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# Identification of four genes involved in suppression of the pre-mRNA splicing defect in the *sng1-1/rhp6*$^{-}$ mutant of fission yeast

ALPANA NARESH and JAGMOHAN SINGH*

*Institute of Microbial Technology, Sector 39A, Chandigarh 160 036, India*

## Abstract

Apart from the global regulators of silencing in the fission yeast *Schizosaccharomyces pombe*, namely *swi6, clr1, clr2, clr3, clr4* and *rik1*, the DNA repair gene *rhp6* plays a unique role in mating-type silencing. Recently, we showed that *sng1-1*, a mutation in the 5′ splice junction of the second intron of the *rhp6* gene, leads to derepression of both the silent loci *mat2* and *mat3* in switching background. To address the mechanism of *rhp6* in silencing, we have isolated several extragenic suppressors of the *sng1-1/rhp6*$^{-}$ mutation. These suppressors fall into four complementation groups and are referred to as suppressor of *rhp6*: *sur1, sur2, sur3* and *sur4*. Interestingly, reverse transcriptase polymerase chain reaction analysis of the *rhp6* transcript shows that in contrast to about > 50% level of unspliced *rhp6* pre-mRNA in the *sng1-1/rhp6*$^{-}$ mutant, there is a restoration of normal splicing to varying degrees in the suppressors. The *sur2* gene belongs to the AAA-ATPase family of proteins, with maximum homology to the SIN1-associated protein SAP1 of *Saccharomyces cerevisiae*. We propose that *sur2*, along with *sur1, sur3* and *sur4*, may play an as yet uncharacterized role in pre-mRNA splicing.

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## Introduction

Position effect control of expression of genes is best exemplified and understood in case of silencing of mating type, centromere and telomere loci in the budding and fission yeasts (Gottschling *et al*. 1990; Allshire *et al*. 1995; Loo and Rine 1995; Nimmo *et al*. 1998; Klar *et al*. 1998). Mating-type silencing in the fission yeast *Schizosaccharomyces pombe* is governed by the action of *cis*-acting silencer elements (Ekwall *et al*. 1991) and *trans*-acting factors like chromodomain protein swi6 (Lorentz *et al*. 1994), zinc finger protein clr1 (Thon and Klar 1992), clr2, clr3 and clr4 (Ekwall and Ruusala 1994), and clr6 (Grewal *et al*. 1998). Among these, swi6 contains the chromodomain and chromo-shadow domain motifs (Lorentz *et al*. 1994), which are widely conserved in evolution among the heterochromatin-associated proteins in *Drosophila*, mice and humans (Paro 1990; Singh 1994). Similarly, clr4 contains the widely conserved SET and chromodomain (Ivanova *et al*. 1998), while the products of *clr3* and *clr6* are histone deacetylases (Grewal *et al*. 1998). Mutations in these genes not only derepress silenced *mat2* and *mat3* alleles but also bring about derepression of marker genes like *ura4* artificially inserted in their vicinity. In addition to causing derepression of silent loci, mutations in *swi6, clr1, clr2, clr3, clr4* and *rik1* also abrogate the cold spot of recombination between *mat2* and *mat3* (Thon and Klar 1992; Thon *et al*. 1994). Thus, these genes play a global, structural role in establishing heterochromatin structure, without directly influencing specific alleles which, in turn, affect both gene expression and recombination.

We recently showed that, in addition to these *trans*-acting factors, *sng1-1*, a mutation in the DNA repair gene *rhp6* (*RAD6* homologue in *S. pombe*), affects silencing of *mat2* and *mat3* loci in a unique manner: the

*For correspondence. E-mail: jag@imtech.res.in.

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derepression of the silent donor loci is observed only if they are in an efficiently switching mode (Singh *et al*. 1998). Since rhp6/rad6 carry out their functions by ubiquitination of proteins and channelling them to the proteosome (Jentsch *et al*. 1987; Varshavsky 1996), we proposed a role of rhp6 in chromatin remodelling and hypothesized the existence of a target/mediator, which helps to reestablish the chromatin structure of the switching donor loci after replication (Singh *et al*. 1998).

We undertook the genetic approach of isolating the extragenic suppressors to identify such a mediator/s. Four classes of extragenic suppressors were identified and analysed for suppression of various phenotypes of the *sng1-1/rhp6*$^-$ mutant. Interestingly, though surprisingly, the suppressors restore the splicing defect of the *sng1-1/rhp6*$^-$ mutant to nearly the wild-type level. These results indicate that the four genes may encode components of the pre-mRNA splicing machinery in *S. pombe.*

## Material and methods

***Yeast media*:** The composition of the media used in this study have been described (Moreno *et al*. 1991). To check temperature sensitivity the strains were streaked on YEA plates and grown at 30ºC and 36ºC for 3–4 days. Serial dilution assay to check UV sensitivity was carried out by spotting 10-fold serial dilutions of overnight cultures on YEA plates, exposing them to known doses of UV, and growing them at 30ºC for 3–4 days. To check level of switching, the strains were grown for single colonies on PMA$^+$ plates and either stained with iodine for 2–3 min or examined microscopically to count the number of zygotic asci or asci with haploid meiosis ('hm'). Normal homothallic strains, which switch efficiently, are referred to as $h^{90}$. The cells of opposite mating type produced efficiently in such strains mate and form zygotes which sporulate to form ascospores. The spores have a starchy compound in their cell wall, which gives dark staining with iodine. Thus the level of staining gives a measure of the level of switching (Moreno *et al*. 1991).

***Complementation analysis*:** Since the suppressors exhibit the dark-staining (spo$^+$) phenotype with iodine due to suppression of the low-staining (spo$^-$) phenotype of the *sng1-1/rhp6*$^-$ mutant, complementation analysis was done by mating one suppressor strain with all the others and doing random spore analysis, followed by screening for spo$^-$ phenotype indicating reappearance of *rhp6*$^-$ recombinants. In case recombinants appeared the mutations involved belonged to separate complementation groups. However, if no recombinants appeared, as indicated by absence of any spo$^-$ segregants, the mutations involved were considered to belong to the same complementation group.

**Table 1.** List of strains used in this study.

| Strain name | Genotype |
|---|---|
| SP837 | $h^{90}$ *leu1-32 ura4D18 ade6-216* |
| SPJ107 | $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$ |
| SPJ132 | $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$ *sur1*$^-$ |
| SPJ133 | $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$ *sur2*$^-$ |
| SPJ320 | $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$ *sur3*$^-$ |
| SPJ176 | $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$ *sur4*$^-$ |

***Mutagenesis*:** The mutant strain *sng1-1/rhp6*$^-$ (SPJ107, table 1) was mutagenized with EMS as described (Moreno *et al*. 1991). Nearly 40,000 colonies were screened for ts$^+$ (temperature-sensitive) phenotype and rechecked for dark iodine staining (Moreno *et al*. 1991). For obtaining the genes complementing the suppressor mutation the double-mutant strain was transformed with the partial *Hin*dIII genomic library of *S. pombe* (Wright *et al*. 1986) and transformants were screened for the ts$^-$ and spo$^-$ phenotypes (phenotype of the *sng1-1/rhp6*$^-$ mutation).

***Isolation of genomic DNA*:** Genomic DNA was isolated from the transformants according to the protocol described earlier (Singh *et al*. 1998). The DNA isolated was introduced into *E. coli* to recover the complementing plasmid DNA.

***RNA isolation and RTPCR analysis*:** RNA was isolated by the method of Schmitt *et al*. (1990). The procedure of reverse transcriptase polymerase chain reaction (RTPCR) to detect the *rhp6* mRNA was carried out according to Singh *et al*. (1998). The oligos used to amplify the mRNA for *rhp6* were 5'AATTCTGCAGTGATATC-TTTTTTTTTTTTTTTT3' and 5′CCAAGGCGATA-TCGATATTTG3′. The PCR products were resolved by agarose gel electrophoresis and subjected to Southern blotting and hybridization (Sambrook *et al*. 1989) with PCR-amplified *rhp6* cDNA which was radiolabelled by the random primer method (Feinberg and Vogelstein 1983).

## Results

### *Identification of four complementation groups of extragenic suppressors of the sng1-1/rhp6*$^-$ *mutation*

A total of 19 extragenic suppressors of the *sng1-1/rhp6*$^-$ mutation were isolated by EMS mutagenesis of the strain SPJ107 (genotype $h^{90}$ *leu1-32 ura4D18 ade6-216 sng1-1/rhp6*$^-$). Since the *sng1-1/rhp6*$^-$ mutant exhibits ts$^-$ and spo$^-$ phenotypes (Singh *et al*. 1998), we selected the EMS-treated cells of strain SPJ107 for growth at 36ºC and, subsequently, screened them for spo$^+$ phenotype. All the suppressors exhibited growth at 36ºC and spo$^+$ pheno-

type. Complementation analysis revealed that the suppressors belong to four complementation groups and accordingly they were denoted as suppressor of *rhp6*$^-$: *sur1, sur2, sur3* and *sur4* (table 2). Figure 1a shows the ts$^+$ phenotype of one of the suppressors compared to the ts$^-$ phenotype of the *sng1-1/rhp6*$^-$ mutant, while figure 1b shows the spo$^+$ phenotype of all the suppressors.

Upon microscopic examination all the suppressor strains (*sur1*, *sur2*, *sur3* and *sur4*) show a higher percentage of zygotic asci reflecting a higher rate of switching compared to the *sng1-1/rhp6*$^-$ mutant (table 3). However, *sur2* and *sur4* still show a residual level of 'hm' asci (6.9 and 4.4%, respectively; hm refers to haploid meiosis phenotype resulting from simultaneous expression of Plus and Minus alleles which triggers meiosis even in a haploid cell; Moreno *et al*. 1991), which was similar to that of the *sng1-1/rhp6*$^-$ mutant (6.7%; table 3). Thus, while *sur1* and *sur3* mutations appear to largely suppress the switching and silencing defect, *sur2* and *sur4* only suppress the switching and/or sporulation defect of the *sng1-1/rhp6*$^-$ mutant. Therefore, our analysis of suppression of the mutant phenotypes of sporulation suggests that two different pathways are affected by the suppressor mutations, one involving *sur1* and *sur3* and the other involving *sur2* and *sur4*. However, no distinct phenotype was exhibited by the suppressor mutants when segregated away from the *sng1-1/rhp6*$^-$ mutation, indicating that these mutations have no distinct defect of their own (data not shown). Because of a lack of phenotype of single mutants *sur1, sur2, sur3* and *sur4*, we could not check the phenotype of double mutants.

### *Differential levels of suppression of the UV sensitivity of the sng1-1/rhp6*$^-$ *mutant by sur mutations*

It has been shown earlier that *rhp6* and *RAD6* play a role in post-replication DNA repair and mutants in these genes exhibit UV sensitivity because of their inability to repair misincorporation of bases across the UV-induced lesions in the DNA (Reynolds *et al*. 1985, 1990). We also showed earlier that the *sng1-1/rhp6*$^-$ mutant also exhibits sensitivity to UV radiation (Singh *et al*. 1998). We used the serial dilution assay to check whether the suppressors *sur1, sur2, sur3* and *sur4* can suppress the UV sensitivity of the *sng1-1/rhp6*$^-$ mutation. We observed that while *sur1* and *sur3* can largely suppress the UV sensitivity of the *sng1-1/rhp6*$^-$ mutant, *sur2* and *sur4* do not suppress it (figure 2). These results match with our earlier results of relative ability of the different suppressor mutations to overcome the silencing defect of the *sng1-1/rhp6*$^-$ mutant. Thus, while *sur1* and *sur3* can suppress both the silencing defect and UV sensitivity of the *sng1-1* mutant, *sur2* and *sur4* suppress these two defects only partially. However, all the four suppressors do suppress the ts$^-$ and the switching defect of the *sng1-1/rhp6*$^-$ mutant (figure 1).

**Table 2.** The four complementation groups of extragenic suppressors of *sng1-1/rhp6*$^-$ mutation.

| No. of suppressor alleles isolated | | | |
|---|---|---|---|
| *sur1* | *sur2* | *sur3* | *sur4* |
| 1 | 2 | 7 | 9 |

**Table 3.** Level of sporulation in wild type, *sng1-1/rhp6*$^-$ mutant and the suppressor double mutants.

| Strain genotype | Per cent sporulation | |
|---|---|---|
| | Zygotic asci | 'hm' asci |
| $h^{90}$ | 91.3 | 0 |
| $h^{90}$ *sng1-1/rhp6*$^-$ | 0.7 | 6.7 |
| $h^{90}$ *sng1-1/rhp6*$^-$ *sur1*$^-$ | 59.2 | 0 |
| $h^{90}$ *sng1-1/rhp6*$^-$ *sur2*$^-$ | 34.7 | 6.9 |
| $h^{90}$ *sng1-1/rhp6*$^-$ *sur3*$^-$ | 58.2 | 0 |
| $h^{90}$ *sng1-1/rhp6*$^-$ *sur4*$^-$ | 64.9 | 4.4 |

### *sur mutations suppress the splicing defect of rhp6 pre-mRNA in the sng1-1/rhp6*$^-$ *mutant*

Earlier we showed that the *sng1-1/rhp6*$^-$ mutant is defective in the splicing of the second intron because of a single point mutation at the fifth base in the 5′ splice site of the second intron of the *rhp6* gene (figure 3a; Singh *et al*. 1998). Therefore, we checked whether the suppression of the *sng1-1/rhp6*$^-$ mutation by *sur1, sur2, sur3* and *sur4* mutations might be due to restoration of splicing of *rhp6* pre-mRNA. We carried out RTPCR for the *rhp6* mRNA with RNA samples derived from wild type, *sng1-1/rhp6*$^-$ mutant, and the *sng1-1/rhp6*$^-$ *sur* double-mutant strains. As shown earlier, in the wild type we observe a single cDNA band of 0.8 kb corresponding to the fully spliced *rhp6* mRNA (figure 3b), while in *sng1-1/rhp6*$^-$ an additional band of 1 kb corresponding to a species of pre-mRNA containing the second intron of 200 bases is also detected (figure 3b; Singh *et al*. 1998). Results of quantitative PCR showed that the unspliced form, represented by the 1-kb band, constituted 45% of total *rhp6* RNA in the *sng1-1/rhp6*$^-$ mutant (figure 3b). Among the suppressors, only *sur4* restored the splicing defect of the *sng1-1/rhp6*$^-$ completely, while *sur1, sur2* and *sur3* still showed some residual level of unspliced pre-mRNA (12–19%; figure 3b), indicating only a partial restoration of the splicing of *rhp6* mRNA by these mutations. Lack of a significant change in phenotype of the *sur* mutants suggests that they may not cause a splicing defect in genes affecting switching and/or sporulation.

### *sur2 gene encodes a protein with AAA-ATPase motif*

The gene at the *sur2* locus was cloned by transforming the *sng1-1/rhp6*$^-$ double mutant with the partial *Hin*dIII library and screening for the ts$^-$ and spo$^-$ phenotypes. Since *sng1-1/rhp6*$^-$ *sur2*$^-$ double mutant is ts$^+$ and spo$^+$

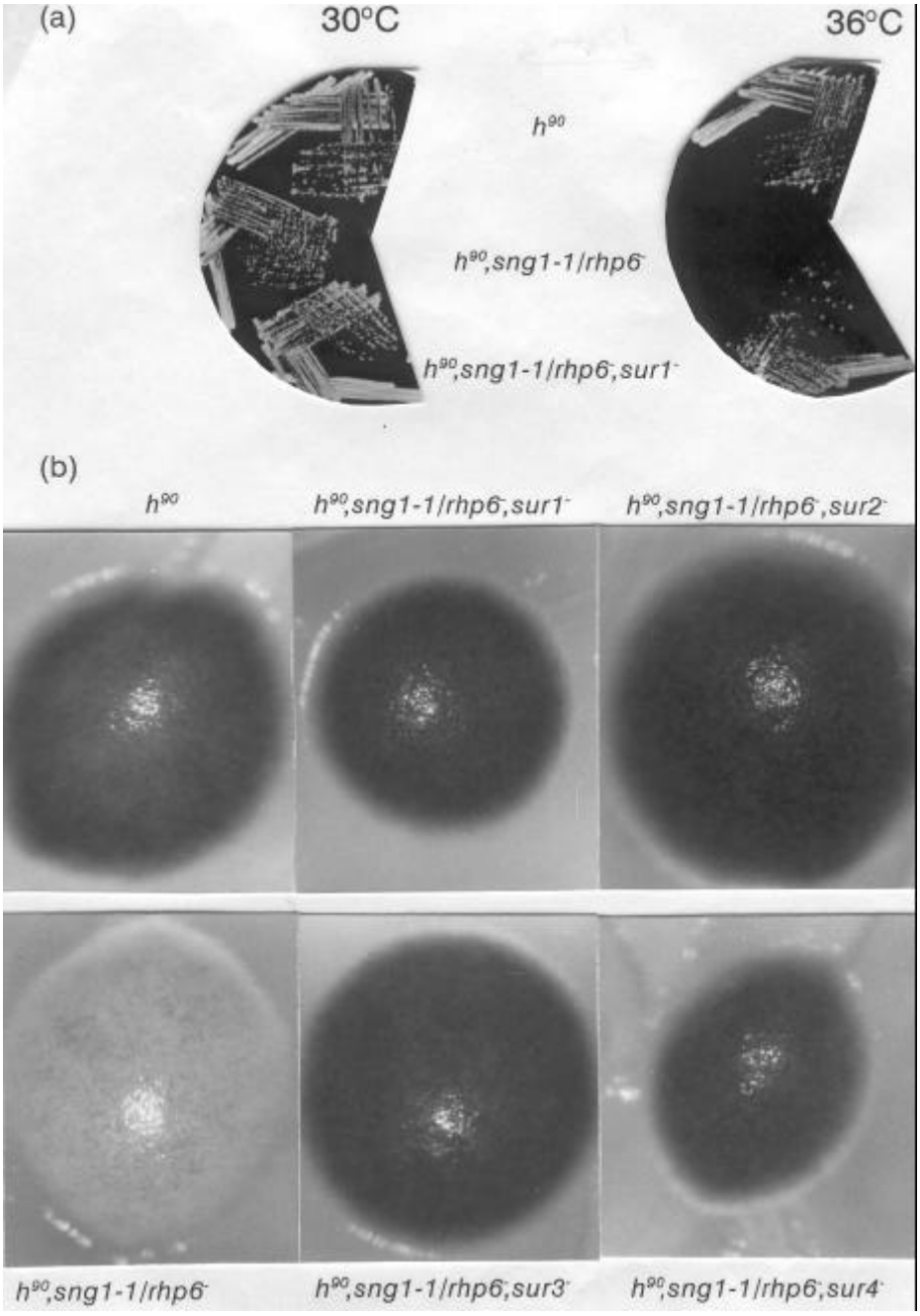


**Figure 1.** Mutations in *sur1–sur4* genes suppress the ts$^-$ and low-iodine-staining (spo$^-$) phenotypes of the *sng1-1/rhp6*$^-$ mutant. (a) Temperature sensitivity was monitored by streaking the wild type, *sng1-1/rhp6*$^-$ mutant and *sng1-1/rhp6*$^-$ *sur1*$^-$ double-mutant strains in the $h^{90}$ background on YEA plates and incubating from at 30ºC and 36ºC for 3–4 days. (b) To monitor the iodine-staining (spo) phenotype cultures were streaked on PMA plates, grown for 4 days at 30ºC, stained with iodine for 2–3 minutes, and photographed.

compared to the ts$^-$ spo$^-$ phenotype of the *sng1-1/rhp6*$^-$ mutant, complementation of the suppressor mutation in the double mutant by the complementing gene would lead to restoration of the ts$^-$ spo$^-$ phenotype of the *sng1-1/rhp6*$^-$ mutant. Therefore, the double mutant *sng1-1/rhp6*$^-$ *sur2*$^-$ was transformed with the genomic library of *S. pombe* and the transformants were screeened for ts$^-$ and spo$^-$ phenotype. The loss of plasmid from the complemented transformants restored the ts$^+$ spo$^+$ phenotype of the original mutant *sng1-1/rhp6*$^-$ *sur2*$^-$ and reintroduction of the plasmid into the *sng1-1/rhp6*$^-$ *sur2*$^-$ double mutant again yielded the ts$^-$ spo$^-$ phenotype. Furthermore, the putative *sur2* gene did not suppress the other *sur* mutations, namely *sur1*, *sur3* and *sur4*,

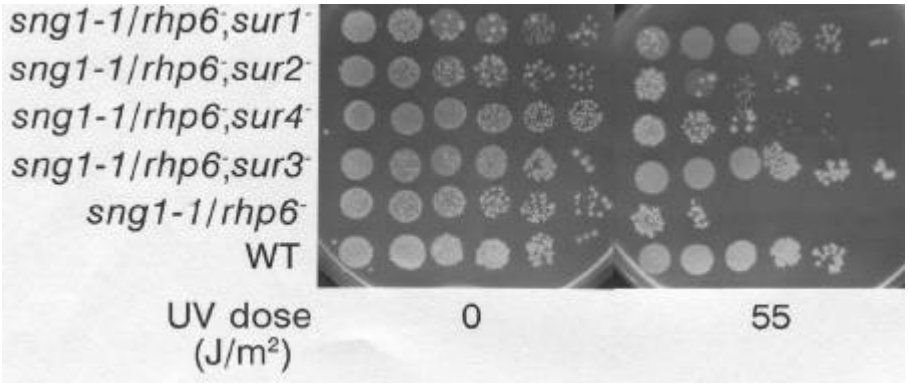


**Figure 2.** Different levels of suppression of the UV sensitivity of the *sng1-1/rhp6*⁻ mutant by *sur1, sur2, sur3* and *sur4* mutations. The strains were grown and 10-fold serial dilutions were spotted on two YEA plates. One of the plates was exposed to the UV dose shown, after which both the plates were incubated at 30ºC for 3 days.

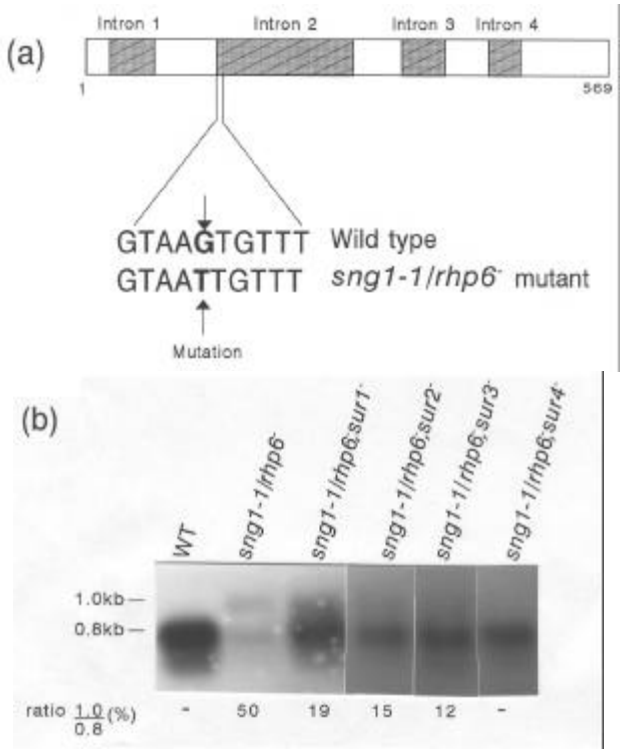


**Figure 3.** Partial suppression of the splicing defect of *rhp6* pre-mRNA in the *sng1-1/rhp6*⁻ mutant by *sur1, sur2, sur3* and *sur4* mutations. (a) The gene map of *rhp6* gene according to Reynolds *et al.* (1990). Also shown is the fifth-base mutation (G to T) in the 5′ splice site of the second intron of *rhp6* gene in *sng1-1/rhp6*⁻ mutant as demonstrated earlier (Singh *et al.* 1998). (b) RTPCR analysis for *rhp6* mRNA in the wild type, *sng1-1/rhp6*⁻ mutant, and *sur* mutants in the *sng1-1/rhp6*⁻ mutant background. RTPCR was carried out as described earlier (Singh *et al.* 1998). The PCR products were resolved by agarose gel electrophoresis and subjected to Southern blotting and hybridization with radiolabelled *rhp6* probe. The ratio of the 1-kb and 0.8-kb bands was estimated by densitometry.

thus confirming the authenticity of the *sur2* clone. The plasmid DNA isolated from the transformants was analysed by restriction analysis and sequencing. A database search with the sequence obtained identified the gene as a putative 26S protease subunit of 660 amino acids and showed that it contains the AAA-ATPase motif, which is found in proteins participating in diverse cellular functions (Patel and Latterich 1998). A BLAST search also revealed that the highest homology of the *sur2* gene product is with the SIN1-associated protein SAP1 from *S. cerevisiae* (Liberzon *et al.* 1996). The *sur2* sequence shows presence of both the Walker motifs A and B and the AAA consensus sequence (figure 4).

## Discussion

In this study we have attempted to isolate the putative mediators of rhp6 in its role in chromatin remodelling at the silent mating-type loci. We used the classical genetic approach of isolating extragenic suppressors that overcome the silencing defect of the *sng1-1/rhp6*⁻ mutant. Because of the fact that the original mutation *sng1-1* is a point mutation at the 5′ splice junction of the second intron of the *rhp6* gene, which reduces the efficiency of splicing of the second intron by nearly 50%, it was likely that we would obtain suppressors that suppressed the splicing defect itself, and we observed this. All the four suppressors overcome the splicing defect to different levels. However, surprisingly, we observed differences in the UV sensitivities and residual silencing defect, as indicated by the persistent haploid meiosis phenotype, especially in *sur2* and *sur4* mutants in the *sng1-1/rhp6*⁻ background; *sur1* and *sur3* restored both the switching/silencing and UV sensitivity to the wild-type level. We infer that *sur2* and *sur4* mutations do not suppress the silencing defect, which results in the haploid meiosis phenotype, but rather suppress only the switching defect of the *sng1-1/rhp6*⁻ mutant. Furthermore, since the single mutants *sur2* and *sur4* do not have a phenotype of their own, these mutations do not directly elicit a silencing defect. Thus, *sur1* and *sur3* may be involved in a pathway affecting UV-induced DNA repair, silencing and

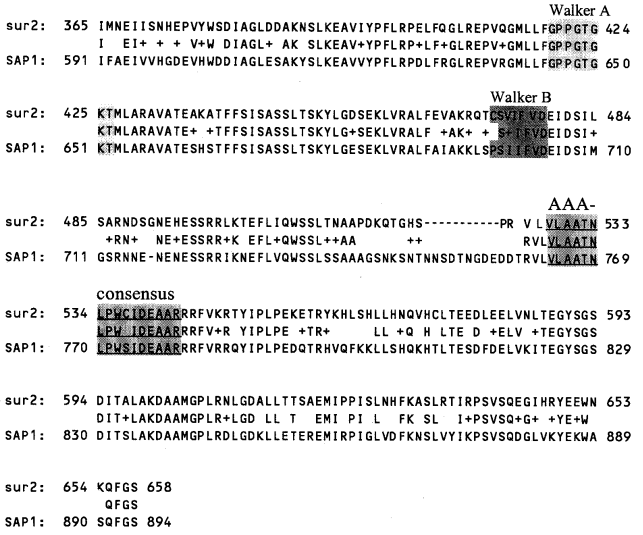


**Figure 4.** Alignment of *S. pombe* sur2 protein sequence with the SIN1-associated protein SAP1 from *S. cerevisiae*. The Walker A, Walker B and the AAA-consensus motifs are highlighted.

sporulation while *sur2* and *sur4* affect only switching and sporulation. In this context, it is surprising that the *sur4* mutant, which suppresses the splicing defect of *sng1-1/rhp6*$^-$ mutant completely, still exhibits UV sensitivity and haploid meiosis. It is possible that the absolute level of *rhp6* mRNA or protein may also be reduced, thus reducing the ubiquitin-mediated function of a certain specific pathway.

The mechanism by which the four *sur* mutations overcome the splicing defect of the *sng1-1/rhp6*$^-$ mutation remains to be addressed. sur2 was identified by BLAST search as a putative 26S protease subunit of *S. pombe* and it showed homology to the AAA motif in several proteins (table 4). This motif is constituted by a 230-amino-acid domain that contains the Walker homology sequences and is associated with $Mg^{2+}$-dependent ATPase activity. AAA proteins are found among both prokaryotes and eukaryotes and perform diverse cellular functions, like cell cycle regulation, protein degradation, organelle biogenesis and vesicle-mediated protein transport (Patel and Latterich 1998). However, the exact biochemical function of the motif is not known. In the BLAST search, the highest homology of sur2 was observed with the SIN1-associated protein SAP1 from *S. cerevisiae* (Liberzon *et al*. 1996). SAP1 was shown earlier to bind to the N-terminal region of SIN1, an HMG1-like protein in *S. cerevisiae* (Liberzon *et al*. 1996). Since SIN1 interacts with the SWI/SNF complex (Laurent *et al*. 1993) SAP1 may play a role in chromatin remodelling. It is not clear how the *sur2* gene functions in splicing since no protein involved in splicing in *S. pombe* and *S. cerevisiae* has been shown to contain the AAA motif so far.

However, these homologies still do not provide a clue to the exact role of *sur* genes in pre-mRNA splicing; this is the first time an AAA protein has been associated with a splicing function. An earlier example showed suppression of a splice site mutation at the fifth base of the 5′ splice junction in the 12S RNA of the E1A gene, which inhibited splicing altogether, by a compensatory base change in the U1 snRNA (Zhuang and Weiner 1986). But we have not isolated any such mutant in our screen.

Compared to work with *S. cerevisiae*, fewer studies have addressed the splicing mechanisms in *S. pombe*. However, comparison of sequences in genes in different species have shown that the splice junction sequences and the frequency and distribution of introns in *S. pombe* are more similar to those in higher eukaryotes than in *S. cerevisiae* (Russell 1989), although introns in *S. pombe* are generally smaller in size (36–129 nucleotides). Furthermore, eukaryotic introns can be spliced in *S. pombe* but not in *S. cerevisiae*, indicating closer similarity of the splicing mechanisms of higher eukaryotes with those in *S. pombe* than with those in *S. cerevisiae* (Kaufer *et al*. 1985) in terms of ability to excise metazoan introns and similarity in the pattern and structure of snRNA components (Russell 1989; Kaufer and Potashkin 2000). Several pre-mRNA processing (*prp*) mutants have been reported in *S. pombe* and some of the proteins involved in splicing have been studied (Urishiyama *et al*. 1996; Kaufer and Potashkin 2000) but the detailed mechanism of splicing still remains to be investigated.

The suppressors we have identified, at the very least, function in pre-mRNA splicing and it would be interesting to check whether they correspond to the known *prp* mutants, although no sequenced *prp* gene has been shown to encode a polypeptide with the AAA motif (Urishiyama *et al*. 1996; Kaufer and Potashkin 2000). Since they overcome the splicing defect caused by a base change in the fifth position of the 5′ splice junction of the second intron (Singh *et al*. 1998), the *sur* mutations may affect the efficiency of the first step of splicing, where the 5′ splice junction sequence is recognized by U1 snRNA, or any subsequent step involved in recognition of this complex and leading to the assembly of the spliceosome. Specifically, because of the presence of the AAA-ATPase motif, the sur2 protein may function as a protein clamp (Confalonieri and Duguet 1998) and utilize the ATPase-driven energy to facilitate the function of the splicing complex. Alternatively, it may affect the proteolysis of a target protein that is involved in splicing. A pertinent example is the report showing impairment of the splicing of transcripts of the mitochondrial genes *COX1* and *COB* in strains of *S. cerevisiae* lacking the m-AAA protease (Arlt *et al*. 1998). Still another possibility is that the AAA motif in sur2 may be associated with RNA helicase activity. For example, in *S. cerevisiae*, a suppressor of the *prp8-1* splicing mutation has been shown to encode a putative ATP-dependent RNA helicase (Jamieson *et al*. 1991). Thus sur2 may function as an RNA helicase, with the mutant sur2 being more

**Table 4.** Proteins showing homology with sur2.

| Protein (Accession number) | Per cent Identity | Per cent Similarity | Function |
|---|---|---|---|
| *S. pombe* AAA family ATPase with similarity to ketanin (AL360054) | 46 | 55 | Putative microtubule severing protein |
| *S. cerevisiae* SAP1 (U18796) | 49 | 70 | Associates with chromatin assembly protein SIN1 (Liberzon *et al*. 1996) |
| *S. cerevisiae* YTA6 (U41849) | 33 | 46 | Putative ATPase (Schnall *et al.* 1994) |
| Mouse SKD1 (U10119) | 29 | 38 | Involved in intracellular transport (Perier *et al*. 1994; Scheuring *et al*. 1999) |
| Human spastin protein (AJ246001) | 36 | 47 | Involved in the assembly or function of nuclear protein complexes (Hazan *et al*. 1999) |

effective in facilitating the splicing of the *sng1-1/rhp6*$^{-}$ mutant pre-mRNA.

Although in this study we have not addressed the mechanism by which rhp6 affects chromatin remodelling and silencing, in our recent work we have also identified two mediators of rhp6 in silencing (A. Naresh and J. Singh, manuscript in preparation). Future studies will address the biochemical mechanism of *sur* genes in pre-mRNA splicing and the mediators of rhp6 in silencing in greater detail.

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