCTFIIB     AAKVGQTLQVTEGTIKSGYKILYEHRDKLVDPQLIANGVVSLDNLPGVEK   344
KLTFIIB     PTRISQTLQVTEGTVKGGYKALYEHKEKVVDPQLIESGVVKWDNLPRVSN   350
HUTFIIB     QKEIGDIAGVADVTIRQSYRLIYPRAPDLFPTDFKFDTPV--DKLPQL--   316
RATFIIB     QKEIGDIAGVADVTIRQSYRLIYPRAPDLFPSDFKFDTPV--DKLPQL--   316
XLTFIIB     QKEIGDIAGVADVTIRQSYRLIYPRAPDLFPADFKFDTPV--DKLPQL--   316
DMTFIIB     QKEIGDIAGVADVTIRQSYKLMYPHAAKLFPEDFKFTTPI--DQLPQM--   315
            .  ....  *.. *.. .*. .* .  ..   .. .. .  *.** .

SCTFIIB     ------K   345
KLTFIIB     DKSEKAN   357
HUTFIIB     -------   316
RATFIIB     -------   316
XLTFIIB     -------   316
DMTFIIB     -------   315
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Figure 1. Alignment of TFIIB sequences. The deduced amino acid sequences for TFIIB from *S.cerevisiae* (SCTFIIB), *K.lactis* (KLTFIIB), human (HUTFIIB), rat (RATFIIB), frog (XLTFIIB), and fly (DMTFIIB) were aligned using the CLUSTAL program of PC/GENE. Identical residues among all six sequences are indicated by asterisks and similar residues (grouped as A,S,T; D,E; N,Q; R,K; I,L,M,V; and F,Y,W) are indicated by dots. SCTFIIB and KLTFIIB are 67% identical and 81% similar.

Table 1. *S.cerevisiae* strains

Strain	Genotype	Source
YDW546	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua7-1*	Ref. 7
YIP363	*MATα cyc1-5000 cyc7-67 leu2-3,112 ura3-52 cyh2 sua7-3*	Ref. 7
MH71-9C	*MATa cyc1-5000 cyc7-67 trp5-48 his5-2 ura3-52 sua7-1*	this study
YIP91-13B	*MATa CYC1 his3-Δ1 leu2-3,112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pDW11:*SUA7 URA3*]	I. Pinto
YMH114	MATa *CYC1 his3-Δ1 leu2-3,112 trp1-289 ura3-52 sua7::LEU2 ade1-100* [pM299: *Sc-SUA7 HIS3*]	this study
YMH115	MATa *CYC1 his3-Δ1 leu2-3,112 trp1-289 ura3-52* sua7::LEU2 ade1-100 [pKL15: *Kl-SUA7 HIS3*]	this study

or low-copy-number plasmid. The complementing activity was subsequently delimited to a 1.5 kb *SpeI*−*XhoI* DNA fragment. We have designated the gene encoding this activity *Kl-SUA7*.

Molecular analysis of *Kl-SUA7*

The DNA sequences of both strands encompassing the *Kl-SUA7* *SpeI*−*XhoI* fragment were determined. A single long open reading frame (ORF) of 357 amino acids, encoding a potential protein with a calculated molecular weight of 39.1 kD, was identified. Comparison of this sequence with the 345 amino acid sequence of the *Sc-SUA7* gene revealed 67% identity with 81% overall similarity (Fig. 1). This similarity extended over the entire lengths of the two proteins. With the exception of two additional amino acids at positions 241−242, the larger size of the *K.lactis* protein is solely a consequence of additional residues at the N- and C-termini.

Transcription start sites at the *Kl-SUA7* locus were determined by primer extension (Fig. 2). Two major initiation sites were identified at positions −102 and −100, which are approximately 25 base-pairs downstream of a potential TATA box. The first ATG from the potential TATA box is the sequence that initiates the 357 amino acid ORF.

Functional analysis of *Kl-SUA7* in *S.cerevisiae*

The ability of the *Kl-SUA7* gene to complement the Csm$^-$ phenotype conferred by an *Sc-sua7* mutation is shown in Figure 3A. The *sua7-3* allele of strain YIP363, like the *sua7-1* allele, confers an extreme Csm$^-$ phenotype on rich medium at 16°C. This phenotype is fully complemented by the *Sc-SUA7* gene borne on a low-copy-number plasmid. Similarly, the *Kl-SUA7* gene, expressed from the same vector, allows growth at 16°C, albeit somewhat more slowly than with *Sc-SUA7*. Thus, *Kl-SUA7* complements the conditional phenotype conferred by both the *sua7-1* and *sua7-3* alleles, which encode different, single amino acid substitutions in TFIIB (I. Pinto and M.H., unpublished results).

The *Sc-SUA7* gene is essential for cell viability (7). We therefore asked whether the *Kl-SUA7* gene would maintain viability of an *S.cerevisiae sua7* null mutant. This was done by plasmid shuffling (see Methods and Materials), using host strain YIP91-13B (Table 1). YIP91-13B was transformed with a low-copy-number *HIS3* vector carrying either no insert (pRS313), the *Sc-SUA7* gene (pM299), or the *Kl-SUA7* gene (pKL15). Resulting strains were transferred to FOA medium, counter-selecting the endogenous plasmid pDW11 (*Sc-SUA7 CEN URA3*), such that only strains encoding functional TFIIB from the *HIS3* vector would grow. As shown in Fig. 3B, YIP91-13B carrying either no plasmid or the *HIS3* vector only failed to grow on FOA medium. In contrast, both the *Sc-SUA7* and *Kl-SUA7* genes sustained growth in the absence of the chromosomal *SUA7* gene. Furthermore, both FOA^r strains grew equally well on rich medium at 30°C (Fig. 3C). Thus, *Kl-SUA7* is able to replace all essential functions encoded by *Sc-SUA7*.

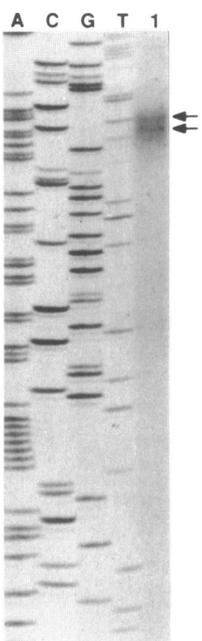

Figure 2. *Kl-SUA7* transcription start sites. Primer extension analysis was performed as described in the Materials and Methods using primer oJN-129 (lane 1). The same primer was used to generate the sequencing ladder from *Kl-SUA7* template DNA (lanes A, C, G and T). The arrows indicate the two major transcription start sites located at positions −102 and −100 relative to the A of the ATG translation start codon.

Effect of *Kl-SUA7* on transcription initiation in *S.cerevisiae*

The *Sc-SUA7* gene is required for normal transcription start site selection *in vivo* (7). The absence of any detectable phenotype associated with substitution of *Sc-SUA7* by *Kl-SUA7* suggested that *K.lactis* TFIIB maintains this function in *S.cerevisiae*. We asked whether this is indeed the case by mapping transcription start sites at the *ADH1* locus using the YIP91-13B strains that express TFIIB from either the plasmid-borne *Sc-SUA7* or *Kl-SUA7* gene (strains YMH114 and YMH115). Transcription at *ADH1* normally initiates at discrete sites located 37 and 27 nucleotides upstream from the ATG start codon (30). Three *sua7* alleles were shown previously to affect initiation at *ADH1* by

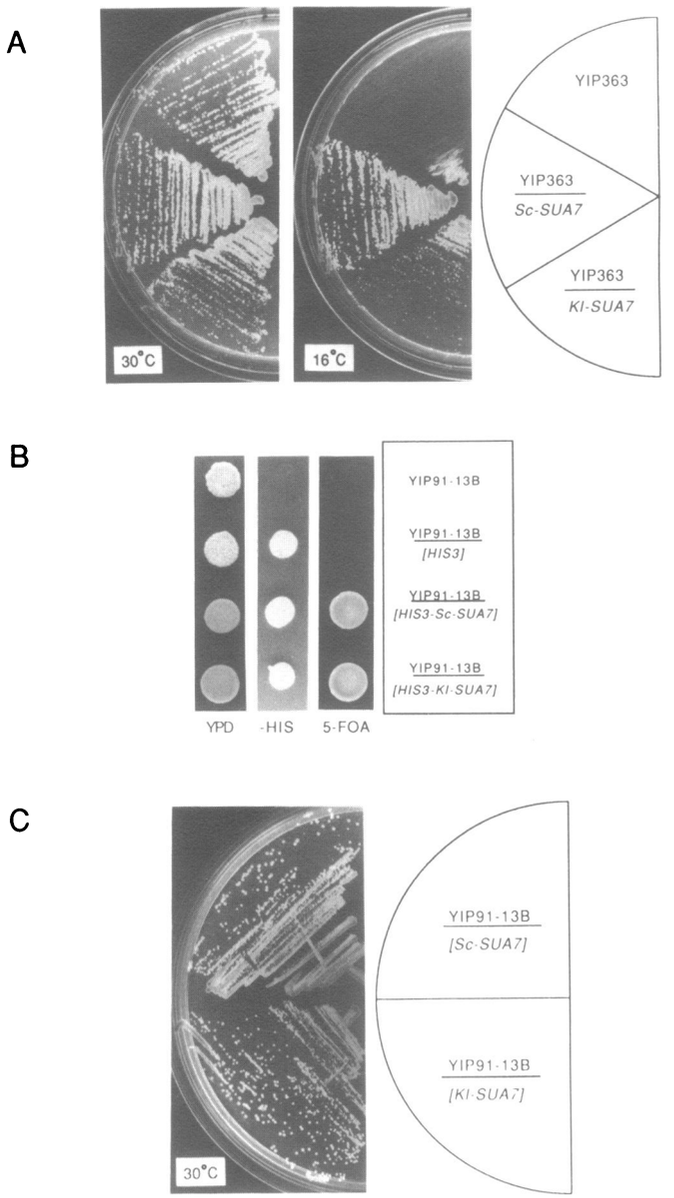

Figure 3. Phenotypes associated with expression of *Kl-SUA7* in *S.cerevisiae*. (**A**) Complementation of the Csm$^-$ phenotype conferred by *sua7-3*. Strain YIP363 (*sua7-3*) exhibits a Csm$^-$ phenotype, defined by extremely impaired growth on rich (YPD) medium at 16°C. The Csm$^-$ phenotype is complemented by expression of either *Sc-SUA7* or *Kl-SUA7* from the low-copy-number vector pRS316 (plasmids pDW11 and pKL25, respectively). YIP363/*Kl-SUA7* grows somewhat more slowly than YIP363/*Sc-SUA7*. (**B**) Replacement of *Sc-SUA7* by *Kl-SUA7*. Strain YIP91-13B, which carries plasmid pDW11 (Table 1), was transformed with the *HIS3* plasmid pRS313 (22), or its derivatives carrying either the *Sc-SUA7* or *Kl-SUA7* gene (plasmids pM299 or pKL15). As expected, all four strains grew on YPD medium at 30°C (left panel) and only the transformed strains grew on −His medium (middle panel). When spotted onto FOA medium, which counter-selects pDW11 (*Sc-SUA7 CEN URA3*), neither the untransformed strain nor the strain carrying the *HIS3* vector grew. In contrast, viability was maintained by the *HIS3* vector carrying either *Sc-SUA7* or *Kl-SUA7*. These two strains have been designated YMH114 (*Sc-SUA7*) and YMH115 (*Kl-SUA7*). (**C**) Relative growth of isogenic strains YMH114 and YMH115 at 30°C. No difference in growth rates is seen, confirming that *Kl-SUA7* replaces all essential TFIIB functions in *S.cerevisiae*.

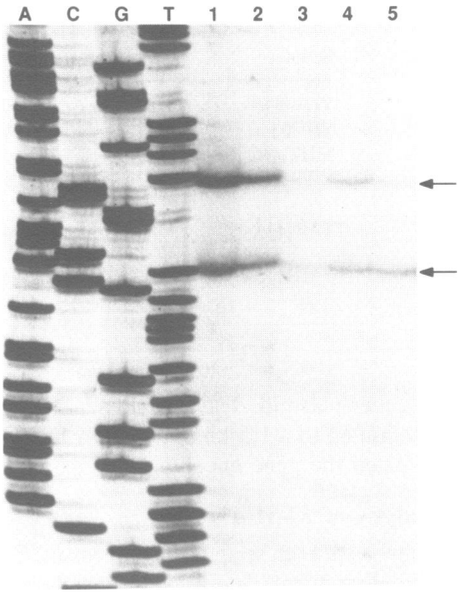

Figure 4. *ADH1* transcription start sites in *S.cerevisiae* strains expressing TFIIB from either *Sc-SUA7* or *Kl-SUA7*. Primer extension reactions were performed as described in the Materials and Methods using the *ADH1*-specific primer, oIP-87. Lane 1, strain T15 (*SUA7*$^+$); lane 2, strain T16 (*SUA7*$^+$); lane 3, strain YIP363 (*sua7-3*); lane 4, strain YMH114 (*Sc-SUA7*); lane 5, strain YMH115 (*Kl-SUA7*). Strains T15 and T16 served as controls and are described in ref. 7. The major *ADH1* initiation sites defined previously (30) are indicated by the arrows. Since strains YMH114 and YMH115 are isogenic, the diminished initiation at the upstream *ADH1* start site (position −37) for strain YMH115 can be attributed solely to an effect of *K.lactis* TFIIB. The sequence ladder (lanes A, C, G, T) is *CYC1* DNA, which served as size markers.

markedly diminishing initiation at position −37 in favor of enhanced initiation at downstream sites, with no apparent effect at position −27 (7; I.Pinto and M.H., unpublished results). As shown by primer extension analysis (Fig. 4), expression of plasmid-borne *Sc-SUA7* maintained the normal pattern of initiation at *ADH1* (lane 4). However, when TFIIB was encoded by *Kl-SUA7* the relative use of the two start sites was altered. Whereas positions −37 and −27 are used at about equal frequency in strain YMH114 (lane 4), initiation at position −37 is significantly diminished with little effect at position −27 in strain YMH115 (lane 5). A similar effect was seen when TFIIB was encoded by the *S.cerevisiae sua7-3* allele (lane 3), a result which is comparable to the effect of the *sua7-1* suppressor on *ADH1* initiation (7). This result can not be attributed to strain differences since YMH114 and YMH115 are otherwise isogenic. These data confirm that TFIIB functions in defining transcription start sites in yeast.

DISCUSSION

Comparative sequence information for homologous proteins and nucleic acids can reveal structural domains that are important for function and can serve as a metric for estimating evolutionary relatedness between organisms. Based on small ribosomal RNA sequences, *S.cerevisiae* and *K.lactis* are estimated to have diverged approximately 10^8 years ago (31,32). Accordingly, these two budding yeasts are related, albeit distantly. A number

of homologous gene-specific transcription factors from *S.cerevisiae* and *K.lactis* have been sequenced and compared. These include, heat shock factor (33), Reb1 (34), Abf1 (35) Gal4/Lac9 (36,37), and Gal11 (38). With the exception of Gal11, these homologous pairs are typically <40% identical with frequent gaps in the alignments; Gal11 homologues are 44% identical with 81% overall similarity. In contrast, ribosomal proteins from these two organisms are 85%–95% identical and are completely collinear (39). The TFIIB homologues are 67% identical with only a two amino acid gap in the alignment (Fig. 1). Thus, the structure required for functional TFIIB is more highly constrained than are the structures of the gene-specific transcription factors, presumably reflecting the requirements for interaction with other components of the multi-subunit preinitiation complex.

Despite the extensive structural similarities between the yeast TFIIBs these sequences have diverged significantly from the metazoan TFIIBs. Nonetheless, the sequence similarities encompass the entire lengths of the proteins, with only a few short gaps required to maintain the alignments (Fig. 1). Also, the TFIIB structural characteristics, including the Zn-finger motif, imperfect amino acid repeats and potential amphipathic helix are conserved. Comparison of all known TFIIB sequences revealed that the most highly conserved region lies immediately downstream of the Zn-finger motif, where 27 of 49 residues are identical and an additional 17 residues are similar (90% overall similarity). Clearly this region of the protein is critical for function. Consistent with this conclusion, the original *sua7* mutations that confer cold-sensitivity and altered start site selection lie within this region (I.Pinto and M.H., unpublished results).

K.lactis TFIIB mimics the effect of the *Sc-sua7* suppressors on start site selection at *ADH1* in *S.cerevisiae* (Fig. 4). However, we do not want to imply that *K.lactis* TFIIB is functionally comparable to the *sua7* suppressors with respect to start site selection. First, *K.lactis* TFIIB has no effect on start site selection at *CYC1*; the normal, heterogeneous transcription initiation pattern at *CYC1* was identical in strains YMH114 and YMH115 (R.W.Berroteran and M.H., unpublished results). This is in marked contrast to the *sua7* suppressors, which dramatically shift initiation at *CYC1* downstream of normal (7). Second, there was no appreciable effect on growth rate when *K.lactis* TFIIB replaced *S.cerevisiae* TFIIB (Fig. 3C), whereas the *sua7* suppressors confer both impaired growth at normal temperature and severe cold-sensitivity (7). Although *Kl-SUA7* did not fully complement the cold-sensitivity of the *sua7-3* mutant (Fig. 3A), strain YMH115 (*Kl-SUA7*) exhibited neither heat- nor cold-sensitivity (not shown). Thus, although *K.lactis* TFIIB has a significant and reproducible effect on start site selection at *ADH1*, thereby supporting a role for TFIIB in start site selection, we do not believe that this effect is occurring at most genes or that it reflects a significant functional difference between *K.lactis* and *S.cerevisiae* TFIIB.

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