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Allelism of *PSO4* and *PRP19* links pre-mRNA processing with recombination and error-prone DNA repair in *Saccharomyces cerevisiae*

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ABSTRACT

The radiation-sensitive mutant *pso4-1* of *Saccharomyces cerevisiae* shows a pleiotropic phenotype, including sensitivity to DNA cross-linking agents, nearly blocked sporulation and reduced mutability. We have cloned the putative yeast DNA repair gene *PSO4* from a genomic library by complementation of the blocked UV-induced mutagenesis and of sporulation in diploids homozygous for *pso4-1*. Sequence analysis revealed that gene *PSO4* consists of 1512 bp located upstream of *UBI4* on chromosome XII and encodes a putative protein of 56.7 kDa. *PSO4* is allelic to *PRP19*, a gene encoding a spliceosome-associated protein, but shares no significant homology with other yeast genes. Gene disruption with a destroyed reading frame of our *PSO4* clone resulted in death of haploid cells, confirming the finding that *PSO4/PRP19* is an essential gene. Thus, *PSO4* is the third essential DNA repair gene found in the yeast *S.cerevisiae*.

INTRODUCTION

At least 50 genes encoding proteins involved in DNA repair are known in the yeast *Saccharomyces cerevisiae* (1,2) and, since molecular studies on many of them have revealed a close relationship to DNA repair enzymes found in humans and mammals, this simple eukaryote has advanced to a model in which the complex mechanisms underlying DNA repair might be unraveled in the near future. The many DNA damage-sensitive yeast mutants were initially grouped into three phenotypic groups (3,4) which, by double mutant analysis that measured synergistic or epistatic interactions, were allocated to three epistasis groups (5–7). This classification more or less holds until today, with group I comprising ~20 genes that are *RAD3*-like and encode proteins for nucleotide excision repair, group II comprising ~12 genes that are *RAD52*-like and which seem to encode proteins involved in repair of DNA double-strand breaks via recombinational mechanisms and the largest, and by far the most heterogeneous, group III, defined by *RAD6*, comprising ~20 genes, many of which are responsible for error-prone DNA repair (1,2,8). Some DNA damage-sensitive mutants, e.g. several of the yeast mutants sensitive to photoactivated psoralens (*pso* mutants; 9) have been allocated to more than one epistasis group, depending on the mutagen applied and the biological end point scored in the respective double mutants (8). A mutant allele of gene *PSO4*, which is involved in error-prone repair and some types of recombination, i.e. gene conversion, crossing-over and intrachromosomal recombination (10,11,12), was originally isolated as X-ray-sensitive mutant xs9 (13); it was found to be particularly sensitive to photoactivated bifunctional 8-methoxypsoralen and was thus renamed *pso4-1* (10). This yeast mutant is phenotypically similar to the *recA* mutant of *Escherichia coli* in that it combines mutagen and radiation sensitivity with a block in recombination and induced mutagenesis. The pleiotropic repair phenotype caused by the singly existing *pso4-1* mutant led to allocation of *PSO4* to more than one epistasis group (8,14).

More recent studies showed that heterologous expression of *recA* increased resistance to UV and ionizing radiation in *S.cerevisiae* wild-type but not in recombination-deficient *rad52-1* mutant cells (15,16). However, *recA*-like yeast mutant *pso4-1* showed restored induced mutability after transformation with a multicopy vector containing the *E.coli recA* gene (17). Biochemical analysis proved normal incision of 8-MOP + UVA-induced interstrand cross-links (ICLs), but failure of DNA strand rejoining in *pso4-1* (16), which seems to depend on a recombinational step. The apparent role of gene *PSO4* in DNA repair processes attributed to genes belonging to epistasis group II (recombination) and III (error-prone) makes it a very interesting candidate for our future understanding of interconnections between these repair processes in yeast. Progress in elucidating the function of Pso4 protein necessitated the molecular cloning of *PSO4*, which we wish to report in this communication.

MATERIALS AND METHODS

Yeast strains and plasmids

The yeast strains used are listed in Table 1. Single copy vector pRS316 (18) and multicopy vector pRS426 (19), both containing *URA3* as selectable marker, were used for subcloning, complementation tests and for sequencing. The original complementing isolate pMG470 is based on the multicopy plasmid YEp24 (20). Hence, subcloning was performed using multicopy vector pRS426

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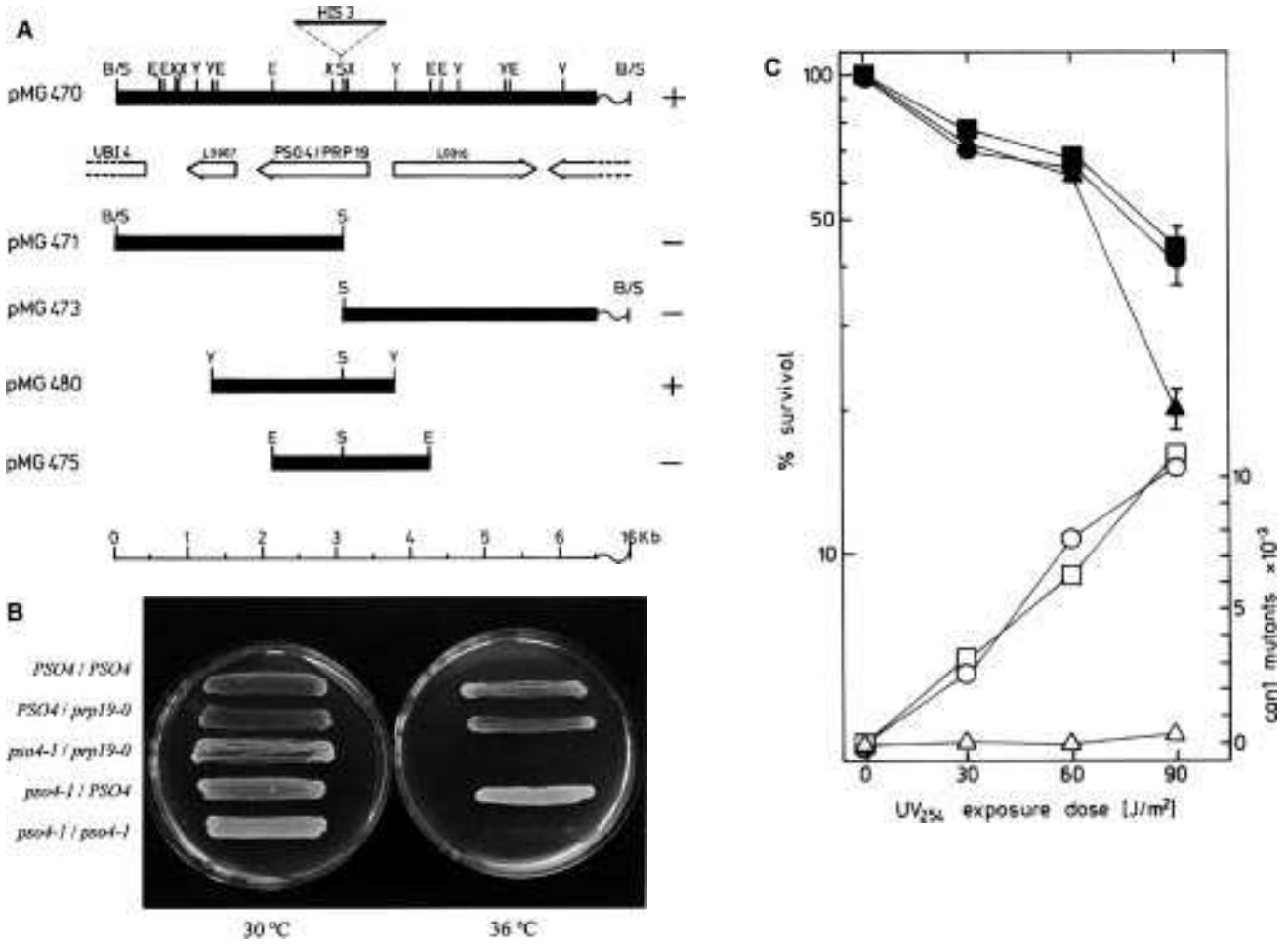

Figure 1. (**A**) Subcloning of the *pso4*-complementing gene from the passenger DNA of pMG470 into multicopy plasmids. The restriction maps of the passenger DNAs of the pMG470-derived plasmids are shown. pMG470: the original clone isolated from the gene bank (20) harboring a 16 kb passenger. Only the 6.5 kb area that overlaps with the second positive clone, pMG490 (not shown), is depicted in detail. pMG471, pMG473 and pMG475: non-complementing subfragments of pMG470. pMG480: fragment containing just one gene, *PRP19*, that is still able to complement all phenotypes of a *pso4-1* mutant. Abbreviations for cleavage sites: B/S, ligation site of *Bam*HI and *Sau*3A; E, *Eco*RI; L, *Sal*I; X, *Xba*I; Y, *Bst*YI. +/–: Plasmid complements/does not complement a *pso4-1/pso4-1* and a *pso4-1/prp19-0* mutant. (**B**) Non-complementation of the *pso4-1* and *prp19-0* alleles. Diploids containing different allele combinations were constructed and tested for survival at 30 and 36°C by streaking cell suspensions on twoYEPD plates which were subsequently incubated at 36 and 30°C respectively. (**C**) Survival and mutagenesis after irradiation with UV_{254} light of heteroallelic *pso4-1/prp19-0* diploid MG5101 transformed with either empty plasmid pRS426 (triangles) or pMG480 containing just *PRP19* (squares) and the heterozygous *pso4-1/PRP19* diploid MG5103 transformed with empty plasmid (circles) as positive control.

to also allow for screening of a possible multicopy suppressor of *PSO4* (Fig. 1A).

Cloning of *PSO4*

Gene *PSO4* was cloned by transforming diploid strain MG5128 homozygous for *pso4-1* with a yeast genomic library (20) according to the lithium acetate-based protocol described by Gietz *et al.* (21). Due to the previously introduced heterzygous *CAN1/can1* marker it was possible to select for forward mutants homozygous for *can1* after replica-plating onto synthetic medium lacking both arginine and uracil and supplemented with 20 mg/l canavanine (SynCo–Arg–Ura+Can). After irradiation with UV_{254} light (20 J/m^2) the plates were incubated at 30°C in the dark for 3–5 days. Clones with more than three papillae on SynCo –Arg–Ura+Can were isolated from the corresponding master plate and tested for the ability to sporulate.

Table 1. *Saccharomyces cerevisiae* strains used in this study

Strain	Genotype	Source
xs9	MAT**a** *lys2 pso4-1*	A.C.Schenberg
W303	MAT**a**/MATα *ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 PSO/PSO*	R.Rothstein
MG5100	MAT**a**/MATα *ura3-1/ura3-1 ade2-1/ade2-1 trp1-1/trp1-1 leu2-3,112/leu2-3,112 his3-11,15/his3-11,15 can1-100/can1-100 PSO/pso4::HIS3*	This study
MG5101	MAT**a**/MATα *ura3-1/ura3 ade2-1/ADE trp1-1/TRP leu2-3,112/LEU his3-11,15/HIS can1-100/CAN pso4::HIS3/pso4-1*	This study
MG5103	MAT**a**/MATα *ura3-1/ura3 ade2-1/ADE trp1-1/TRP leu2-3,112/LEU his3-11,15/HIS can1-100/CAN PSO4/pso4-1*	This study
MG5128	MAT**a**/MATα *ura3-52/ura3 ade2/ADE can1/CAN pso4-1/pso4-1*	This study

Mutagenicity assay and survival

Exponentially growing cells cultivated in liquid synthetic medium supplemented with the appropriate amino acids and bases were washed and resuspended in phospate buffer (67 mM, pH 7) to a final density of 10^7 cells/ml and irradiated with different UV_{254} doses. For the determination of survival the cells were plated on complete medium (SynCo) and for the determination of forward mutations (*can1*) on SynCo without arginine and plus canavanine (20 mg/l) respectively.

DNA sequencing

DNA sequencing was performed as part of the framework of the European Union BIOTECH program to sequence the entire chromosome XII. A set of random subclones in plasmid cloning vector pUC18 was generated from the insert region of cosmid 1F17. These subclones served as the starting points for a primer walking strategy. Double-stranded template DNA of the cosmid and DNA of the subclones was purified using the QIAwell Purification System (Qiagen). Cycle sequencing reactions were carried out using Taq DNA polymerase and fluorescent terminators. Gel electrophoresis and automated base calling were done on an ABI 373A Stretch DNA Sequencer (Applied Biosystems). Contig assembly and DNA sequence editing were done using the DNASTAR software package (Lasergene).

RESULTS

Cloning of DNA repair gene *PSO4*

Diploids homozygous for mutant allele *pso4-1* show higher than wild-type UV sensitivity, lower than wild-type UV-induced mutation and mitotic recombination and sporulate very poorly (<1% asci) on appropriate medium (8). *PSO4* was molecularly cloned by screening for transformants in diploid MG5128 homozygous for *pso4-1* and heterozygous for *CAN1* (Table 1), in which inducibility of mutagenesis by UV_{254} had been restored (*CAN1*→*can1*^{r}). Amongst 6000 transformants two were found to show wild-type-like UV-induced mutagenesis and furthermore restored sporulation (40% in the transformants and the respective wild-type versus a maximal number of <1% asci in the mutant) and restored resistance to DNA cross-linking mutagens. Restriction analysis of the two complementing plasmids, named pMG470 and pMG490, showed that the both passengers contained an identical 6.5 kb overlapping fragment (depicted in Fig. 1A). Hybridization of a ^{32}P-labeled 2 kb *Eco*RI fragment from this area (Fig. 1A, pMG475) revealed that the passengers were part of chromosome XII (not shown). Two subfragments obtained by restriction of plasmid pMG470 at the singular *Sal*I site yielded the two plasmids pMG471 and pMG473 (Fig. 1A), both unable to restore the *PSO* phenotype (Table 2A), indicating that *Sal*I is located within the *pso4-1* complementing open reading frame (ORF) or its promoter. We determined the DNA sequence in this area and found a match with gene *PRP19* (22), containing the *Sal*I site. Further sequencing revealed that *PSO4*/*PRP19* is located ~1.5 kb upstream of *UBI4* and that it shares a promoter region of ~320 bp with another ORF (L0916, Fig. 1A) upstream of *PRP19* encoding a hitherto uncharacterized protein containing a putative ATP/GTP binding site. Subcloning of a 2.4 kb fragment containing only gene *PRP19* (pMG480, Fig. 1A) revealed that this gene alone is sufficient to restore the wild-type-like phenotype when transformed into a *pso4* mutant (Fig. 1C and Table 2A).

The putative Pso4 protein

We determined the size of the *PSO4/PRP19* gene to be 1512 bp, which corresponds to a 56.7 kDa protein consisting of 503 amino acids, instead of 502, as originally described for *PRP19* by Cheng *et al*. (22), owing to one additional proline at position 239. Sequence analysis of the putative protein revealed one myb-like DNA binding domain (W[ST]X_2E[DE]X_2[LIV]) at the C-terminus of the Pso4/Prp19 protein (positions 457–465, WTKDEESAL). The retroviral oncogene v-*myb* and its cellular counterpart c-*myb* encode nuclear DNA binding proteins. In *S.cerevisiae*, the *myb*-related genes include the DNA-binding proteins *REB1* (23) and *BAS1* (24). However, the Pso4/Prp19 protein does not share any considerable homology with the putative proteins encoded by these two other yeast genes. The nucleotide sequence of *PSO4* will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession no. X99770.

Construction and phenotype of a *pso4* null allele

After *in vitro* disruption of the Pso4/Prp19-encoding ORF at the *Sal*I site by inserting *HIS3* (cf. Fig. 1), we performed one-step disruption experiments in haploid and diploid wild-type cells. While no haploid transformants with correct insertion could be found, diploid disruptants (MG5100, Table 2) showed only two surviving His auxotrophic ascospores upon sporulation and tetrad analysis. The absence of any His prototrophic spores confirmed the previously reported essentiality of the *PSO4/PRP19* gene (22). However, it has never been demonstrated whether this essentiality is restricted to spore germination. Therefore, the heterozygous diploid MG5100 was transformed with pMG480 (*URA3*; *PSO4*), resulting in four viable spores after sporulation. Plasmid loss experiments with spores containing the *HIS3* disruption marker did not yield any viable Ura auxotrophs, while haploid His auxotrophic non-disruptants showed plasmid loss of ~20% within 36 h. This indicates that the essentiality of *PSO4* is not restricted to spore germination, since loss of the plasmid results in lethality of haploid disruptants. As essentiality could be verified with another construct using *URA3* as reporter gene (not shown), the disrupted alleles will be generally named *pso4-0* for the purpose of simplification during discussion.

PSO4 is allelic to *PRP19*

Besides the fact that gene *PRP19* alone was sufficient to complement the *pso4-1* mutation, final evidence for the allelism of the cloned ORF with *PSO4* was obtained by showing that null allele *prp19*::*HIS3* cannot complement any phenotype of the *pso4-1* mutant in a heteroallelic diploid which was constructed by crossing the original *pso4-1* mutant xs9 with the haploid *prp19*::*HIS3* disruptant MG5100-1C of opposite mating-type, the latter containing *PSO4*-harboring plasmid pMG480 to ensure viability of the haploid disruptant. After plasmid loss, the resulting Ura auxotrophic diploid MG5101, heteroallelic for *pso4-1/prp19*::*HIS3*, was viable and showed the pleiotropic phenotype typical of a homozygous *pso4-1* diploid, including a strong reduction in sporulation and in induced mutagenesis together with sensitivity to several DNA cross-linking mutagens (Table 2 and Fig. 1C). Since the *prp19-0* allele is unable to complement a *pso4-1* mutant phenotype, we have final proof that both genes are allelic. Thus the terms *pso4*::*HIS3* (*pso4-0*) and *prp19*::*HIS3* (*prp19-0*) can be considered as synonymous.

Table 2. (**A**) Complementation analysis of different subclones of pMG470 (see also Fig. 1A); (**B**) complementation analysis of the same set of plasmids transformed into the heteroallelic diploid MG5101; (**C**) non-complementation of the *prp19* and *pso4* mutant alleles (MG5101) as evidence for their allelism (see also Fig. 1B and C)

Strain	Relevant genotype	Plasmid	Sporulation	HN2	30°C	36°C	Mutability
(**A**)							
MG5128	*pso4-1/pso4-1*	pRS426	<1%	S	+	–	–
		pMG470	45%	R	+	+	+
		pMG471	<1%	S	+	–	–
		pMG473	<1%	S	+	–	–
		pMG480	35%	R	+	+	+
(**B**)							
MG5101	*prp19-0/pso4-1*	pRS426	0.1%	S	+	–	–
		pMG470	40%	R	+	+	+
		pMG471	0.1%	S	+	–	–
		pMG473	0.1%	S	+	–	–
		pMG480	35%	R	+	+	+
(**C**)							
W303	*PSO/PSO*	None	35%	R	+	+	+
MG5100	*prp19-0/PSO*	None	33%	R	+	+	+
MG5128	*pso4-1/pso4-1*	None	<1%	S	+	–	–
MG5103	*pso4-1/PSO*	None	32%	R	+	+	+
MG5101	*prp19-0/pso4-1*	None	0.1%	S	+	–	–

R, wild-type-like resistance; S, *pso4* mutant-like sensitivity to HN2.
+/– corresponds to viability/non-viability at 30 or 36°C and mutability/reduced mutability respectively.
Only the plasmids pMG470 and pMG480 can complement the *pso4-1* mutation.
Sporulation efficiency, sensitivity the DNA cross-linking mutagen nitrogen mustard (HN2), and mutability was determined at 30°C.

pso4-1 is a non-lethal allele of the essential gene *PSO4/PRP19*

Although a disruption of *PSO4/PRP19* is lethal, mutants containing the *pso4-1* allele are viable but show the above-mentioned phenotype. Often, viable mutants of essential genes are isolated as sensitve to temperatures >30°C. We therefore tested our diploids harboring different alleles of *PSO4* for possible temperature sensitivity. In fact, both diploids either homoallelic for *pso4-1* or heteroallelic (*pso4-1*/*prp19-0*) were sensitive to 36°C. Again, the *prp19-0* allele failed to complement this sensitivity, thus once more confirming allelism of both genes (Fig. 1B). When comparing the growth yield of our diploid strains at 30°C we found that heteroallelic *pso4-1/pso4-0* diploid MG5101 can grow at 30°C (cf. above) but has a severely retarded growth rate with a generation time >3 h in YEPD medium, while the isogenic *PSO4/pso4-0* diploid MG5103 showed a generation time of ~1.5 h, comparable with other wild-types (not shown).

DISCUSSION

Repair of DNA double-strand breaks in *S.cerevisiae* involves genes of the *RAD52* epistasis group. The corresponding proteins are supposed to act via a recombinational mechanism and some of them have been shown to constitute a protein complex which has been termed a recombinosome (25,26). A *pso4-1* mutant has previously been shown to exhibit a pleiotropic phenotype, i.e. sensitivity to many DNA damaging agents, nearly blocked induced mutagenesis, lowered spontaneous and induced recombination and nearly totally blocked pre-meiotic DNA synthesis and sporulation (8,12,27). We have now isolated gene *PSO4*, which is a member of the *RAD52* epistasis group by complementation of the *pso4-1* mutant's drastically reduced induced mutability and blocked sporulation. Characterization of DNA repair gene *PSO4* showed its allelism to the yeast gene *PRP19*, which encodes a 502 amino acid spliceosome-associated protein (22,28). In contrast, our *PSO4* sequence contains one additional proline-coding CCC triplet and thus encodes a 503 amino acid protein. However, this discrepancy may be due to a strain-specific difference and is presently most likely irrelevant for discussion of the putative protein.

The allelism of *PSO4* and *PRP19* implicates that this yeast gene encodes a protein that has functions in RNA splicing and in error-prone DNA repair, recombination and sporulation in this organism. It is thus the first DNA repair gene with a function linked to the processing of RNA and its essentiality underscores the vital function in the latter process. Next to *RAD3* and *SSL2/RAD25*, *PSO4/PRP19* is the third DNA repair gene that is essential for growth and viability of *S.cerevisiae*.

The Prp19 protein has been demonstrated to be essential for splicing of pre-mRNA (28). However, biochemical characterization revealed that it is not tightly associated with snRNAs, but is associated with the spliceosome during the splicing reaction (22). Interestingly, Cheng *et al*. discussed the fact that Prp19 is distinct from other Prp proteins or other spliceosomal components regarding the protein sequence and that it does not contain any of

the four motifs found in other Prp sequences. It could be further demonstrated that the Prp19 protein is associated with a protein complex different from the spliceosome, consisting of at least seven proteins in addition to Prp19, itself most likely present in an oligomeric form (29). However, the Prp19-associated complex, itself about as big as the spliceosome, is unlikely to bind to the latter complex (29). The authors suggest that the Prp protein may be released to become associated with the splicing complex. This event was found to be concomitant with or just after dissociation of the U4 snRNA and an ATP-dependent conformational change before formation of a functional spliceosome, suggesting that Prp19 may function in this step of spliceosome assembly (30).

What could be, on the other hand, the function of the *PRP19/PSO4*-encoded protein in recombinational and error-prone repair? All evidence gathered from genetic and biochemical experiments using allele *pso4-1* point to a late function in repair of DNA lesions missing in the mutant strain. The excision of 8-MOP + UVA-induced ICLs from DNA is thought to proceed normally, with production of DNA double-strand breaks as repair intermediates which, however, are not rejoined in *pso4-1* (14). It has been suggested that repair of DNA lesions of the ICL type would require two modes of repair (31,32), the first being removal of the damage by the repairosome postulated for nucleotide excision repair (33) and the second another enzyme complex for repair of strand breaks, in which the *PSO4/PRP19* encoded protein would have a function. The existence in *S.cerevisiae* of such a DNA double-strand repair-specific recombinosome, a complex containing at least proteins encoded by *RAD51*, *RAD52*, *RAD55* and *RAD57*, has recently been suggested (26). We suggest that the Prp19/Pso4-associated protein complex found by Tarn and co-workers (29) might be the above-mentioned recombinosome.

The fact that both existing viable alleles of the essential gene *PSO4*/*PRP19*, as represented by the previously described *prp19* mutant (22,34) and by the *pso4-1* mutant respectively, exhibit a temperature sensitivity implies that the 503 amino acid protein encoded by *PSO4/PRP19* might have two or more functional domains, of which one or more would be still active in the protein expressed by haploid and diploid *pso4-1* mutants: since there is no obvious growth retardation at 30°C in *pso4-1* haploid and homoallelic diploid mutants we must assume that RNA splicing proceeds with an efficiency close or identical to that of the wild-type. This hypothesis is underscored by the fact that the viable *prp19* mutant accumulates unprocessed pre-mRNA at the non-permissive but not at the permissive temperature (34), suggesting that viability of a *pso4*/*prp19* mutant is dependent on functional splicing. Survival of a *pso4-1* mutant but not of *pso4-0* therefore suggests that the former mutant allele still expresses a partially functional protein at 30°C; while its role in spliceosomal assembly and function would be about normal its function in repair of DNA single- and double-strand breaks via recombinational processes would be significantly perturbed. We suggest that these phenotypes could be due to disturbed binding of the other proteins to Pso4 in the multienzyme complex found by Tarn *et al*. (29), indicating that at least one of these hitherto unidentified proteins may belong to the *RAD52* epistasis group.

An alternative, but in our opinion highly unlikely, explanation for the repair phenotype of the *pso4-1* mutant would be the incorrect processing of transcripts of DNA repair genes. Amongst the many repair genes in yeast only one, namely *RAD14*, which is involved in incision of damaged DNA, is intron-containing (35) and hence would require splicing for proper function. Since *pso4-1* mutants are able to incise DNA (14), the only known splicing-dependent repair gene is functional, once again pointing to normal splicing activity in *pso4-1* mutants at 30°C. Also, if non-functional splicing always resulted in repair deficiency phenotypes, at least some of the ~50 DNA repair genes cloned ought to encode proteins needed for splicing, i.e. they should have turned out to be allelic to one of the many other *PRP* genes.

The severe handicap in growth in complete medium at 30°C of heteroallelic *pso4-1/pso4-0* but not of heterozygous *pso4-1/PSO4* diploids indicates that the quantity of protein produced from one *pso4-1* mutant allele is not sufficient for normal spliceosome activity, whereas the protein encoded in one *PSO4* wild-type allele of a normal growing *PSO4/pso4-0* diploid apparently satisfies cellular demand for the spliceosome-associated protein function. In this context the partial complementation of the yeast *pso4-1* mutant's sensitivity to photoactivated 8-MOP + UVA by overexpression of RecA protein (17) has new significance. RecA protein can bind to single-and double-stranded DNA, thereby facilitating initiation of recombination (36). Interstrand cross-link repair in *E.coli* as well as in yeast has been shown to proceed via double-strand breaks (cf. 2,31); these secondary lesions would be a substrate for RecA or RecA-like proteins which, after helical filament formation, would initiate recombinational repair. The RecA and Rad51 proteins share considerable sequence homology and there is strong evidence that Rad51 binds to the Rad52 protein (26). The sensitivity of the *pso4-1* mutant to 8-MOP + UVA, its partial complementation by overexpressed RecA protein and the epistatic interactions of the *pso4-1* mutant with *rad51* and *rad52* mutant alleles (17) has lead to the hypothesis that it functions in the repair of primary or secondary induced DNA strand breaks via a recombinational mechanism. The association with a functional protein complex, perhaps in forming a recombinosome (26), could be one function of the Pso4/Prp19 protein; the other, more essential to the yeast cell, would be its role as a spliceosome-associated protein in pre-mRNA processing. Recently, a similar two-way association has been described for yeast transcription factor TFIIH: it participates in formation of a holocomplex active in RNA polymerase II transcription initiation and can also be found in the repairosome, which is specific for nucleotide excision repair (37,38).

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REFERENCES

1 Haynes,R.H. and Kunz,B. (1981) In: Strathern,J.N., Jones,E.W. and Broach,J.R (eds), *The Molecular Biology of the Yeast Saccharomyces cerevisiae*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 341–414.
2 Friedberg,E.C., Walker,G.C. and Siede,W. (1995) *DNA Repair and Mutagenesis.* ASM Press, Washington, DC.
3 Brendel,M., Khan,N.A. and Haynes,R.H. (1970) *Mol. Gen. Genet.*, **106**, 289–295.

4 Game,J.C. and Cox,B.S. (1971) *Mutat. Res.*, **12**, 328–331.
5 Game,J.C. and Cox,B.S. (1972) *Mutat. Res.*, **16**, 353–362.
6 Brendel,M. and Haynes,R.H. (1973) *Mol. Gen. Genet.*, **125**, 197–216.
7 Game,J.C. and Cox,B.S. (1973) *Mutat. Res.*, **20**, 35–44.
8 Henriques,J.A.P. and Brendel,M. (1990) *Curr. Genet.*, **18**, 387–393.
9 Henriques,J.A.P. and Moustacchi,E. (1980) *Genetics*, **95**, 273–288.
10 Henriques,J.A.P., Vicente,E.J., daSilva,K.V.C.L. and Schenberg,A.C.G. (1989) *Mutat. Res.*, **218**, 111–124.
11 Andrade,H.H.R., Marques,E.K., Schenberg,A.C.G. and Henriques,J.A.P. (1989) *Mol. Gen. Genet.*, **217**, 419–426.
12 Meira,L.B., Fonseca,M.B., Averbeck,D., Schenberg,A.C.G. and Henriques,J.A.P. (1992) *Mol. Gen. Genet.*, **235**, 311–316.
13 Benathen,I.A. and Beam,C.A. (1977) *Radiat. Res.*, **69**, 99–116.
14 Morais,M.A.,Jr, Vicente,E.J., Brozmanová,J., Schenberg,A.C.G. and Henriques,J.A.P. (1996) *Curr. Genet.*, **29**, 211–218.
15 Brozmanová,J., Cernáková,L., Vlcková,V., DurajJ. and Fridrichová,I. (1991) *Mol. Gen. Genet.*, **227**, 473–480.
16 Cernaková,L., Fridrichová,I., Pirsel,M., Kleibl,K., Duraj,J. and Brozmanová,J. (1991) *Biochimie*, **73**, 285–288.
17 Morais,M.A.,Jr, Brozmanová,J., Benfato,M., Duraj,J., Vlcková,V. and Henriques,J.A.P. (1994) *Mutat. Res.*, **314**, 209–220.
18 Sikorski,R.S. and Hieter,P. (1989) *Genetics*, **122**, 18–27.
19 Christianson,T.W., Sikorski,R.S., Dante,M., Shero,J.H. and Hieter,P. (1992) *Gene*, **110**, 119–122.
20 Carlson,M. and Botstein,D. (1982) *Cell*, **28**, 145–154.
21 Gietz,D., St Jean,A., Woods,R.A. and Schiestl,R.H. (1992) *Nucleic Acids Res.*, **20**, 1425.
22 Cheng,S.-C., Tarn,W.-Y., Tsao,T.Y. and Abelson,J. (1993) *Mol. Cell. Biol.*, **13**, 1876–1882.
23 Ju,Q., Morrow,B.E. and Warner,J.R. (1990) *Mol. Cell. Biol.*, **10**, 5226–5234.
24 Tice-Baldwin,K., Fink,G.R. and Arndt,K.T. (1989) *Science*, **246**, 931–935.
25 Firmenich,A.A., Elias-Arnanz,M. and Berg,P. (1995) *Mol. Cell. Biol.*, **15**, 1620–1631.
26 Hays,S.L., Firmenich,A.A. and Berg,P. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 6925–6929.
27 daSilva,K.V.C.L, Morais,M.A.,Jr, and Henriques,J.A.P. (1995) *Curr. Genet.*, **27**, 207–212.
28 Tarn,W.-Y., Lee,K.R. and Cheng,S.-C. (1993) *Mol. Cell Biol.*, **13**, 1883–1891.
29 Tarn,W.Y., Hsu, C.H., Huang,K.T., Chen,H.R., Kao,H.Y., Lee,K.R. and Cheng,S.C. (1994) *EMBO J.*, **13**, 2421–2431.
30 Tarn,W.Y., Lee,K.R. and Cheng,S.C. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 10821–10825.
31 Jachymczyk,W.J., von Borstel,R.C., Mowat,M.R.C. and Hastings,P.J. (1981) *Mol. Gen. Genet.*, **182**, 196–205.
32 Magaña-Schwencke,N., Henriques,J.A.P.,Chanet,P. and Mousacchi,E. (1982) *Proc. Natl. Acad. Sci. USA*, **79**,1722–1726.
33 Bardwell,A.J., Bardwell,L., Iyer,N., Svefstrup,J.Q., Feaver,R.D., Kornberg,R.D. and Friedberg,E.C. (1994) *Mol. Cell. Biol.*, **14**, 3569–3576.
34 Vijayraghavan,U., Company,M. and Abelson,J. (1989) *Genes Dev.*, **3**, 1206–1216.
35 Bankmann,M., Prakash,L. and Prakash,S. (1992) *Nature*, **355**, 555–558.
36 Radding,C.M. (1991) *J. Biol. Chem.*, **266**, 5355–5358.
37 Schaeffer,L., Roy,R., Humbert,S., Moncollin,V., Vermeulen,W., Hoeijmakers,P., Chambon,P. and Egly,J.M. (1993) *Science*, **260**, 58–63.
38 Wang,Z., Buratowski,S., Svejstrup,J.Q., Feaver, W.J., Wu,X., Kornberg,R.D., Donahne,T.F. and Friedberg,E.C. (1995) *Mol. Cell. Biol.*, **15**, 2288–2293.