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Rpc19 and Rpc40, two α-like subunits shared by nuclear RNA polymerases I and III, are interchangeable between the fission and budding yeasts

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Abstract The cDNAs and genes encoding the common subunits Rpc19 and Rpc40 of nuclear RNA polymerases I and III of *Schizosaccharomyces pombe* were isolated from cDNA and genomic libraries of the fission yeast and tested for their ability to substitute for the homologous genes in *Saccharomyces cerevisiae* by heterospecific complementation of corresponding null alleles and temperature-sensitive mutations. The results obtained indicate that both *Sz. pombe* genes (*rpc19*$^+$ and *rpc40*$^+$) are able to replace their *S. cerevisiae* counterparts in vivo. The primary structure and general organization of both genes were established: *rpc40*$^+$ is an intronless gene, while *rpc19*$^+$ contains three introns (73, 48 and 77 bp long); *rpc19*$^+$ is situated on the long arm of chromosome I and *rpc40*$^+$ on the long arm of chromosome II.

Key words *Schizosaccharomyces pombe* · Nuclear RNA polymerases I and III · *rpc19*$^+$ and *rpc40*$^+$ genes · α-motif

Introduction

Eukaryotic cells have a very complex basal transcription apparatus consisting of three nuclear RNA polymerases (I, II and III). These three enzymes are organized around a nucleating core which includes subunits related to the components of the core of bacterial RNA polymerase ($\alpha_2\beta'\beta$) and a set of five subunits (Rpb5, Rpb6, Rpb8, Rpb10 and Rpc10) which are common to all three nuclear RNA polymerases (Sentenac et al. 1992; Thuriaux and Sentenac 1992, and references therein). The two largest subunits are homologous to the β′ and β subunits of bacterial RNA polymerase (Allison et al. 1985; Sweetser et al. 1987; Riva et al. 1990; Zaychikov et al. 1996). The role of the prokaryotic α_2 homodimer is probably performed by the Rpc40-Rpc19 heterodimer in the case of enzymes I and III (Lalo et al. 1993) and by the Rpb3-Rpb11 heterodimer in RNA polymerase II (Ulmasov et al. 1996; Kimura et al. 1997). The present work deals with the *Schizosaccharomyces pombe* genes encoding two α-related subunits, Rpc19 and Rpc40, which are shared between nuclear RNA polymerases I and III of the fission yeast. This study completes the initial characterization of the seven components shared between nuclear RNA polymerases I and III of the fission yeast (Shpakovski 1994; Shpakovski et al. 1995; Miyao et al. 1996; Shpakovski and Lebedenko 1997; Shpakovski et al. 1997, 1998).

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Materials and methods

Plasmids, strains and media. Yeast strains and plasmids constructed in the present work are listed in Table 1. The yeast strain YPH499 and shuttle vectors pGEN and pRS314 were described previously (Sikorski and Hieter 1989; Shpakovski et al. 1995). Growth conditions and the media used correspond to standard laboratory practice for *Escherichia coli* and *Saccharomyces cerevisiae* (Maniatis et al. 1982; Sherman 1991). Minimal SD medium supplemented with 0.1% casein hydrolysate, 0.002% adenine sulphate, and 0.002% uracil or tryptophan was used as tryptophan or uracil omission medium. 5-FOA (5-fluoroorotic acid monohydrate, product of PCR Inc., Gainesville, Florida, USA)-containing media was prepared as described in Ausubel et al. (1990).

Screening of libraries. The cDNA and genomic libraries of the wild-type strain 972h$^-$ were previously described (Becker et al. 1991; Weaver et al. 1993). Library screening was performed by means of PCR with use of the subsequent dilution method (Shpakovski 1994; Shpakovski et al. 1997). The following PCR-primers were employed for isolation plasmids with the *rpc40*$^+$ gene: oGXW31 [5′-CAT*GCTAGC*AAAAATGGCAGCGGTTGAC], oGVS352 [5′-CG*GGATCC*AAAATGGCAGCGGTTGAC] (upstream prim-

Table 1 Shuttle plasmids and yeast strains used

Plasmid or strain	Yeast genetic markers or genotype	Construction, origin or reference
pESH83	2 μm ORI, *URA3*, *rpc19*$^+$	Clone with full-length *rpc19*$^+$ cDNA of *Sz. pombe* isolated from a cDNA library. The clone, unlike pESH38, contains correct orientation of *Sz. pombe rpc19*$^+$ gene allowing its expression from ADC1 promoter.
pRS-rpc19$^+$	*CEN6*, *ARSH4*, *TRP1*, *rpc19*$^+$	Cloning of *Bam*HI-*Bam*HI fragment of pESH83 (containing ADC1 promoter, *rpc19*$^+$ cDNA and ADH1 terminator) in pRS314.
pYUL23	2 μm ORI, *ura4*$^+$, *rpc19*$^+$	Clone isolated from genomic library (Weaver et al. 1993) and containing 6.4 kb DNA insert with *rpc19*$^+$ gene of *Sz. pombe*.
pYUL18	2 μm ORI, *ura4*$^+$, *rpc40*$^+$	Clone isolated from the genomic library (Weaver et al. 1993) and containing 7.4 kb DNA insert with *rpc40*$^+$ gene of *Sz. pombe*.
pESH10	2 μm ORI, *TRP1*, *rpc40*$^+$	Directional cloning of the *Sz. pombe rpc40*$^+$ gene between *Sma*I and *Eco*RI sites of pGEN by PCR amplification of pYUL18 with use of oGXW31 and oGVS347 primers in front of ATG and behind stop codon.
pGSH9	2 μm ORI, *TRP1*, *rpc40*-R106G	Directional cloning of the *Sz. pombe rpc40*$^+$ gene between *Bam*HI and *Eco*RI sites of pGEN by PCR amplification of pYUL18 using oGVS352 and oGVS347 primers in front of ATG and behind stop codon.
pESH1	2 μm ORI, *URA3*, *rpc40*$^+$	Clone with the full-length *rpc40*$^+$ cDNA of *Sz. pombe* isolated from cDNA library.
DLY200	MATα *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc19-Δ*::*HIS3* [pLS135 (*URA3*, *CEN4*, *ARS1*, *RPC19*)]	Lalo et al. 1993
YSHS1 & YSHS2	MATα *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc19-Δ*::*HIS3* [pRS-*rpc19*$^+$]	Plasmid shuffling in DLY200
DLY202 (*ts*)	MATα *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc19-Δ*::*HIS3* [DlpAC135 (*URA3*, *CEN4*, *ARS1*, *rpc19*-G73D)]	Lalo et al. 1993
YSHS3	MATα *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc19-Δ*::*HIS3* [DlpAC135 (*URA3*, *CEN4*, *ARS1*, *rpc19*-G73D) + pESH83]	Transformant of DLY202
YSHS4 (*ts*)	MATα *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc19-Δ*::*HIS3* [DlpAC135 (*URA3*, *CEN4*, *ARS1*, *rpc19*-G73D) + pESH38]	Transformant of DLY202
CMY217	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-Δ*::*HIS3* [YCp50-RPC40 (*URA3*, *CEN4*, *ARS1*, *RPC40*)]	Mann et al. 1987
YSHS5	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-Δ*::*HIS3* [pESH10]	Plasmid shuffling in CMY217
YSHS6 (*ts*)	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-Δ*::*HIS3* [pGSH9]	Plasmid shuffling in CMY217
DLY7c (*ts*)	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-V78R*	Lalo et al. 1993
YSHS7	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-V78R* [pESH10]	Transformant of DLY7c
YSHS8 (*ts*)	MAT**a** *ura3-52 his3-Δ200 trp1-Δ1 lys2-801 ade2-101 rpc40-V78R* [pGSH9]	Transformant of DLY7c

ers), and oGXW32 [5′-AG*GCTAGC*AAGTAGTGATT-GAAAACC], oGVS347 [5′-C*GAATTC*AGTCAGAAGAAAT-ATTC] (downstream primers). The *rpc19*$^+$ gene was re-cloned with the following set of primers: oGVS324 [5′-CG*GGATCC*ACT-TTAGACATGGCGGC], oGXW33 [5′-CA*GCTAGC*GA-CATGGCGGCAATGACAG] (upstream primers), and oGVS325 [5′-GG*GAATTC*AT-AACCAAATTTATCCATC], oGXW34 [5′-CA*GCTAGC*TAAACTGCAGCTGATGGAATG (downstream primers). The italicized nucleotides in both sets of oligos define the *Bam*HI, *Eco*RI and *Nhe*I sites used for PCR-cloning.

Hybridization onto the cosmid libraries and DNA sequencing. High-density cosmid filters (Hoheisel et al. 1993; Mizukami et al. 1993) were provided by Rhian Gwilliam (The Sanger Centre, UK). The hybridization conditions employed were mostly as described in Hoheisel et al. (1993) and Mizukami et al. (1993). Filters were hybridised usually overnight at 42 °C, washed twice for 15 min at room temperature (2 × SSC/0.1% SDS) and once at 60 °C (6 × SSC/1% sarkosyl), and then exposed overnight at room temperature. The sequencing reactions were performed on alkaline-treated double-stranded DNA templates with Sequenase 2.0 (United States Biochemicals, USA), and/or the dsDNA Cycle Sequencing System (BRL/Life Technologies Inc., USA) and the fmolTM DNA Sequencing System of "Promega" (USA) with sequencing grade *Taq* DNA polymerase following instructions supplied by the manufacturers. Both strands of the DNAs were sequenced in all cases.

Plasmid-shuffling and other yeast genetic techniques. *Sz. pombe rpc19*$^+$ and *rpc40*$^+$ cDNAs were tested for their ability to complement the *S. cerevisiae* null alleles *rpc19-Δ::HIS3* of strain DLY200 (Lalo et al. 1993) and *rpc40-Δ::HIS3* of strain CMY217 (Mann et al. 1987) on rich medium YPD at 16 °C, 30 °C and 37 °C, using a plasmid shuffle assay (Shpakovski et al. 1995). Null alleles of both haploid tester strains were complemented by the corresponding wild-type genes borne on the *URA3*$^+$ plasmids pLS135 and YCp50-RPC40 (see Table 1). Plasmid loss, which is a lethal event and can be relieved only by heterospecific complementation, was monitored by the formation of fluoro-orotate-resistant colonies. The temperature-sensitive (*ts*) phenotypes of the *S. cerevisiae* strains DLY202 and DLY7c (Lalo et al. 1993) were tested for complementation by the *Sz. pombe rpc19*$^+$ and *rpc40*$^+$ genes by transforming these *ts*-strains with plasmids pESH83 and pESH10 (see Table 1), respectively, and then testing for growth at 37 °C for 3–4 days.

Nucleotide sequence accession numbers. The sequences of the *rpc19*$^+$ cDNA and gene, and the *rpc40*$^+$ gene of *Sz. pombe* established in this work were deposited into the GenBank/EMBL database under the following accession numbers: AF079779 (*rpc19*$^+$ cDNA), AF116919 (*rpc19*$^+$ gene) and AF082512 (*rpc40*$^+$ gene).

Results and discussion

The primary structure, organization and chromosomal location of the *Sz. pombe rpc19*$^+$ gene

The *rpc19*$^+$ cDNA of *Sz. pombe* was originally isolated in a search for fission yeast gene products that disrupt mitotic chromosome segregation when overexpressed (Javerzat et al. 1996). We have re-cloned this cDNA from a previously described cDNA library (Becker et al. 1991). The sequence of the corresponding cDNA clones pESH83 and pESH38 matches the previously reported data (Javerzat et al. 1996) except for eight positions outside the coding sequence (cf. EMBL/GenBank accessions U50769 and AF079779). Using a genomic library (Weaver et al. 1993), we isolated a clone, pYUL23, bearing an insert of approximately 6.4 kb. Sequencing this insert showed that the *rpc19*$^+$ gene consists of four exons (131-, 98-, 77- and 316-bp long) and three introns (73-, 48- and 77-bp long) (see GenBank accession number AF116919). The pYUL23 insert contains at one end about 4.9-kb of cosmid c22H12 (http://www.sanger.ac.uk; EMBL accession number AL034565) and at the other end an approximately 1.2-kb fragment of cosmid c1687 (EMBL accession number AL035064), thus closing the gap between these two neighbouring cosmids on the long arm of chromosome I of *Sz. pombe* (see Hoheisel et al. 1993; Mizukami et al. 1993). This was confirmed by hybridization of the *rpc19*$^+$-specific probe with high-density cosmid filters (data not shown).

The *Sz. pombe rpc19*$^+$ gene is functionally interchangeable with its *S. cerevisiae* counterpart

Comparison of the primary structure of the *Sz. pombe* Rpc19 with homologous subunits from *S. cerevisiae* (Dequard-Chablat et al. 1991), *Arabidopsis thaliana*

Fig. 1 Comparison of amino-acid sequences of the subunit Rpc19 of RNA polymerases I and III of *Sz. pombe* (Javerzat et al. 1996; this work) and its homologues from *S. cerevisiae* (accession no. M64991), *M. musculus* (accession no. D86609), *A. thaliana* (accession no. U35050) and *Yarrowia lipolytica* (deduced from accession no. AJ001302). Here and below (in Fig. 3) the programme Advanced Gapped BLAST 2.0 (Altschul et al. 1997) was used. The invariant amino acids are given *in bold*; the amino acids that are conserved between *S. cerevisiae* and *Sz. pombe* and/or between at least three species are *shadowed*. The conserved α-motif is indicated. The intron positions in the *rpc19*$^+$ gene of *Sz. pombe* are marked by *arrows*, and the corresponding amino-acid residues are *underlined*

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A. thaliana      1                                                    MEHGS   5
S. cerevisiae    1  MTEDIEQKKTATEVTPQEPKHIQEEEEQDVDMTGDEEQEEEPDREKIKLLTQAT  54
Sz. pombe        1                                MAAMTDVTDPSSVAMESATEKIILP-GH  28
Y. lipolytica    1       MDEDVAPEISEEERLAQEKAERKQELNQKADDFHAEFMANKFTILP-GA  48
M. musculus      1                          MEDDQELERKISGLKTSMAEGERKTALEMVQAA  33
INVARIANTS

                                    //////////α-motif\\\\\\\\\\
A. thal.     6  FTNVSHASFTLSEEDHTLANAVRFVLNQDPRVTVAAYTIPHPSLEQVNIRVQTTGD-PA  63
S. cer.     55  SEDGTSASFQIVEEDHTLGNALRYVIMKNPDVEFCGYSIPHPSENLLNIRIQTYGETTA 113
Sz. pom.    29  SADLTSVTFQIQKEDHTLGNSLRYVIMKNPEVEFCGYSIPHPSEAKMNFRIQTAPSTTA  87
Y. lip.     49  SADKTACSFQFTEEDHTIGNALQYIIMKNPEVEFCGYSIPHPSEAKLNIRIQTYGDITA 107
M. mus.     34  GTDRQCVTFVLHEEDHTLGNCLRYIIMKNPEVEFCGYTTTHPSESKINLRIQTRGALPA  92
INVARIANTS            F    EDHT  N         P V    Y   HPS    N R QT     A

A. thal.    64  REVFKDACQELMQMNRHVRSVFDKAVAEYKDEQKRKEEAEEEELKRQRDLFGSMDIENN 122
S. cer.    114  VDALQKGLKDLMDLCDVVESKFTEKIKSM                               142
Sz. pom.    88  VDVLRKGLDDLIDLCDAVTEKFTEQLPRDTSTTMEVDG                      125
Y. lip.    108  IEALQKGLDDLIQACDVVKNKFTDAVTDFTSKN                           140
M. mus.     93  VEPFQKGLNELLNVCQHVLVKFEASIKDYKAKKASKKEPTF                   133
INVARIANTS            L     V   F
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(Larkin and Guilfoyle 1996) and *Mus musculus* (Yao et al. 1996) show that the most-conserved region of the subunits is the α-motif (Lalo et al. 1993), whereas their N- and C-terminal parts are quite variable (Fig. 1). We have previously observed that the *AtRPAC14* cDNA of *A. thaliana* can not substitute for the homologous gene of *S. cerevisiae* (G.V.S.; M. Vigneron and P. Thuriaux, unpublished results). The *rpc19*$^+$ gene of *Sz. pombe* has a much-higher homology with the *RPC19* from *S. cerevisiae* (58 and 35% identity, respectively) and can replace its budding yeast counterpart in vivo (Fig. 2). Complementation was observed when the *Sz. pombe* gene was supplied on a centromeric (low-copy number) plasmid pRS314 or on the high-copy vector pGEN. It also complemented the *ts* mutant *rpc19*-G73D of *S. cerevisiae* (strain DLY202, Lalo et al. 1993) (Fig. 2). Thus, the corresponding subunit of fission yeast is able to function in both RNA polymerases I and III of *S. cerevisiae*. The main structural differences between *Sz. pombe* and *A. thaliana* RPC19 homologues are in the middle of the α-motif (see Lalo et al. 1993) and in the C-terminal parts of the molecules (Fig. 1).

The *rpc40*$^+$ gene of *Sz. pombe*, an intron-less gene located near the right telomere of chromosome II

Open reading frame (ORF) segments that are highly homologous to the *RPC40* gene of *S. cerevisiae* are present on the nucleotide sequence of cosmid c1289 of *Sz. pombe* sequenced by the Sanger Centre. Based on this structural information, specific primers were used to screen genomic and cDNA libraries of the fission yeast (see Materials and methods), yielding clones pYUL18 and pESH1, respectively. The corresponding sequences (GenBank accession number AF082512) showed that the *Sz. pombe rpc40*$^+$ gene has no introns. A good candidate for the TATA box (TATAAA) of the *rpc40*$^+$ gene is located 225 nt upstream of the ATG start codon, followed after 8 nt by a stretch of $(A)_{12}$ which can serve as a constitutive promoter. Our hybridization data using a *rpc40*$^+$ probe (data not shown) confirm the assignment of the gene to cosmid c1289. In concordance with the cosmid map of the *Sz. pombe* genome (Mizukami et al. 1993), this means that the *rpc40*$^+$ gene is located in contig 9 on the long arm of chromosome II, close to the right telomere.

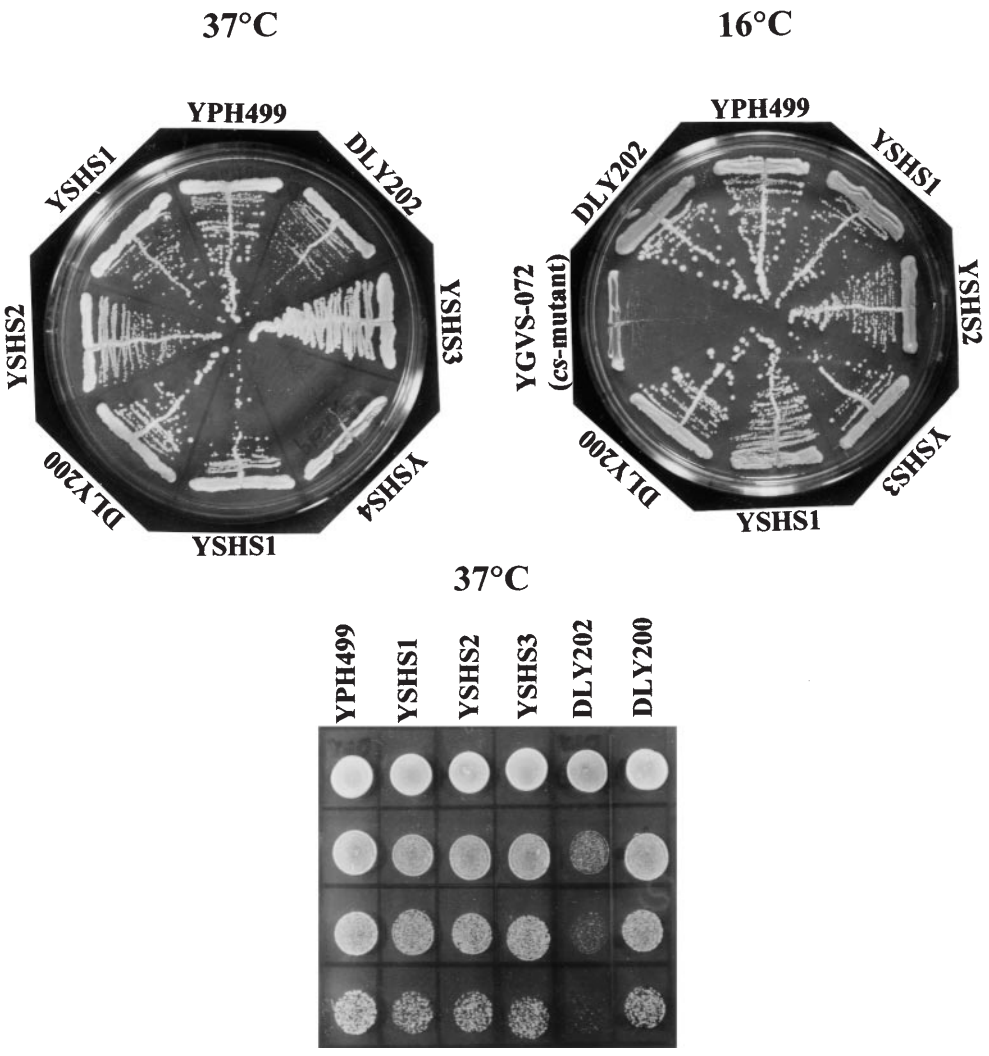

Fig. 2 Inter-specific complementation of the *rpc19-Δ::HIS3* null allele and the *ts*-phenotype of the G73D mutation in subunit RPC19 by *rpc19*$^+$ cDNA of *Sz. pombe*. (*Upper panels*) yeast strains were streaked on YPD plates and incubated for 3 days at 37 °C (on the left) and for 7 days at 16 °C (on the right). YSHS1 (*rpc19*$^+$) and YSHS2 (*rpc19*$^+$), bearing pRS-rpc19$^+$ with a different orientation of the *rpc19*$^+$ expression module regarding vector pRS314, are isogenic derivatives of DLY200 (wild-type *RPC19*); YSHS3 (*rpc19*$^+$) and YSHS4 (*rpc19*$^-$) harboring *rpc19*$^+$ cDNA in expressed and unexpressed orientation with respect to the ADC1 promoter, respectively, are isogenic to DLY202 (*tsRPC19*-G73D); YGVS-072 (*csRPB11*) was used as a control. (*Lower panel*) the strength of the inter-specific complementation is illustrated for strains with *Sz. pombe rpc19*$^+$ by their growth after 3 days on complete medium YPD at 37°C in comparison with the tester strains, using a serial dilution technique

The primary structure of the Rpc40 subunit of fission yeast, deduced from the genetic sequence, is 348 aa long (Mr 39 141 kDa; pI 5.40). A comparison with the homologous subunits of *S. cerevisiae* (Mann et al. 1987), *A. thaliana* (Ulmasov et al. 1995), *M. musculus* (Song et al. 1994) and *Homo sapiens* (Dammann and Pfeifer 1998) indicate that they are evolutionarily well-conserved, especially within three regions (aa 60–116, 197–238 and 299–334 on the primary structure of the *Sz. pombe* Rpc40 subunit) (see Fig. 3). Curiously, the EMBL/GenBank database contains what is described as a cDNA fragment of Chinese hamster (*Cricetulus griseus*) (accession number Y10542) which is almost identical to the *Sz. pombe* sequence (only three nucleotide and one amino-acid substitutions) and is therefore almost certainly due to some contamination artefact.

Heterospecific complementation between the *Sz. pombe rpc40*$^+$ gene and its *S. cerevisiae* orthologue

The inter-specific complementation of the *Sz. pombe rpc40*$^+$ gene in *S. cerevisiae* cells was tested by introducing plasmid pESH10 into the haploid strain CMY217 (Mann et al. 1987) as described in Materials and methods. *S. cerevisiae* colonies bear the deleted chromosomal allele (as monitored by histidine prototrophy) together with the complementing copy of the wild-type *Sz. pombe rpc40*$^+$ gene supplied by pESH10. As in the case of the Rpc19 subunit, complementation was observed at all temperatures tested (16, 30, and 37 °C) (Fig. 4). We also obtained a serendipitous PCR-generated mutation of the *Sz. pombe rpc40*$^+$ gene (plasmid pGSH9, see Table 1) which complements the *S. cerevisiae rpc40-Δ::HIS3* null allele only at 30 °C, but not at the restrictive temperature 37 °C (Fig. 4). The *rpc40* insert from pGSH9 contains three nucleotide changes compared to the *Sz. pombe rpc40*$^+$ wild-type allele. Two of the substitutions are in the third positions of codons no. 12 (TCT → TCc) and no. 56 (GAA → GAg) and have no effect on the amino-acid sequence. The third mutation (AGA → gGA) affects an invariant arginine residue (R106G) and is located inside the first region of strong conservation between Rpc40 subunits from different eukaryotes, but outside the α-motif (see Fig. 3). Unlike *ts*-mutants at the α-motif (Lalo et al. 1993), *rpc40*-R106G was not suppressed by genes encoding other subunits of *S. cerevisiae* RNA polymerases (*RPC19*, *RPB10*, *RPC10*, *RPB8*, *RPB6*, *RPB5*, *RPA43*).

In conclusion, the *Sz. pombe* equivalents of the five common subunits shared by all three nuclear RNA

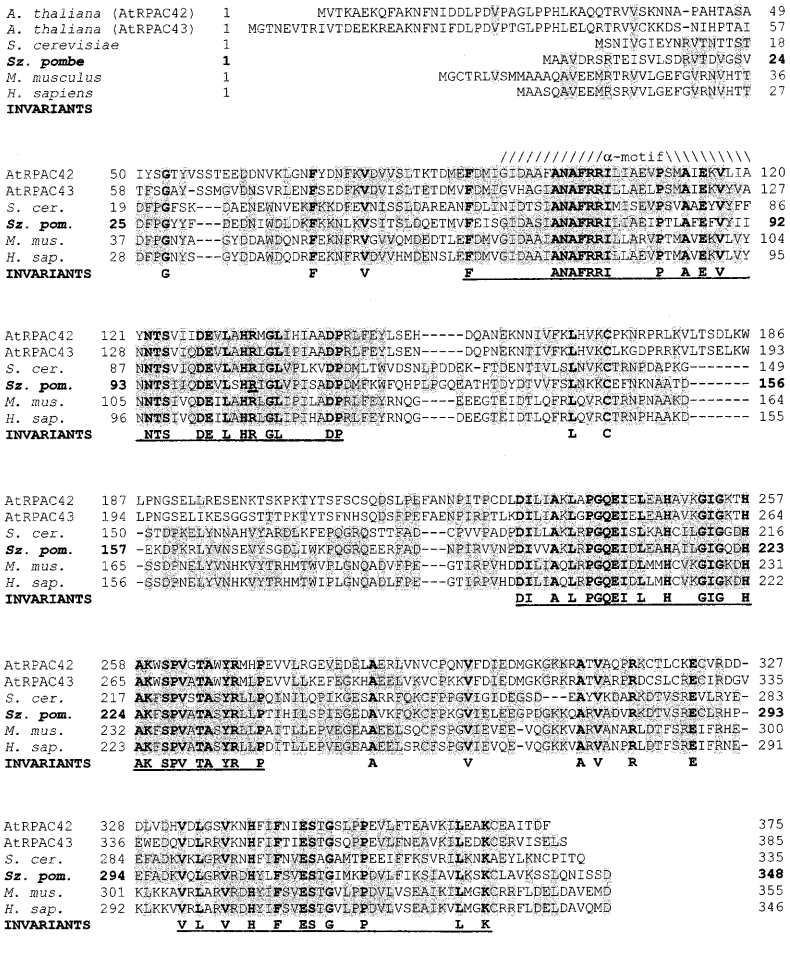

Fig. 3 Comparison of amino-acid sequences of the subunit Rpc40 of RNA polymerases I and III of *Sz. pombe* and its homologues from *S. cerevisiae* (accession no. M15499), *A. thaliana* (accession nos. L34772 and L34773), *M. musculus* (accession no. D31966) and *H. sapiens* (accession no. AF008442). The invariant amino acids are given *in bold*; the amino acids that are conserved between *S. cerevisiae* and *Sz. pombe* and/or between at least three sequences are *shadowed*. Three of the most-highly conserved regions discussed in the text, and the α-motif are indicated. The invariant arginine residue mutated in the *ts*-allele of *Sz. pombe rpc40*-R106G (plasmid pGSH9) is *underlined*

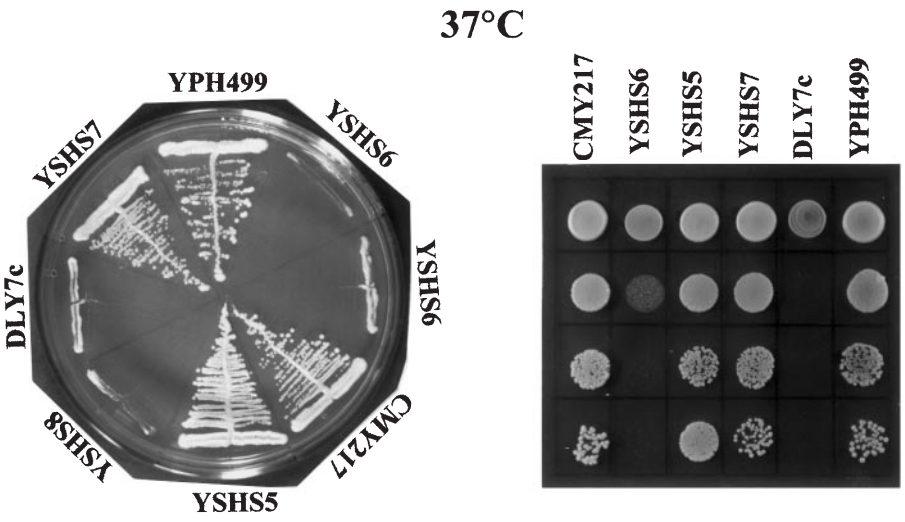

Fig. 4 Inter-specific complementation of the *rpc40-Δ::HIS3* null allele and the *ts*-phenotype of the V78R mutation in subunit RPC40 by *rpc40*$^{+}$ cDNA of *Sz. pombe*. (*Left panel*) yeast strains were streaked on YPD plates and incubated for 4 days at 37°C. YSHS5 (*rpc40*$^{+}$) and YSHS6 (*rpc40*-R106G) are isogenic derivatives of CMY217 (wild-type *RPC40*); YSHS7 (*rpc40*$^{+}$) and YSHS8 (*rpc40*-R106G) are isogenic to DLY7c (*tsRPC40*-V78R). (*Right panel*) the strength of the inter-specific complementation is illustrated for strains with *Sz. pombe rpc40*$^{+}$ by their growth after 4 days on complete medium YPD at 37 °C in comparison with the tester strains, using a serial-dilution technique

polymerases in *S. cerevisiae* (Shpakovski 1994; Shpakovski et al. 1995; Miyao et al. 1996; Shpakovski and Lebedenko 1997; Shpakovski et al. 1997, 1998; Voutsina et al. 1999) and of the two subunits shared by RNA polymerases I and III have now been identified (Javerzat et al. 1996; this work). Except for Rpb8 (which in *S. cerevisiae* cells can not support Pol III-specific transcription without simultaneous overexpression of the *S. cerevisiae RPC160* gene encoding the largest subunit of RNA polymerase III, Voutsina et al. 1999), all of them efficiently complement the corresponding null mutants of *S. cerevisiae* (Shpakovski 1994; Shpakovski et al. 1995, 1997; Shpakovski et al., in preparation). Moreover, partial heterospecific complementation was also observed for one RNA polymerase III-specific subunit, RPC11, and revealed that this subunit is crucial to transcription termination (Chédin et al. 1998). The current sequencing of the *Sz. pombe* genome has revealed several other regions of homology to RNA polymerase I- or RNA polymerase III-specific subunits, and it will be interesting to see if they are all functionally equivalent to their *S. cerevisiae* counterparts.

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Note added in proof. Whilst this manuscript was under revision, a paper by Imai et al. appeared in Mol Gen Genet (261: 364–373) describing results essentially similar to ours for the heterospecific complementation of *rpc19*$^+$.