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Cloning and characterisation of the *Schizosaccharomyces pombe rad32* gene: a gene required for repair of double strand breaks and recombination

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ABSTRACT

A new *Schizosaccharomyces pombe* mutant (*rad32*) which is sensitive to gamma and UV irradiation is described. Pulsed field gel electrophoresis of DNA from irradiated cells indicates that the *rad32* mutant, in comparison to wild type cells, has decreased ability to repair DNA double strand breaks. The mutant also undergoes decreased meiotic recombination and displays reduced stability of minichromosomes. The *rad32* gene has been cloned by complementation of the UV sensitive phenotype. The gene, which is not essential for cell viability and is expressed at a moderate level in mitotically dividing cells, has significant homology to the meiotic recombination gene *MRE11* of *Saccharomyces cerevisiae*. Epistasis analysis indicates that *rad32* functions in a pathway which includes the *rhp51* gene (the *S.pombe* homologue to *S.cerevisiae RAD51*) and that cells deleted for the *rad32* gene in conjunction with either the *rad3* deletion (a G2 checkpoint mutation) or the *rad2* deletion (a chromosome stability and potential nucleotide excision repair mutation) are not viable.

INTRODUCTION

DNA repair processes are fundamentally important if cells are to maintain their genetic integrity. Organisms have thus evolved a number of processes by which DNA damage is recognised and repaired, and these include a well characterised nucleotide excision repair pathway and a damage tolerance mechanism involving recombination. Recent studies have begun to yield important data on the mechanisms associated with nucleotide excision repair and the highly conserved nature of the proteins involved (e.g. 1–3). In contrast, somewhat less is known about the molecular mechanisms involved in recombination in eukaryotes.

The fission yeast, *S.pombe*, has proved to be a good model system for the study of eukaryotic processes, such as the cell cycle (e.g. 4) and more recently nucleotide excision repair (e.g. 5,6). Radiation sensitive mutants in *S.pombe* were originally assigned to 23 complementation groups (7,8), although this number has been expanded to include several new G2 checkpoint mutants (9). Categorization of the corresponding genes into epistasis groups has been limited and effective only for those genes involved in nucleotide excision repair and a G2 checkpoint pathway. Of the original *S.pombe* mutants which are sensitive to gamma irradiation, only *rad21* and *rad22* are now categorized as being defective in recombination (10,11). A more recent search for meiotic recombination mutants has led to the identification of 10 new complementation groups, mutants in three of which are sensitive to DNA damaging agents (12).

Recombination is not only required for the tolerance of DNA damage, but is also central to meiosis. In *S.cerevisiae*, recombination mutants have been isolated in a number of screens e.g. the *rad52* series of mutants which are sensitive to gamma radiation (e.g. 13) as well as several mutants defective in meiotic recombination (e.g. 14). Recent studies have shown that some of the *RAD52* series of genes are highly conserved between the yeasts, *S.cerevisiae* and *S.pombe*, and higher eukaryotes. e.g. *RAD51* (15), *RAD52* (16) and *RAD54* (17).

We report here the isolation and characterization of a new gamma radiation sensitive *S.pombe* mutant *rad32*, and the cloning and sequence analysis of the corresponding gene. The mutant is defective in meiotic recombination, displays spontaneous chromosome instability and is defective in repairing DNA double strand breaks. The *rad32* gene has homology to the *S.cerevisiae* meiotic recombination gene *MRE11* and is not essential for cell viability. Epistasis analysis indicates that Rad32 is likely to function in a pathway that includes Rhp51 (the *S.pombe* homologue to *S.cerevisiae* Rad51).

MATERIALS AND METHODS

Plasmids, strains and growth conditions

The *S.pombe* plasmid pUR19 and the genomic library used in this study have been described elsewhere (18). The *S.pombe* cDNA library was a gift from L. Guarente (19). *S.pombe* strains used in

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this study are shown in Table 1; strain GP24 was a gift from G. Smith (Seattle, 12). Plasmids were grown in *E.coli* strain DH5α, [*endA1, hsdR17, (rk⁻, mk⁻), supE44, thi-1, recA1, lacIqZ-M15 (lacproAB)*]. M13 derivatives were grown in DH5αF′ (as DH5α except that it contains an integrated F′). Procedures and media used for the routine growth and maintenance of *S.pombe* strains were as reported in our previous work (20).

Table 1.

972 h^-	
sp.011	*ade6-704, leu1-32, ura4-D18, h⁻*
sp.139	*rad3, ura4-D18, h⁻*
sp.150	*rhp51::ura4, ade6-704, leu1-32, h⁺*
sp.216	*rad2::ura4, ade6-704, leu1-32, h⁻*
sp.222	*rad13::ura4, ade6-704, leu1-32, h⁻*
sp.315	*ade6-469, h⁺*
sp.1235	*Ch^{16}(ade6-M216), ade6-M210, ura4-D18, leu1-32*
GP24	*ade6-M26, h⁺*
Strains created during this study	
sp.132	*rad32-1, ura4-D18, ade6-704, h⁺*
sp.276	*rad32::ura4, ade6-704, leu1-32, h⁺*
sp.277	*rad32::ura4, ade6-704, leu1-32, h⁻*
sp.282	*rad32::ura4, rhp51::ura4, ade6-704, ura4-D18, leu1-32*
sp.280	*rad32::ura4, Ch^{16}(ade6-m216)′, ade6-m210, ura4-D18*
sp.283	*rad32::ura4, rad13::ura4, ade6-704, ura4-D18, leu1-32*
sp.321	*rad32::ura4, ade6-M26, h⁻*
sp.331	*rad32::ura4, ade6-469, h⁻*

Irradiation of *S.pombe* cells

Gamma irradiation was carried out using a ^{137}Cs gamma source, with a dose rate of 12 Gy/min. UV irradiation was carried out directly on freshly plated cells using a Stratagene 'Stratalinker'.

Pulsed field gel electrophoresis

Preparation of DNA plugs was as described by Noolandi and Turmel (21). Electrophoresis using 0.7% agarose gels (Seakem, FMC) in 0.25 × TBE buffer was carried out using a Rotophor apparatus (Biometra). The following settings were used: 45 V, interval 4000–2000 s log, field angle 105–95°, at 11°C for 90 h. The DNA was stained by immersing the gel in 0.25 × TBE containing 1 μg/ml ethidium bromide for 40 min and destained in water for 5 h.

Genetics and molecular biology methods

S.pombe genetic and molecular biology methods have been described in our previous work (20). Meiotic recombination frequencies were measured using mutant alleles of the *ade6* gene, namely *ade6-704*, *ade6-469* and a hotspot mutation, *ade6-M26*. Crosses were carried out using standard methods and wild type recombinants were scored as white colonies on minimal medium containing 7.5 mg/l adenine (*ade6* mutant colonies were pink). The chromosome segregation assay was carried out as described by Niwa *et al.* (22). General molecular biology protocols were as detailed by Sambrook *et al.* (23).

RESULTS

Isolation of the *rad32* mutant

In order to identify new *S.pombe* genes required for the repair of DNA double strand breaks, a search was initiated for additional gamma radiation-sensitive mutants; *rad32-1* is one of two mutants isolated in such a screen. Wild type *S.pombe* (972 h^-) cells were subjected to a UV dose of 150 Jm^{-2}, a dose sufficient to give 10% cell survival. Approximately 5×10^4 cells were plated onto YES agar and grown at 29°C for 4 days. Colonies were then replica plated and the replicas exposed to gamma radiation at a dose of 1000 Gy. Colonies failing to grow after irradiation were re-tested for sensitivity to gamma radiation, resulting in two independently isolated mutants. These mutants, named *rad31-1* and *rad32-1*, were outcrossed at least three times with the *rad*⁺ strain sp.011. Further characterization of *rad31-1* will be described elsewhere (Shayeghi *et al.* manuscript in preparation).

Phenotype of the *rad32* mutant strain

To investigate the radiation sensitivity of the *rad32-1* mutant, its survival was analysed after exposure to both gamma and UV radiation (Fig. 1a and b). In addition to being sensitive to gamma radiation, the *rad32-1* strain (sp.132) was also found to be moderately sensitive to UV irradiation. *rad32-1* is more sensitive to gamma irradiation than a typical nucleotide excision repair mutant and less sensitive to UV radiation than the G2 checkpoint mutants and excision repair mutants (20,24). Its phenotype thus closely resembles those of the *S.pombe* mutants previously shown to be required for repair of DNA strand breaks e.g. *rad21* (10).

The ability of the *rad32* deletion mutant (see below) to repair DNA double strand breaks was investigated using pulsed field gel electrophoresis. Cells were exposed to 125 Gy, a dose sufficient to give 85 and 7% survival of wild type and *rad32* mutant cells respectively (Fig. 1b). Analysis of genomic DNA extracted from both wild type and *rad32* mutant cells immediately following exposure to ionizing radiation indicated the presence of substantial DNA damage which, after 4.5 h had been almost completely repaired in the wild type cells (Fig. 2). However the *rad32d* mutant strain showed only very limited repair of the strand breaks after 4.5 h, confirming that this mutant is defective in double strand break repair.

Table 2. Meiotic recombination at *ade6*

Cross	*ade*⁺ recombinants/10^6 viable spores	
	M26 × 704	469 × 704
wt × wt	6931 (14/2.02 × 10^3)	273 (9/3.3 × 10^4)
rad32d x *rad32d*	560 (7/1.25 × 10^4)	14 (1/7 × 10^4)

The effect of the *rad32* deletion mutation on meiotic recombination frequency was investigated using mutant alleles of the *ade6* gene. The alleles used were *ade6-607*, *ade6-469* and *ade6-m26*, of which the latter displays recombination 'hotspot' activity by stimulating recombination at the *ade6* locus 10–15-fold (25). The effect of the *rad32d* mutation was analysed on both hotspot and non-hotspot recombination (Table 2). The

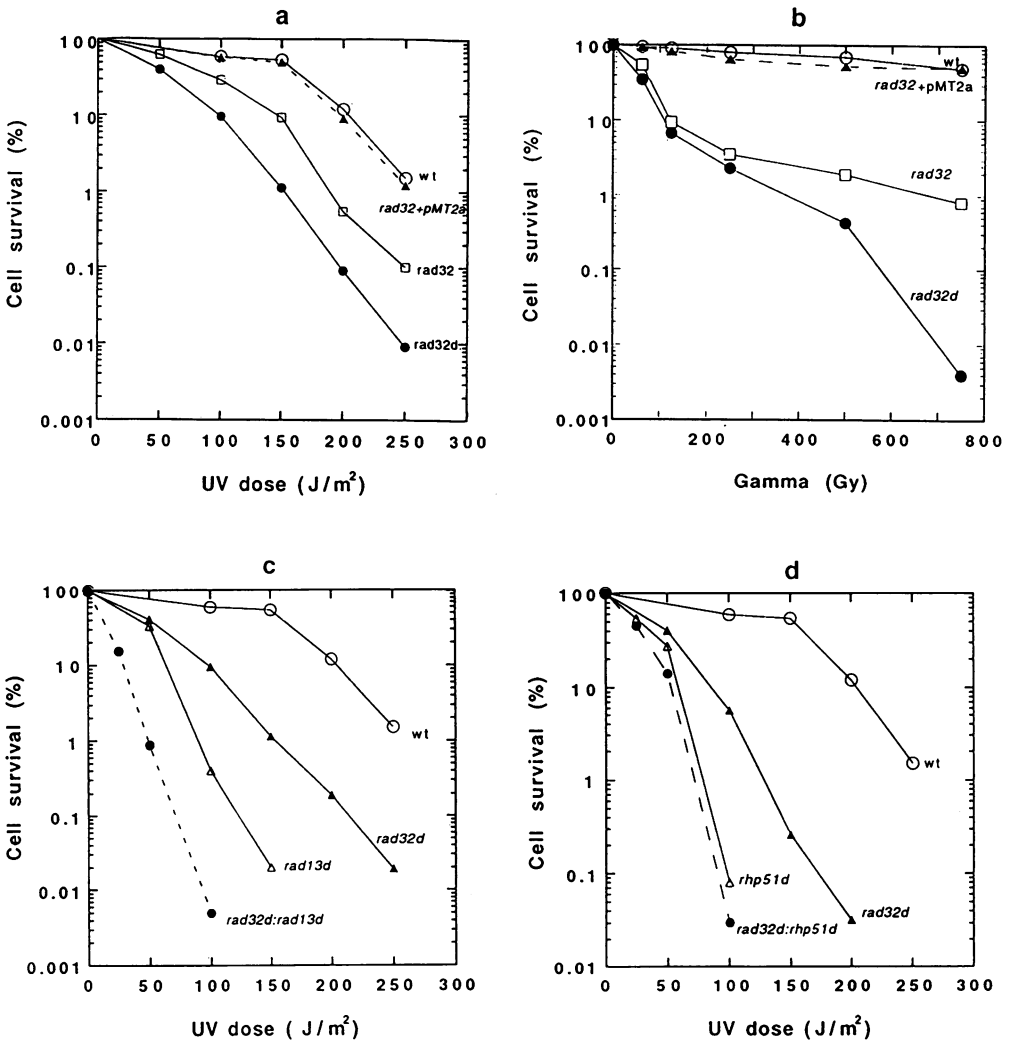

Figure 1. Survival curves of *rad32* mutant strains following UV or gamma-irradiation. (**a**) and (**b**) survival of *rad*$^+$ strain sp.011 (wt), the *rad32-1* strain sp.132 (*rad32*), the *rad32* deletion strain sp.276 (*rad32d*) and the *rad32-1* strain sp.132 containing the complementing plasmid pMT2a (*rad32* + pMT2a) after UV and gamma irradiation respectively. (**c**) and (**d**) survival of double mutants of the *rad32* deletion in combination with other *rad* mutations. The *rad*$^+$ strain sp.011 (wt), the *rad32* deletion (*rad32d*), the *rad13* deletion strain (*rad13d*), the *rad32/rad13* double deletion (*rad32d:rad13d*), the *rhp51* deletion strain (*rhp51d*), the *rad32/rhp51* double deletion (*rad32d:rhp51d*).

results indicate that in both cases the *rad32* deletion mutation reduces meiotic recombination by at least 15-fold in comparison to wild type.

Table 3. Fidelity of chromosome transmission

rad locus	% Loss/generation	Fold increase over wild type
wild type	0.005	1
rad32d	1.52	304
rad2d[a]	0.63	126
rad13d[a]	0.1	20
rad21d[a]	0.01	2

[a]Data from Murray *et al.* (6).

Chromosome segregation was also measured in the *rad32* deletion strain using the minichromosome assay of Niwa *et al.* (22). Table 3 shows that of all the *S.pombe rad* mutants tested to date, *rad32* displays the greatest loss of the minichromosome.

Isolation of the *rad32* gene

An *S.pombe* genomic library (18) was used to transform the *rad32-1* mutant strain sp.130 to uracil prototrophy. 30 000 *ura*$^+$ colonies were pooled and subjected to three rounds of UV irradiation at a dose of 100 Jm^{-2} for the first round followed by 300 Jm^{-2} for the second and third rounds as previously described (24). Individual colonies were tested for co-instability of the *rad*$^+$ and *ura*$^+$ phenotypes. Two plasmids (pMT2a and pMT2d) were isolated from two independently isolated radiation-resistant colonies. These were retransformed into sp.130 (the *rad32-1* mutant strain) and found to complement both the UV and gamma radiation sensitive phenotypes of the *rad32-1* mutant (Fig. 1a and

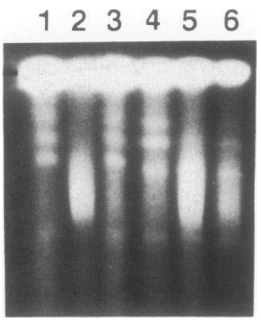

Figure 2. Repair of double strand breaks in wild type and *rad32* deletion strains. Genomic DNA of wild type cells (sp.011, lanes 1–3) and *rad32* deletion cells (sp.276, lanes 4–6) were subjected to pulsed field gel electrophoresis. Lanes 1 and 4, samples from unirradiated cells; lanes 2 and 5, from cells immediately after exposure to 125 Gy; lanes 3 and 6, from cells 4.5 h after irradiation with 125 Gy. Note that DNA from unirradiated *rad32d* cells shows a low level of DNA strand breaks as witnessed by the slightly reduced size of the chromosomes.

b). The plasmids also complement the slow growth and elongated cell phenotypes of the mutant (data not shown). Restriction mapping and Southern blot analysis (data not shown) indicated that the two plasmids contain overlapping fragments (Fig. 3a).

Mapping of the chromosomal locus of *rad32*

The 2.2 kb *Hin*dII fragment of the *rad32* gene was hybridised to the gridded *S.pombe* cosmid array of Lehrach and colleagues (26), and its position on the physical map was determined as being on chromosome 1 between *rad29* and *cdc25* on cosmid 13c5c.

DNA sequence analysis

The sequence of the 3.7 kb fragment of plasmid pMT2a that complemented the *rad32* defect was determined in both directions using a series of overlapping clones created using exonuclease III deletion in M13 mp19 and mp18 (27). Sequence analysis revealed an open reading frame of 2154 nucleotides which is interrupted by four small introns of 48, 70, 43 and 46 bp (Fig. 3b). The four introns were confirmed by sequence analysis of a PCR product generated from an *S.pombe* cDNA library. The predicted *rad32* gene product comprises 648 amino acids with a M_r of 73 582 Da and isoelectric point of 5.52.

Computer searches revealed that the predicted Rad32 protein has significant homology to the *S.cerevisiae* meiotic recombination protein, Mre11 (14, GenBank database) (Fig. 4). The sequence similarity between the two proteins is 44% over the first 528 amino acids, with the most highly conserved sequences within the N-terminal 410 amino acids. A search for motifs failed to identify any characteristic functional or structural domains.

Gene deletion

In order to determine whether *rad32* is essential for cell viability, the gene was disrupted by replacing the 2.2 kb *Hin*dII fragment by a 1.7 kb fragment containing the *ura4* gene (28) (Fig. 3c). A 4.7 kb linear DNA fragment containing the disrupted gene was released from the vector and used to transform a diploid strain (sp.101) to uracil prototrophy. An h^{90}/h^{+} derivative was then isolated and induced to sporulate. The spores were then subjected to tetrad analysis. Ten tetrads were dissected and the individual spores allowed to form colonies. The ura^{+} phenotype was always associated with radiation sensitivity, showing that the *rad32* gene is not essential for mitotic growth. The radiation sensitivity of the null allele was investigated and is shown in Figure 1a and b. The phenotype is similar to that of the original *rad32-1* mutant with a slight increase in sensitivity to both UV and gamma irradiation,

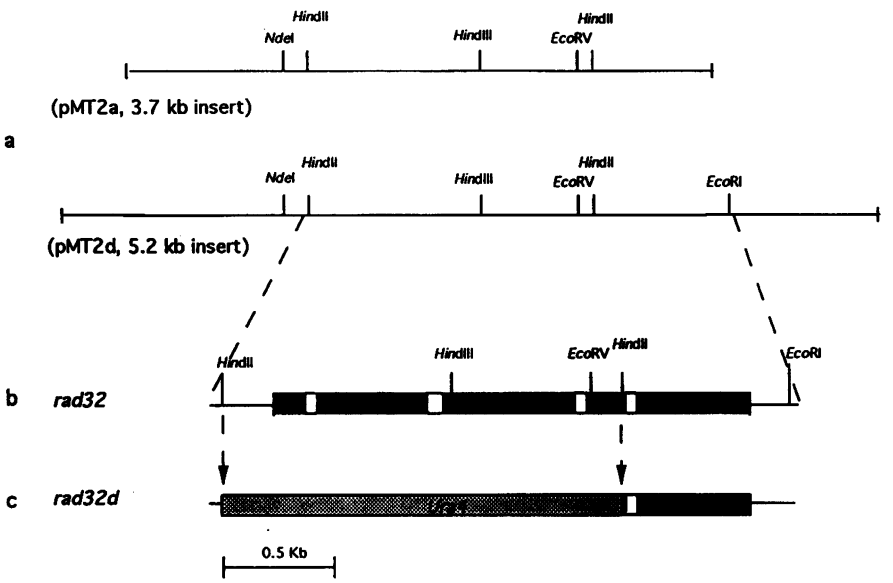

Figure 3. (**a**) Restriction map of the *rad32* region. (**b**) Position of the *rad32* open reading frame. The closed boxes represent exons, the open boxes denote position of introns, the N-terminus is on the left hand side of the diagram. (**c**) Construction of the *rad32* deletion (*rad32d*). The 2.2 kb *Hin*dII fragment was replaced by the *ura4* gene, deleting the first three exons and the majority of the fourth exon of the *rad32* gene.

```
rad32                                                                MPNDPSDMN   9
rad32   NELHNENTIRILISSDPHVGYGEKDPVRGNDSFVSFNEILEIARERDVDMILLGGDIFHDNKPSRKALYQ  79
          : .: :TIRILI::D HVGY. E: DP:  G: DS.  : F: E: :  : A: . : VDM: :  : GD: FH  NKPS: K: LYQ
MRE11   MDYPDPDTIRILITTDNHVGYNENDPITGDDSWKTFHEVMMLAKNNNVDMVVQSGDLFHVNKPSKKSLYQ  70

rad32   ALRSLRLNCLGDKPCELELLSDTSLTTGDTAVCNINYLDPNINVAIPVFS-HGNHDDPSGDGRYSALDIL 148
        . L: : LRL  C: GDKPCELELLSD. S .    . .    N: NY  DPN: N: : IPVF:    GNHDD: SGD:    . : : DIL
MRE11   VLKTLRLCCMGDKPCELELLSDPSQVFHYDEFTNVNYEDPNFNISIPVFGISGNHDDASGDSLLCPMDIL 140

rad32   QVTGLVNYFGRVPENDNIVVSPILLQKGFTKLALYGISNVRDERLYHSFRENKVKFLRPDLYRDEWFNLL 218
          :. TGL: N. FG: V  E: D: I  V  P: L: QKG  TKLALYG: : . VRDERL: : : F: : .   V. F   P. :    : EWFNL:
MRE11   HATGLINHFGKVIESDKIKVVPLLFQKGSTKLALYGLAAVRDERLFRTFKDGGVTFEVPTMREGEWFNLM 210

rad32   TVHQNHSAHTPTSYLPESFIQDFYDFVLWGHEHECLIDGSYNPTQKFTVVQPGSTIATSLSPGETAPKHC 288
          VHQNH: : HT  T: : LPE  F: . DF  D. V: WGHEHEC:  :    . NP. : : F. V: QPGS: : ATSL.  : E: . PK.
MRE11   CVHQNHTGHTNTAFLPEQFLPDFLDMVIWGHEHECIPNLVHNPIKNFDVLQPGSSVATSLCEAEAQPKYV 280

rad32   GILNIT-GKDFHLEKIRLRTVRPFIMKDIILSEVSSIPPMVENKKEVLTYLISKVEEEAITEAN---AQWY 354
          IL: I.  G. .  .: .  I. L  T: R. F  MK. I  L  : V:  : . P   : : K. . .  . YLI. : VEE  I  : AN     Q
MRE11   FILDIKYGEAPKMTPIPLETIRTFKMKSISLQDVPHLRP--HDKDATSKYLIEQVEEMIRDANEETKQKL 348

rad32   EAQGTVPVVENEKPPLPLIRLRVDY--------TGGYQTENPQRFSNRFVGRVANATDVVQFYLKKK-YT 415
          . . : G.   : V  . .  P  PLIRLRVDY          . : YQ. ENP: RFSNRFVGRVAN: . : VVQFY  K: .    T
MRE11   ADDGEGDMV--AELPKPLIRLRVDYSAPSNTQSPIDYQVENPRRFSNRFVGRVANGNNVVQFYKKRSPVT 416

rad32   RSKRNDGLYTSAVEDIKINSL--------RVESLVNEYLKTNRLECLPEDSLGEAVVNFVEKDDRDAIKE 477
          RSK: :  G:  . : : : . D  . : : . L           V: : LVN:  L: .  : L.  LPE  : L. EAV  : FV: KD: : . A: KE
MRE11   RSKKS-GINGTSISDRDVEKLFSESGGELEVQTLVNDLLNKMQLSLLPEVGLNEAVKKFVDKDEKTALKE 485

rad32   CVETQLNKQINLL-VKKRVTEENLEQEISSIINDLPKISTTKRKDYEELPEEVSETSINIAEHT-PVLKH 545
          :. : : : : : : . : L . : .      . :  . : E: . : : I: : : : : : : : .  .       P. E . ET: : . : . : .     : :
MRE11   FISHEISNEVGILSTNEEFLRTDDAEEMKALIKQVKRANSVRPTP----PKENDETNFAFNGNGLDSFRS 551

rad32   TSSLLDHHSP-LATSSSEHE--MEATPSPALLKKTNKRRELPSSLTKKNTRTPQRSKEVKKVPARKLSQS 612
          : : . :    SP : :  S  : : E  : .  . : : .    K  T: K. : . : : : . KK .   : : . :    .  .  .    : : .
MRE11   SNREVRTGSPDITQSHVDNESRITHISQAESSKPTSKPKRVRTATKKKIPAFSDSTVISDAENELGDNND 621

rad32   TKKSDKNTQSTLLFYDPSSTTEAQYLDNEDDEILDD                                  648
          : : : .  . : : : : : .  . . . . . .
MRE11   AQDDVDIDENDIIMVSTDEEDA                                                643
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Figure 4. Comparison of Rad32 protein with *S.cerevisiae* Mre11. Conserved amino acids are indicated between the Rad32 and Mre11 sequences, conservative substitutions are indicated :, gaps (–) have been introduced to maximise the alignment.

indicating that the *rad32* gene had been inactivated. Exponentially growing cultures of *rad32d* cells contain high proportions (~75%) of non-viable cells.

During the construction of the null allele, it was noted that the appearance of the *rad32* deletion mutation (*rad32d*)-containing spores was infrequent. The viability of *rad32d* spores was low compared to that of wild type spores. The results of the crosses are shown in Table 4. Compared to the products of a cross between two wild type strains where the spore viability is 93%, the viability of spores resulting from a cross between two *rad32* deletion strains is dramatically reduced (0.5%). Intermediate values are observed if *rad32d* is crossed with either wild type cells (sp.011) or the *rhp51d* strain (sp.150). These results indicate that Rad32 is likely to play an important role in either meiosis or spore germination or both.

Table 4. Spore viability

Cross	Spore viability (%)
wt × wt	93
rad32d × *rad32d*	0.5
rad32d × wt	58
rad32d × *rhp51d*	52

Epistasis analysis

To determine whether Rad32 functions in a pathway with any other Rad proteins, double mutants were constructed and analysed for their sensitivity to UV or gamma irradiation (Fig. 1c and d). The *rad32* deletion mutation in conjunction with the *rad13* deletion mutation (defective in excision repair, 5) shows increased sensitivity to radiation when compared to any of the single mutants, suggesting that Rad32 does not function in excision repair. Analysis of a *rad32d/rhp51* double mutant shows no increase in radiation sensitivity over that of the *rhp51* deletion strain, suggesting that Rad32 functions in a pathway requiring Rhp51. Despite extensive random spore and tetrad analyses no double mutants were obtained between the *rad32* deletion mutant and either the *rad2* mutant (defective in chromosome segregation, 6) or the *rad3* mutant (defective in a G2 checkpoint, 29), suggesting that these combinations may be lethal.

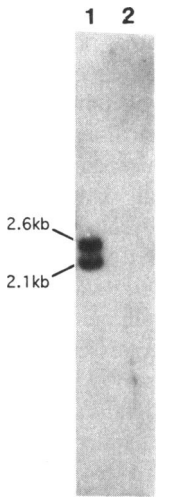

Figure 5. Northern analysis of *S.pombe* total RNA hybridised with a *rad32* specific probe (1.7 kb of the *rad32* cDNA derived by PCR). Lane 1, RNA from a *rad*$^{+}$ strain (sp.011); lane 2, RNA from the *rad32* deletion strain (sp.276). Two transcripts are observed in the wild type strain, which are both absent from the deletion strain.

Northern analysis

Northern blot analysis (Fig. 5) detects two transcripts of 2.1 and 2.6 kb, and indicates that the gene is expressed at moderate levels. The 2.1 kb transcript is consistent with the size expected from the Rad32 open reading frame of 648 amino acids. To ensure that both transcripts are encoded by the *rad32* gene and to confirm that the *rad32* gene had been disrupted, Northern analysis was carried out on RNA from the *rad32* deletion strain. This indicated the loss of both transcripts in the *rad32* null allele (Fig. 5, lane 2). The existence of two transcripts may be due to the presence of unspliced RNA (the size would be consistent with this possibility) or could be a due to the requirement for Rad32 in meiosis, as large 5′ untranslated regions have been identified in several mRNAs required for sexual development in fission yeast (e.g. 30,31).

DISCUSSION

Two new gamma radiation sensitive *S.pombe* mutants, *rad31-1* and *rad32-1*, have been isolated. Using pulsed field gel electrophoresis and measurement of recombination frequencies we have shown that the *rad32* mutant is defective in the repair of double strand breaks and in meiotic recombination. Detailed analysis of *rad31* will be presented elsewhere (Shayeghi *et al.*, manuscript in preparation). The level of the reduction in meiotic recombination in *rad32d* and the sensitivity to DNA damaging agents suggest that *rad32* is a Class III-type recombination mutant as defined by

DeVaux and co-workers (12). The low spore viability resulting from a *rad32d* × *rad32d* cross is consistent with the observation in *S.cerevisiae* that the absence of recombination in meiosis results in a low proportion of viable cells due to severe disjunction of the homologs during meiosis to produce aneuploid spores (32). The high frequency of minichromosome loss suggests that Rad32 also has a role in mitotically dividing cells.

We have cloned and analysed the *rad32* gene by complementation of the radiation sensitive phenotype. The gene has significant homology to the *S.cerevisiae MRE11* gene which was isolated by complementation of the meiotic recombination mutation *mre11* (14). The *rad32* gene is not essential for cell viability, although *rad32d* spores germinate poorly. The presence of two differently sized transcripts is consistent with a role for Rad32 in meiosis, since a number of genes required for both mitotic and meiotic growth have been shown to encode two differently sized transcripts.

The precise role of Rad32 in recombination or double strand break repair is unknown, since there are no obvious motifs in the protein. Epistasis analysis suggests that it functions in a pathway requiring Rhp51, the *S.pombe* homologue to *S.cerevisiae* Rad51 and *E.coli* RecA. Rad51, which is also required for mitotic recombination and repair of DNA damage, is proposed to act in concert with the Rad52 protein in a step which converts DSBs to the next intermediate in recombination (33). The phenotype of the *rad32* mutant described here is consistent with Rad32 having a role in a pathway involving Rhp51 and Rad22 (the *S.pombe* homologue to *S.cerevisiae* Rad52). In *S.cerevisiae*, Mre11 along with Rad50, has been proposed to be involved in an early step of meiotic recombination while Rad52 is thought to be involved in an intermediate step (14). Our data from pulsed field gel electrophoresis indicate that the Rad32 protein is involved in a step subsequent to double strand break formation. Further experiments are currently underway to investigate the role of the Rad32 protein and whether it interacts with any of the other known recombination proteins in *S.pombe* such as Rad22 (the homologue of *S.cerevisiae* Rad52, 11), Rhp51 or Rad21.

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REFERENCES

1 Friedberg, E.C., Bardwell, A.J., Bardwell, L., Wang, Z. and Dianov, G. (1994) *Mutat. Res.*, **307**, 5–14.
2 Feaver, W.J., Svejstrup, J.Q., Bardwell, L., Bardwell, A.J., Buratowski, S., Gulyas, K.D., Donahue, T.F., Friedberg, E.C. and Kornberg, R.D. (1993) *Cell*, **75**, 1379–1387.
3 Schaeffer, L., Moncollin, V., Roy, R., Staub, A., Mezzina, M., Sarasin, A., Weeda, G., Hoeijmakers, J.H.J. and Egly, J.M. (1994) *EMBO J.*, **13**, 2388–2392.
4 Nurse, P. (1990) *Nature*, **344**, 503–507.
5 Carr, A.M., Sheldrick, K.S., Murray, J.M., Al-Harithy, R., Watts, F.Z. and Lehmann, A.R. (1993) *Nucleic Acids Res.*, **21**, 1345–1349.
6 Murray, J.M., Tavassoli, M., Al-Harithy, R., Sheldrick, K.S., Lehmann, A.R., Carr, A.M. and Watts, F.Z. (1994) *Mol. Cell. Biol.*, **14**, 4878–4888.
7 Phipps,J., Nasim, A. and Miller, D.R. (1985) *Adv. Genet.*, **23**, 1–72.
8 Leiberman, H.B., Riley, R. and Martel, M. (1989) *Mol. Gen. Genet.*, **218**, 554–558.
9 Al-Khodairy, F., Fotou, E., Sheldrick, K.S., Griffiths, D.J.F., Lehmann, A.R., and Carr, A.M. (1994) *Mol. Biol. Cell*, **5**, 147–160.
10 Birkenbihl, R.P. and Subramani, S. (1992) *Nucleic Acids Res.*, **20**, 6605–6611.
11 Ostermann, K., Lorentz, A. and Schmidt, H. (1993) *Nucleic Acids Res.*, **21**, 5940–5944.
12 DeVeaux, L.C., Hoagland, N.A. and Smith, G.R. (1992) *Genetics*, **130**, 251–262.
13 Friedberg, E.C. (1988) *Micro.Revs.*, **52**, 70–102.
14 Ajimura, M., Leem, S-H. and Ogawa, H. (1992) *Genetics*, **133**, 51–66.
15 Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K. and Ogawa, T. (1993) *Nature Genet.*, **4**, 239–243.
16 Bezzubova, O.Y., Schmidt, H., Ostermann, K. Heyer, W-D. and Buerstedde, J-M. (1993) *Nucleoc Acids Res.* ,**21**, 5945–5949.
17 Bezzubova, O.Y. and Buerstedde, J.-M. (1994) *Experientia*, **50**, 270–276.
18 Barbet, N., Muriel, W.J. and Carr, A.M. (1992) *Gene*, **114**, 59–66.
19 Fikes, J., Becker, D. Winston, F. and Guarente, L. (1990) *Nature*, **346**, 291–294.
20 Murray, J.M., Carr, A.M., Lehmann, A.R. and Watts, F.Z. (1991) *Nucleic Acids Res.*, **19**, 3525–3531.
21 Noolandi, J. and Turmel, C. (1992) *Methods Mol. Biol.*, **12**, 73–89.
22 Niwa, O., Matsumoto, T. and Yanagida, M. (1986) *Mol. Gen. Genet.*, **203**, 397–405.
23 Sambrook,J., Fritsch, E.F. and Maniatis, T.E. (1989) *Molecular Cloning, A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor, NY.
24 Murray, J.M., Doe, C.L., Schenk, P., Carr, A.M., Lehmann, A.R. and Watts, F.Z. (1992) *Nucleic Acids Res.*, **20**, 2673–2678.
25 Gutz, H. (1971) *Genetics*, **69**, 317–337.
26 Maier, E., Hoheisel, J.D., McCarthy, L., Mott, R., Grigoriev, A.V., Monaco, A.P. Larin, Z. and Lehrach, H. (1992) *Nature Genet.*, **1**, 273–277.
27 Henikoff, S. (1984) *Gene*, **28**, 351–359.
28 Grimm, C., Kohli, J., Murray, J.M. and Maundrell, K. (1988) *Mol. Gen. Genet.*, **215**, 81–86.
29 Al-Khodairy, F. and Carr, A.M. (1992) *EMBO J.*, **11**, 1343–1350.
30 Benton, B.K., Reid, M.S. and Okayama, H. (1993) *EMBO J.*, **12**, 135–143.
31 Forsburg, S.L. and Nurse, P. (1994) *J. Cell Sci.*, **107**, 601–613.
32 Klapholz, S., Waddell, C.S. and Esposito, R.E. (1985) *Genetics*, **110**, 187–216.
33 Shinohara, A., Ogawa, H. andOgawa, T. (1992) *Cell*, **69**, 457–470.