4932–4936 Nucleic Acids Research, 1994, Vol. 22, No. 22

Role of a small RNA pol II subunit in TATA to transcription start site spacing

Elizabeth M.Furter-Graves, Benjamin D.Hall[1] and Rolf Furter*
Institute for Cell Biology, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland and [1]Department of Genetics, SK-50, University of Washington, Seattle, WA 98195, USA

Received September 6, 1994; Revised and Accepted October 17, 1994

ABSTRACT

The yeast *shi* mutation affects the spacing between the TATA promoter element and transcription initiation sites; for the *H2B* and *ADH1* genes, a series of start sites located ~50–80 bp downstream of TATA is used in addition to the wild-type initiation sites located at around 100 bp from TATA (1). Here, the yeast *SHI* wild-type gene has been isolated by complementation and shown to be identical to *RPB9*, the gene encoding a small subunit of RNA polymerase II. A point mutation in the *shi* gene, changing a cysteine residue in a putative zinc ribbon motif into a phenylalanine residue, was demonstrated to permit the observed usage of upstream initiation sites. Deletion of the non-essential *SHI* gene also results in usage of upstream initiation sites and causes conditional growth defects.

INTRODUCTION

The major positioning signal for initiation of pre-mRNA transcription by eukaryotic RNA polymerase II (pol II) is the TATA sequence. In addition to the well-documented binding of TBP to this element, pol II start site selection involves other proteins, including the initiation factor TFIIB and one or more integral pol II subunits (reviewed in 2). Recently, Li *et al.* (3) analyzed biochemically the interplay between these components in specifying the transcription start. They took advantage of the inherent difference in TATA to start distance between *Saccharomyces cerevisiae* (40–120 bp) and *Schizosaccharomyces pombe* (~30 bp). The species-specific determinants of transcription starting proved to be the transcription factor TFIIB and RNA polymerase II itself. When TFIIB and RNA polymerase II from *S.cerevisiae* were replaced with their *S.pombe* homologs in a system in which the remaining components were derived from *S.cerevisiae*, *S. pombe*-specific starts were seen. The role of TFIIB was not unexpected, since it was shown earlier that a mutation in the *SUA7* gene, which was identified as a yeast homolog of human TFIIB, affects the location of start sites (4). Since RNA polymerase II consists of at least 11 subunits in yeast (5), these *in vitro* studies raise interesting questions regarding the identity and mode of function of pol II apoenzyme subunits that participate in transcription initiation and start site specification. In view of their strong evolutionary conservation in prokaryotic and eukaryotic RNA polymerases, the two largest pol II subunits are likely participants in these steps. Indeed, mutations in *RPB1*, the yeast gene encoding the largest pol II subunit, have recently been reported to shift transcription initiation sites away from the TATA element (6). We describe here mutant effects upon the TATA to start distance that act in the opposite manner, by decreasing the TATA to start distance. Surprisingly, the protein encoded by the gene which is mutated is a small and non-essential subunit of RNA polymerase II. The yeast gene we have previously named *SHI* is shown to be identical to *RPB9*. We demonstrate that a single base pair mutation in a cysteine motif as well as deletion of the gene lead to the observed shift in RNA initiation sites.

MATERIALS AND METHODS

Yeast strains, media and transformation

The originally isolated *shi* strain (EG1), used for cloning the wild-type *SHI* gene and for primer extension analysis, has genotype *MATa shi ade2 ade4 trp1 adh1Δ adh2Δ adh3 SUP4::URA3-adhΔ50*. Except for the *shi* mutation, this strain is otherwise identical to the wild-type *SHI* parent strain (EG2) used for primer extension analysis. [Identical to strains designated *shi* and Δ50, respectively, as described in (1).] For determining the minimal complementing piece of the *SHI* clone, a *MATα shi cyh2 leu2 trp1 adh1 adh2 adh3 SUP4::URA3-adhΔ50* strain (EG3), derived by a cross of the original *shi* strain EG1, was used. A diploid strain (EG5) derived from a cross of *SHI* strains EG2 and EG4 (*MATα SHI cyh2 leu2 trp1 ura3*) was used for disruption of the *SHI* allele. Strain EG6, containing a deletion of the *SHI* locus, is a Ura$^+$ haploid segregant of strain EG5, with genotype *MATa ade2 trp1 leu2 cyh2 adh1Δ adh2Δ adh3 SHI::URA3*. Yeast media, matings, sporulation, transformation and genetic techniques were as described in (1). Medium lacking inositol was prepared as described in (7).

Plasmids

Plasmid pCEN-SHI/1.5 was constructed by inserting the 1.5 kb *Pst*I–*Hin*cII fragment encoding the putative *SHI* gene (see Fig.

*To whom correspondence should be addressed at: Department of Biochemistry and Molecular Biology, Lederle Graduate Research Center, University of Massachusetts, Amherst, MA 01003, USA

2) into the corresponding restriction sites of single copy vector pRS314 (8). pCEN-SHI/4.1 is a corresponding construct containing the 4.1 kb *Sal*I–*Bam*HI fragment of the wild-type *SHI* gene. The *Sna*BI–*Bgl*II fragment of the wild-type *SHI* allele contained in pCEN-SHI/4.1 was replaced by the corresponding fragment of the *shi* mutant allele, containing the mutation of cysteine 7 to phenylalanine, thereby generating plasmid pCEN-shi/4.1. The two plasmids differ only by a single base pair change, as verified by sequencing both strands in the region *Sna*BI–*Bgl*II for both plasmids. For testing the ability of the putative *SHI* gene to target homologous recombination to the correct locus, the 3.2 kb *Pst*I–*Bam*HI fragment was fused next to the *TRP1* selective marker in integrating plasmid pRS304 (8) to create plasmid pINT-SHI/3.2.

Construction of the *shi* deletion mutant strain

The *URA3* marker gene was fused between the two *Bgl*II sites within the *SHI* gene, thereby removing the coding sequence for the N-terminal 69 amino acids along with 290 bp of 5′-upstream sequences (Fig. 2B and C). A linear fragment containing the inserted *URA3* marker flanked by remaining *SHI* sequences was used to transform *ura3/ura3* diploid strain EG5. Eight Ura$^+$ diploids were sporulated and the resulting tetrads dissected. For each of the dissected diploids, 4 spored tetrads could be obtained; the *URA3* marker segregated 2:2. Southern blot analysis on Ura$^+$ haploid segregants from 4 of the dissected diploids showed that the *SHI* locus in these segregants was disrupted and that the deleted *Bgl*II fragment was indeed absent (not shown).

RNA isolation, primer extension, and PCR reactions

Preparation of total RNA and primer extensions were performed as described previously (9). Two oligonucleotides were used for primer extension experiments. One is complementary to the *S.cerevisiae PGK* gene between nucleotides +27 and +48 (5′-GTCCTTCAAGTCCAAATCTTGG-3′) and the other is complementary to the *S.cerevisiae* histone *H2B-1* gene in the region between +29 and +46 (5′-CAGCTGGGGCTTTGG-AGG-3′). Primer extensions with the *PGK*-specific oligonucleotide were done with 400 units M-MLV reverse transcriptase (BRL) at 37°C for 1 h. Extensions with the *H2B*-specific oligonucleotide were done with 15 units AMV reverse transcriptase (Promega) at 42°C. For the PCR amplification of the *shi* allele, an oligonucleotide 5′-GGGAAACA-CATTATTGG-3′ homologous to the region 180 bp downstream of the *Dra*I site, and a second oligonucleotide 5′-CCAGATA-GAAGAGGATCCCTTCGCTAGTATACG-3′, spanning the *Bgl*II and *Sna*BI sites, were used.

RESULTS

The isolated *SHI* gene is identical to *RPB9*

The *shi* mutation was recovered in a genetic selection designed to identify proteins involved in initiation site selection in *S.cerevisiae*. The selection relied on the use of a defective reporter gene for which the location of the initiation sites must be shifted upstream in order to produce a functional mRNA. For this purpose, we used an alcohol dehydrogenase allele (*adhΔ50*) with an artificially short distance between the TATA sequence and ATG. In wild-type *S.cerevisiae*, this gene yielded only non-functional message that initiated from sites downstream of the first ATG codon (Fig. 1; see also 1). *Trans*-acting mutations were identified that activated the expression of an *adhΔ50* allele integrated in a *S.cerevisiae* strain devoid of its endogenous *ADH* genes. The resulting mutant strains could then be tested for their effects on transcription initiation. In strains containing the *shi* gene, named for shift of initiation, RNA start sites for *adhΔ50* are shifted upstream, closer to TATA (Fig. 1). In addition to its effects on initiation sites chosen for the *adhΔ50* allele, the *shi* gene permits initiation sites closer to TATA to be used for several other genes (1).

The wild-type *SHI* gene could be cloned by complementation, due to the recessivity of the *shi* allele. Transformation of a *shi adhΔ50* strain with a plasmid containing the dominant wild-type *SHI* allele would be expected to change the cellular phenotype from Adh$^+$ to Adh$^-$. A plasmid with an 8.5 kb DNA insert (pCEN-SHI/8.5) that behaved in this manner was identified by transforming a *trp1 shi adhΔ50* mutant strain (Adh$^+$ phenotype, strain EG1) with a library of yeast sequences contained on a *CEN TRP1* vector (10), and screening for an Adh$^-$ transformant. After a passage through *Escherichia coli*, pCEN-SHI/8.5 was retransformed into strain EG1, resulting in Adh$^-$ transformants only. The 8.5 kb insert included a 1.5 kb *Pst*I–*Hin*cII fragment (Fig. 2) which conferred the dominant Adh$^-$ phenotype when expressed from the single copy plasmid pCEN-SHI/1.5 in a *leu2 trp1 shi adhΔ50* (Adh$^+$) strain (strain EG3).

To verify that the isolated DNA fragment was indeed derived from the *SHI* locus, we showed that the putative *SHI* gene could target homologous integration at or very near to the *SHI* locus. Integrating plasmid pINT-SHI/3.2 containing the putative *SHI* gene fused next to a *TRP1* marker (see Materials and Methods)

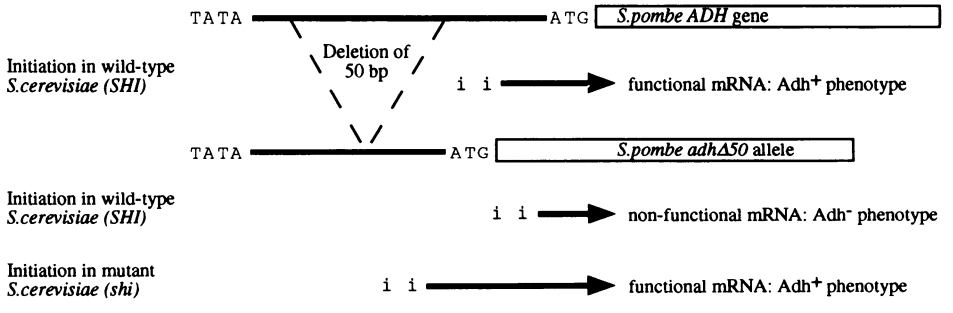

Figure 1. Schematic representation of the selection scheme for the isolation of yeast mutants with new positioning of RNA initiation sites. TATA marks the promoter element, ATG the translation initiation signal. i denotes the position of mRNA initiation sites; multiple start sites were used in each case depicted; for details see (1).

was linearized at the unique *Hin*cII restriction site within the *Pst*I–*Bam*HI piece. After integrative transformation of *shi trp1 adhΔ50* strain EG1, Adh$^-$ Trp$^+$ prototrophs were obtained. Such a transformed strain was mated to *shi trp1 cyh2 adhΔ50* strain EG3, and the segregation of the integrated *TRP1* marker was monitored by tetrad dissection with respect to the *cyh2* (scored by resistance to cycloheximide) and *shi* loci (scored by growth on antimycin-containing plates). No recombination was observed between the integrated *TRP1* marker and the *SHI* locus in a total of 30 dissected, viable tetrads, indicating that the cloned DNA could target homologous recombination to the *SHI* locus. Furthermore, the *TRP1* marker mapped 25 cM away from the *cyh2* locus, in agreement with the map distance between *shi* and *cyh2* determined previously (1).

DNA sequencing of the *Pst*I–*Hin*cII fragment revealed a single open reading frame that encodes a predicted protein of 122 amino acids (Fig. 2B and D). Comparison with known sequences showed that the *SHI* gene is identical to *RPB9*, the yeast gene encoding the 12.6 kDa subunit of RNA pol II. The *RPB9* gene had previously been cloned using oligonucleotides designed from tryptic fragments of the purified subunit (11).

Deletion of the *SHI* gene produces the same effects as the *shi* mutation

To produce a yeast strain lacking the chromosomal *SHI* allele, a defective *SHI* allele lacking codons for the first 69 amino acids and marked with a *URA3* gene (Fig. 2C) was integrated by homologous recombination in one homolog of diploid strain EG5. After sporulation and tetrad dissection, the resulting haploid strain harboring the *shi* deletion allele was able to grow at 30°C, indicating that the *SHI/RPB9* gene is not essential at this temperature, as has also been reported earlier (11). However, several growth related phenotypes were observed in the *shi* deletion strain. Germination of tetrads from strains heterozygous for the disrupted *shi* locus was reduced. In 48% of the tetrads from strains in which the *SHI* gene was disrupted on one homolog, only 1–3 of the spores were able to germinate; in the 52% of tetrads showing germination of all 4 spores, microcolonies were frequently observed from one or two of the spores. Furthermore, haploid strains with the disrupted *shi* allele grew more slowly on complete medium at 30°C, were severely defective for growth at 37°C and 18°C, and were auxotrophic for inositol. Interestingly, the analogous deletion in *Drosophila* of the fly homolog of *RPB9*, which is 46% identical to its yeast counterpart at the amino acid level, caused lethality (12). This suggests that the *Drosophila* homolog has either acquired some additional, essential functions within the polymerase holoenzyme that the yeast subunit lacks, or, more likely, that the effect of abolishing the function of this polymerase subunit is more deleterious to *Drosophila* than to yeast, due to the stricter spacing regimen between TATA and start sites in higher eukaryotes.

The absence of the *SHI* protein led to a similar effect on RNA initiation sites as was seen with the *shi* allele. Start sites for the endogenous yeast *PGK* and *H2B* genes were mapped using the

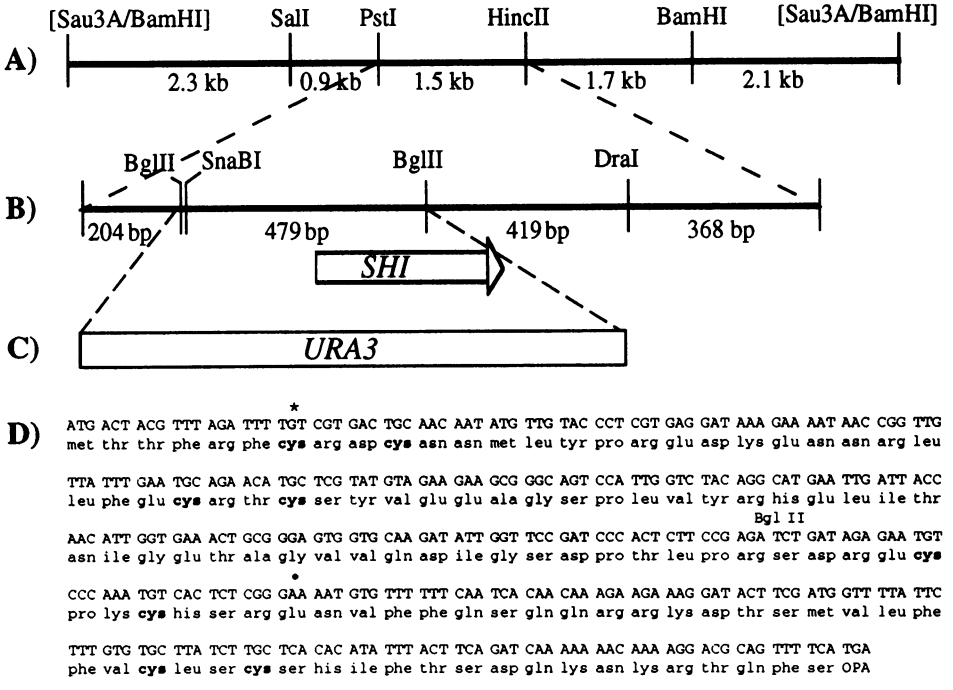

Figure 2. Restriction maps and sequence information for the *shi* complementing DNA. (**A**) Originally isolated DNA piece which complemented the *shi* mutation when expressed from single copy vector M111. (**B**) Detailed map of the sequenced, complementing *Pst*I–*Hin*cII piece. The open reading frame of the *SHI* gene is indicated by an open arrow. (**C**) The *URA3* gene was used to replace part of the *SHI* gene and its promoter to construct a *SHI:URA3* deletion mutant by homologous recombination. (**D**) The nucleotide sequence and corresponding amino acid sequence of the 122 amino acid open reading frame of the SHI/RPB9 protein. The *SHI* and *RPB9* DNA sequences were identical throughout the coding region, with the exception of the third position of codon glutamine 82 (marked with •; GAG in *RPB9*), not resulting in an amino acid change. Upstream of the ATG (not shown), 5 scattered nucleotide differences were found, reflecting differences in the yeast strains from which the respective genes were isolated. Cysteine residues are shown in bold. The site of the TGT→TTT, Cys→Phe change in the *shi* allele is shown with an asterisk.

primer extension technique for RNA isolated from the *shi* and *shi* deletion strains. In both strains, prominent upper bands were seen for the *PGK* gene (Fig. 3A, position −81) as well as for the *H2B* gene (Fig. 3B, positions −90 and −76), corresponding to start sites that were only weakly used in wild-type strains. These initiation sites, which are located between 48 and 69 bp downstream of TATA, are used in addition to the wild-type sites at distances of 102 (*H2B*) and 112 (*PGK*) bp downstream. By inference, transcription starts on the *adhΔ50* allele are also shifted upstream by the deletion of *SHI*; like the *shi* strain, the *shi* deletion strain was Adh$^+$ in the presence of the *adhΔ50* allele.

The mutation in the *shi* allele is a Cys to Phe change in a Cys_4 zinc ribbon

To find the molecular basis for the observed shift of RNA initiation caused by the *shi* allele, we used the polymerase chain reaction to amplify the coding region of the *shi* gene from several independent DNA samples prepared from the *shi* strain. The entire amplified *Sna*BI−*Dra*I fragment (Fig. 2B) was sequenced for one PCR product and was found to be identical to the wild-type *SHI* gene with the exception of a single base pair change.

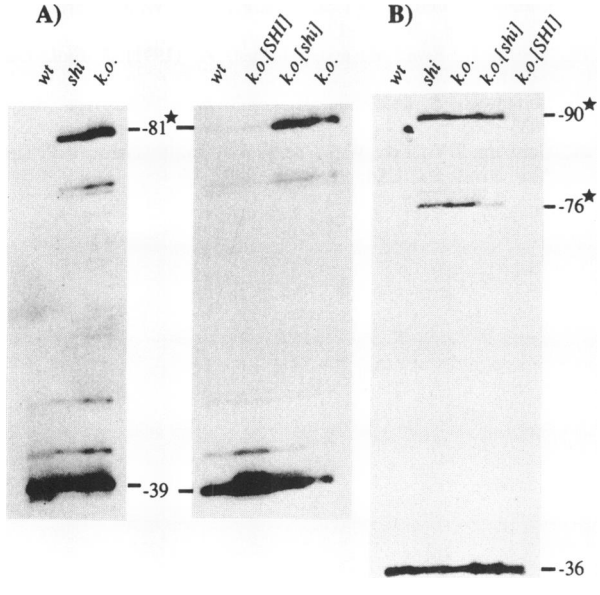

Figure 3. Total RNA was used for primer extension with radioactively end-labeled oligonucleotides. The asterisks mark the new prominent start sites caused by mutations in the *SHI* gene, and numbers indicate the distances of start sites from the ATG initiation codon. (**A**) A *PGK*-specific primer was used with RNA isolated from the yeast strains described above the individual lanes. (**B**) RNA isolated from the indicated strains was extended using an *H2B*-specific primer. For both (A) and (B), *wt* indicates the wild-type strain EG2, *shi* indicates strain EG1 containing the *shi* point mutation, *k.o.* indicates the *shi* deletion strain EG6, whereas k.o.[shi] and k.o.[SHI] indicate strain EG6 transformed with plasmids pCEN-shi/4.1 and pCEN-SHI/4.1, respectively. (**C**) DNA sequences of the *PGK* and *H2B* genes, showing the region TATA to the ATG, with initiation sites used in wild-type strains marked with # and those used in *shi* or *shi* deletion strains marked by *.

Partial sequencing of the other independently isolated PCR products showed that all contained the identical single base pair change, altering the cysteine residue at amino acid position 7 (encoded by a TGT codon) to a phenylalanine residue (encoded by a TTT codon). This cysteine residue is located in the first of two cysteine-rich (Cys_4) domains with sequences $CX_2CX_{18}CX_2C$ and $CX_2CX_{24}CX_2C$ (where C denotes cysteine and X any other amino acid) as noted by Woychik *et al.* (11) for the *RPB9* gene.

To show that this sequence alteration is solely responsible for the shift of initiation sites seen in the *shi* mutant strain, we demonstrated that a plasmid containing this single base pair change could not restore the wild-type growth phenotype or RNA initiation pattern when transformed in the *shi* deletion strain. The *shi* deletion mutant strain was transformed with plasmids pCEN-SHI/4.1 and pCEN-shi/4.1, which are identical except for the mutation causing the cysteine to phenylalanine residue change. Transformation of the wild-type *SHI* gene into the *shi* deletion strain complemented not only the inositol requirement for growth but also reversed the ability of the *shi* deletion strain to activate the *adhΔ50* allele. In addition, the RNA initiation patterns for the *PGK* and *H2B* genes in these transformants were very similar to those seen in the wild-type strains (Fig. 3); the prominent upper start site (position −81) of the *PGK* gene and the upper start sites (positions −76 and −90) for *H2B* were essentially unused. Transformation of the wild-type *SHI* gene into the *shi* mutant strain led to a similar restoration of the wild-type RNA initiation patterns for these genes (not shown). This demonstrates that the wild-type *SHI* gene complements the shift in initiation site phenotype. In contrast, transformants of the mutant *shi* gene in the *shi* deletion strain could not grow in the absence of inositol, and could not complement the Adh$^+$ phenotype of the *shi* deletion strain that was due to activation of the *adhΔ50* allele. Additionally, the pattern of start sites was unaffected by introduction of the mutant *shi* gene by transformation (Fig. 3). This result identifies the cysteine to phenylalanine change as responsible for the observed shift of initiation sites in the *shi* mutant strain.

DISCUSSION

Mutations in the *S.cerevisiae* TFIIB gene (*SUA7*) and the gene encoding the largest subunit of pol II (*SUA8*) have been identified in a selection scheme requiring a downstream shift in RNA initiation sites (4, 6). Our independent selection for proteins involved in RNA start site selection in *S.cerevisiae* yielded a mutation in another subunit of RNA polymerase II, the 12.6 kDa subunit. The wild-type *SHI* gene was cloned through complementation of the *shi* mutant phenotype, and the mutant *shi* allele was subsequently isolated by PCR amplification. The mutant allele was found to contain a single base pair mutation, changing a cysteine to a phenylalanine. This single base pair mutation led to the same molecular phenotype as deletion of the entire gene, resulting in increased usage of upsteam initiation sites for the *PGK* and *H2B* genes.

The finding that substitution of a cysteine residue in one Cys_4 motif leads to an apparent loss of function of the *SHI* protein underscores the functional importance of this motif. The two Cys_4 motifs in *SHI/RPB9* belong to a family of sequences analogous to the Cys_4 motif seen in the transcription elongation factor TFIIS; the solution structure of TFIIS has been shown by

3D NMR to exhibit a novel 3-stranded antiparallel β-sheet, designated the Zn ribbon, in which the four cysteines coordinate a single Zn^{2+}. This Cys_4 Zn ribbon is not related to the classical Cys_2-His_2 Zn finger shown to be involved in protein–DNA binding nor to other Cys_4 or Cys_6 domains (13). Other proteins containing an analogous $CX_2CX_{(8-25)}CX_2C$ motif include the transcription factors TFIIE (13) and TFIIB (4), and the adenovirus transactivator E1A (14). In addition, the second largest subunits of RNA polymerases I (15), II (16) and III (17), as well as of archaebacterial RNA polymerases (18), contain a Cys_4 motif. The second largest subunits of all three yeast RNA polymerases have been shown to bind zinc (19). The RPB9 subunit, which could potentially bind two zinc ions, was in fact shown to produce the strongest Zn binding signal when purified pol II was examined by a blotting assay (19).

There is growing evidence that the Zn ribbon facilitates protein–protein interactions and that the cysteines are required for this function. Substitutions of any of the cysteines within the Cys_4 motif of the second largest subunit of RNA pol II reduced Zn^{2+} binding *in vitro* 2- to 5-fold and resulted in either a lethal or thermosensitive phenotype (19). These authors propose that the thermosensitive phenotype reflects the role the zinc-bound structure plays in stabilization and possibly subunit–subunit interaction in the assembly of the holoenzyme complex. Substitution of either one of two cysteine residues in TFIIB by serine resulted in mutant proteins that could no longer recruit RNA polymerase II–TFIIF into the initiation complex, suggesting that the putative Zn ribbon motif of TFIIB might act as a metal-linked interaction domain that allows protein–protein interaction (20). In the case of the adenovirus E1A protein, it has recently been directly demonstrated that the zinc ribbon is required for binding of this protein to TBP. Furthermore, replacing any of the 4 cysteines in the adenovirus E1A Cys_4 motif produces a protein no longer capable of activating transcription, with a corresponding reduction in TBP binding ability (21). The fact that a substitution of a cysteine in SHI/RPB9 produces effects on RNA initiation pattern identical to those seen in the absence of this subunit argues that the SHI/RPB9 Cys_4 zinc ribbon is needed for this polymerase subunit to interact with other proteins needed for proper initiation site selection.

The RPB1/SUA8, RPB9/SHI and TFIIB/SUA7 proteins, each identified in genetic screens to find proteins involved in initiation site selection, may well comprise all the necessary components for setting the TATA to start site spacing in *S.cerevisiae*, since biochemical studies showed that RNA polymerase II and TFIIB are sufficient for setting the spacing *in vitro* (3). An open question is how the task of choosing initiation sites is divided between these three proteins, and whether they directly interact. Synthetic lethality of *sua7 sua8* double mutants suggests an interaction between these two proteins (6). Since both the RPB9 and the TFIIB proteins contain putative zinc ribbons with a postulated function in protein–protein interaction, it is intriguing to speculate whether these proteins can also contact each other in the context of an initiation complex.

ACKNOWLEDGEMENTS

We thank T.Wallimann and H.M.Eppenberger (Cell Biology, ETH) for their most generous support towards the completion of this work. The help of E.Zanolla with photographic work is appreciated.

REFERENCES

1. Furter-Graves, E.M., Furter, R. and Hall, B.D. (1991) *Mol. Cell. Biol.*, **11**, 4121–4127.
2. Buratowski, S. (1994) *Cell*, **77**, 1–3.
3. Li, Y., Flanagan, P.M., Tschochner, H. and Kornberg, R.D. (1994) *Science*, **263**, 805–807.
4. Pinto, I., Ware, D.E. and Hampsey, M. (1992) *Cell*, 68, **977**–988.
5. Young, R. A. (1991) *Annu. Rev. Biochem.*, **60**, 689–715.
6. Berroteran, R.W., Ware, D.E. and Hampsey, M. (1994) *Mol. Cell. Biol.*, **14**, 226–237.
7. Lawrence, C.W. (1991) *Methods Enzymol.*, **194**, 273–281.
8. Sikorski, R. S. and Hieter, P. (1989) *Genetics*, **122**, 19–27.
9. Furter-Graves, E.M. and Hall, B.D. (1990) *Mol. Gen. Genet.*, **223**, 407–416.
10. Whiteway, M., Hougan, L., Dignard, D., Thomas, D.Y., Bell, L., Saari, G.C., Grant, F.J., O'Hara, P. and MacKay, V.L. (1989) *Cell*, **56**, 467–477.
11. Woychik, N.A., Lane, W.S. and Young, R.A. (1991) *J. Biol. Chem.*, **266**, 19053–19055.
12. Harrison, D.A., Mortin, M.A. and Corces,V.G. (1992) *Mol. Cell. Biol.*, **12**, 928–935.
13. Qian, X., Gozani, S. N., Yoon, H., Jeon, C., Agarwal, K. and Weiss, M.A. (1993) *Biochemistry*, **32**, 9944–9959.
14. Berg, J.M. (1990) *J. Biol. Chem.*, **265**, 6513–6516.
15. Yano, R. and Nomura, M. (1991) *Mol. Cell. Biol.*, **11**, 754 -764.
16. Sweetser, D., Nonet, M. and Young, R.A. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 1192–1196.
17. James, P., Whelen, S. and Hall, B.D. (1991) *J. Biol. Chem.*, **266**, 5616–5624.
18. Leffers, H., Gropp, F., Lottspeich, F., Zillig, W. and Garrett, R. A. (1989) *J. Mol. Biol.*, **206**, 1–17.
19. Treich, I., Riva, M. and Sentenac, A. (1991) *J. Biol. Chem.*, **266**, 21971–21976.
20. Buratowski, S. and Zhou, H. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 5633–5637.
21. Geisberg, J.V., Lee, W.S., Berk, A.J. and Ricciardi, R.P. (1994) *Proc. Natl. Acad. Sci. USA*, **91**, 2488–2492.