Cloning and characterization of *rad21* an essential gene of *Schizosaccharomyces pombe* involved in DNA double-strand-break repair

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ABSTRACT

Analysis of the *Schizosaccharomyces pombe* chromosomes by pulsed field gel electrophoresis showed that the fission yeast has a very efficient DNA double-strand-break (dsb) repair system, which properly restores the three chromosomes after they are degraded by γ -irradiation. The radiation-sensitive mutant *rad21-45* is deficient in this repair pathway but is capable of cell-cycle arrest in G2 following DNA damage. We cloned the *rad21* gene by complementing the radiation sensitivity of the *rad21-45* mutant. The plasmid-borne gene completely reestablished the DNA dsb repair pathway. The *rad21* gene was localized to chromosome III by hybridization. The transcript is 2.5 kb long and expressed at a moderate level. The 1884-bp open reading frame encodes a 628 amino acid, very acidic peptide with a calculated molecular mass of 67,854 D. The *rad21* gene shows no significant homology to other known nucleotide or peptide sequences. The inability of the mutant to perform efficient DNA repair is caused by a single base substitution, which changes wild-type isoleucine⁶⁷ into threonine in the mutant. Deletion of the genomic *rad21* gene showed that it is essential for mitotic growth of *S.pombe*.

INTRODUCTION

Despite the fact that the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* have about the same genome size, *S.pombe* is approximately ten times more resistant to DNA-damaging irradiation with UV or γ -rays (1). This presumably is the consequence of a very efficient DNA repair machinery and a much longer G2-phase in *S.pombe*. Both yeasts are much more resistant to radiation in G2-phase than during the rest of the cell cycle (2, 3). Radiation-sensitive (*rad*) mutants of *S.pombe* belonging to 20 complementation groups have been isolated (4, 5, 6). Originally the *rad* mutants were sorted into three DNA repair pathways by virtue of their specific sensitivity to UV rays alone, UV and ionizing rays or ionizing rays alone (1). A revised grouping of the *rad* mutants has been

proposed (7, 8), based on the limited data available from the analysis of epistasis groups and the sensitivity of the mutants to caffeine after radiation. Caffeine inhibits mitotic recombination in *S.pombe* (9) and is used as an indicator for the recombinational DNA repair pathway (10).

The *rad5* group contains the *rad* mutants which are supposed to act in DNA excision repair. They are sensitive to UV rays and to postirradiation caffeine treatment. The members of the *rad1* group are insensitive to caffeine after irradiation and therefore thought to act in recombinational repair and in checkpoint controls. The *rad21* group contains only two mutants, *rad22*, which has also been reported as a *swi* gene involved in mating type switching (11), and *rad21* the mutant analyzed in this paper. Both are sensitive to caffeine treatment after irradiation (1, 12) indicating that the recombinational repair pathway is still intact. Both mutants are sensitive mainly to ionizing radiation which supports the idea that they play a role in the repair of DNA dsb.

It has been reported for some members of the *rad1* group, which are also very sensitive to γ -rays, that their sensitivity to radiation is not a consequence of deficient DNA repair alone, but due to an inability to arrest cells in the G2 phase after irradiation to provide time for DNA repair (13, 14, 15). The *rad21-45* mutant has been reported to exhibit a partial defect in G2-arrest after UV irradiation (13), implying that its sensitivity to ionizing radiation could also result from a G2-arrest deficiency rather than from a defect in a DNA repair function.

We asked whether the γ -ray sensitivity of the *rad21-45* mutant is linked to a deficiency in the DNA dsb repair pathway. To monitor DNA damage and repair after irradiation we employed pulsed field gel electrophoresis (PFGE) which, in recent years, has become a powerful tool for the analysis of high molecular weight DNA (16). Using PFGE, Smith *et al.* (17) separated the three chromosomes of *S.pombe* with sizes of 3.5, 4.7 and 5.7 Mb. PFGE has also been used to investigate DNA damage and recombination in yeast (18, 19). In this study we used the advanced pulsed field technique CHEF (clamped homogenous electric fields) electrophoresis (20) to visualize DNA damage in *S.pombe* caused by irradiation, and the subsequent repair of the introduced DNA dsb. We also characterized the *rad21-45* mutant for cell-cycle checkpoint defects.

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Table 1. *S.pombe* strains

SPR11	972 h^- (wild type)	A.Nasim
SPR15	<i>ura4-D6</i> , h^+	
SPR6	<i>rad21-45</i> , h^-	A.Nasim
SPR7	<i>rad21-45</i> , <i>ura4-D6</i> , h^+	A.Nasim
SPR14	<i>ura4-D6</i> , <i>leu1-32</i> , <i>his3</i> , h^-	
SPR21	<i>rad21-45</i> , <i>ura4-D6</i> , <i>leu1-32</i> , h^+	
SPR55	<i>ura4-D6/ura4-D6</i> , <i>leu1-32/leu1-32</i> , <i>his3/his3</i> , h^-/h^-	P.Russell
SPR54	<i>ura4-D6/ura4-D6</i> , <i>leu1-32/leu1-32</i> , <i>his3/his3</i> , h^+/h^+	P.Russell
SPR56	<i>rad21::ura4/ura4-D6</i> , <i>leu1-32/leu1-32</i> , <i>his3/his3</i> , h^-/h^-	

Some of the genes of the *rad1* group have been sequenced and analyzed recently (*rad1* [21]; *rad3* [22]; *rad4* [23]; *rad9* [24]). in order to contribute to the understanding of the interaction between the *rad* genes, and to learn more about their gene structure and function, we cloned and analysed the *rad21*⁺ gene.

MATERIALS AND METHODS

S.pombe strains and culture conditions

The strains used in this study are listed in Table 1. *S.pombe* strains were grown and mated as described previously (21).

Measurement of survival of yeast strains after UV or γ -irradiation

A 15 W Sylvania G15T8 germicidal lamp emitting primarily 254 nm light was used for UV irradiation. The dose rate was 4 W/m². For γ -irradiation we used ¹³⁷Cs as the radiation source. The dose rate was 2 krad/min.

(i) *UV spot test*: For qualitative recognition of UV sensitivity, 3 μ l of exponentially growing cultures were spotted on YPD plates and irradiated with 120 and 160 J/m². After two days of growth, sensitivity was determined by comparison to survival of wild-type cells.

(ii) *Quantitative measurement of survival to UV or γ -radiation*: Exponentially growing cells were plated on YPD at appropriate dilutions and irradiated with increasing doses of UV light. Colonies were counted after 3 days at 30°C. For experiments involving γ -irradiated cells, exponentially growing cells at a density of 5×10⁶ cells/ml were irradiated, plated at appropriate dilutions and colonies counted after 3 days.

CHEF electrophoresis

(i) *Separation of *S.pombe* chromosomes*: Samples of 1×10⁸ cells were washed once in 50 mM EDTA pH 8.0, resuspended in ice cold 50 mM EDTA pH 8.0, 1 mg/ml sodium azide, and stored at 4°C until all samples were collected. Preparation of DNA plugs was done as described previously (25). Electrophoresis in a 0.6% agarose gel (FastLane agarose, FMC) was done with a CHEF-DR II apparatus (BIO-RAD) using 0.5×TBE buffer. The following settings were used: 45 V, 3600 sec constant pulse time, 80 hours. The gel was stained in 0.5×TBE buffer containing 5 μ g/ml ethidium bromide and destained for 3 hours in water.

(ii) *Separation of irradiated DNA*: For separation of *S.cerevisiae* chromosomes the altered electrophoresis conditions were: 1% agarose, 200 V, 15 hours at a 70 sec pulse time, followed by 10 hours at a 120 sec pulse time.

Figure 1. Repair of dsb in wild type, the *rad21-45* mutant, and the mutant transformed with the complementing plasmid p21F. Genomic DNAs of wild-type cells (SPR11, lanes 1–3), *rad21-45* (SPR6, lanes 4–6), and the mutant transformed with plasmid p21F (lanes 7–9) were subjected to CHEF electrophoresis. Samples were from unirradiated cells (lanes 1, 4, and 7), from cells immediately after irradiation with 60 krad γ -rays (lanes 2, 5, and 8), and 4 h after irradiation (lanes 3, 6, and 9). The sizes of the *S.pombe* chromosomes are 3.5, 4.7, and 5.7 Mb.

Preparation of DNA and RNA

S.pombe genomic and plasmid DNA and RNA were isolated by vortexing cells in the presence of glass beads, phenol and lysis buffer (100 mM Tris–HCl pH 7.5, 40 mM LiCl, 25 mM DTT). After a second phenol extraction of the aqueous phase, nucleic acids were precipitated with 1/25 vol. 5 M sodium acetate and 2.5 vol. ethanol. Plasmids amplified in *E.coli* were isolated by the alkaline lysis method (26).

Transformations

Transformation of *S.pombe* strain SPR7 (*rad21-45 ura4-D6*) with the *S.pombe* genomic library (gift of J. Carbon) was performed following the protocol of Beach *et al.* (27). Standard transformations with plasmids were done by electroporation (28). For transformation of *E.coli* the 'one minute protocol' of Golub (29) was used.

DNA sequence analysis

DNA fragments obtained by restriction digests were cloned into vector pUC19 (30). The sequence of both ends was determined from double-stranded templates by the dideoxy chain-termination method of Sanger *et al.* (31) using Sequenase (USB) and ([³⁵S]thio)dATP.

Cloning the *rad21-45* gene

Genomic DNA of the *rad21-45* mutant (SPR6) was cut to completion with *Eco*RI and *Bgl*II. Fragments ranging in size from 2.2 kb to 2.8 kb were isolated from an agarose gel and ligated into pUC19 cut with *Eco*RI and *Bam*HI. Clone p21-45U was isolated by colony hybridization with the *rad21* specific *Hind*III–*Bgl*II fragment. p21-45U was subcloned for sequencing and complementation studies.

RESULTS

The *rad21-45* mutant is deficient in DNA dsb repair but not in G2-arrest

CHEF electrophoresis is capable of resolving DNA fragments in the megabase range. Therefore we used this technique to demonstrate the introduction of dsb in *S.pombe* chromosomes following irradiation with γ -rays. The reconstitution of chromosomes by DNA dsb repair was then monitored after an appropriate time of recovery. By this we were able to compare

Figure 2. CHEF electrophoresis to determine the size distribution of *S.pombe* DNA after irradiation. Genomic DNA of unirradiated wild-type cells (lane 1) and of cells directly after irradiation (lane 3) (same samples as in lanes 1 and 2 of Figure 1) was separated side by side with *S.cerevisiae* chromosomes as markers (lane 2).

the repair efficiency of the wild-type strain and the *rad21-45* mutant.

Wild-type (SPR11) and *rad21-45* (SPR6) cells were γ -irradiated with 60 krad at a dose rate of 2 krad/min. Half of the cells were collected for CHEF electrophoresis immediately after irradiation, the other half incubated at 30°C for recovery, and some cells plated to check the survival. To monitor G2 arrest and start of the next round of cell division, samples of the recovering cells were collected at time points and stained with DAPI (stains nuclei) and Calcofluor (stains cell walls and septa) for fluorescence microscopy. DNA of unirradiated cells, and cells corresponding to time points 0 h and 4 h after irradiation were examined by CHEF electrophoresis (Figure 1). The ethidium bromide-stained gel shows clearly the three chromosomes of unirradiated cells (lanes 1 and 4), and the degraded DNA of the cells directly after irradiation (lanes 2 and 4). After a 4 h recovery under growing conditions, most of the wild-type chromosomes had been restored (lane 3) while in the mutant only a light shift to higher molecular weight could be observed (lane 6).

Because the degraded DNA could not be resolved under the electrophoresis conditions used for the *S.pombe* chromosome separation (3.5–5.7 Mb) we ran a CHEF-gel under different conditions, to calculate the number of dsb introduced by the irradiation procedure, using the *S.cerevisiae* chromosomes (220–1900 kb) as markers. Figure 2 shows that DNA of unirradiated cells (same sample as lane 1 in Figure 1) hardly enters the gel under these conditions whereas the DNA after irradiation (same sample as lane 2 in Figure 1) actually consists of fragments between 150 and 800 kb. Considering that more molecules in the lower part of the smear generate the same intensity of staining as those in the higher molecular weight range, we estimated the average molecule size in the smear at about 300 kb, leading to a number of 40 to 50 dsb per 14 Mb genome.

Examination of the mitotic index (fraction of binucleate or septated cells) during recovery after irradiation, revealed that both the wild-type and the *rad21-45* strain arrested simultaneously and completely in G2 to allow DNA repair (Figure 3). This was confirmed by FACS analysis (not shown). After 3 h the wild-type cells resumed growth followed by the mutant cells 0.5 h later. Fluorescence microscopy showed that 1 h after irradiation the cells in both cultures were unseptated and mononuclear. At

Figure 3. G2-arrest of strains after γ -irradiation. Wild-type and *rad21-45* cells were exposed to 60 krad ionizing radiation and incubated at 30°C for recovery. At time points indicated the cultures were examined for their mitotic index. The arrested cells were judged to be in G2 on the basis of their 2n DNA content examined by FACS analysis.

Figure 4. Survival of *S.pombe* strains after UV (A) or γ -irradiation (B). Cells were grown and irradiated as described in Materials and Methods. Symbols: SPR15 (*rad21*⁺) transformed with pFL20 (○); SPR7 (*rad21-45*) transformed with pFL20 (●), p21-5 (●), and p21F (□), respectively. Each point represents the average of two plates.

time point 4 h, the wild-type and mutant cells were very elongated, which also indicated that cell cycle arrest had taken place. However, while most of the wild-type cells divided normally, many mutant cells showed missegregation of nuclear material or the 'cut' phenotype with septa laid through nuclei. The cell survival in this experiment after the 60 krad dose was 61% for the wild-type and 6% for the *rad21-45* mutant.

Cloning the *rad21* gene by complementation

S.pombe rad21-45 (SPR7) cells were transformed with a genomic *S.pombe* DNA library (32) based on the vector pFL20. By two rounds of selection, first irradiation with 120 krad γ -rays and second UV spot assay, we isolated 6 radiation-resistant clones, which all contained the same plasmid. Retransformation into the mutant confirmed the ability of this plasmid to complement the UV and γ -sensitivity of the *rad21-45* mutant. Mutant cells transformed with vector pFL20 only were still sensitive (Figure 4).

A restriction map of the complementing clone p21-5 was constructed (Figure 5) and colinearity with the relevant genomic region confirmed by Southern blotting. To show that p21-5

Figure 5. Restriction map and clones of the *S. pombe rad21* region. The restriction sites shown along a kb-scale are: *Bam*HI (B), *Cla*I (C), *Eco*RI (E), *Bgl*III (G), *Hind*III (H), *Kpn*I (K), *Pst*I (P), *Spe*I (S), *Eco*RV (V), *Xba*I (X). The *rad21* ORF is shown as a white box interrupted by the intron in black. p21-5 and p21F are the complementing genomic clones. p21F::ura4 was used in the gene disruption of *rad21*. The box represents the deleted region in the *rad21* gene, which was replaced by the *S. pombe ura4* gene.

contained the *rad21* gene and not, for example, a high copy number suppressor, we integrated the *S. cerevisiae LEU2* gene into the genome of *S. pombe* strain SPR14 (*ura4-D6 leu1-32 his3h⁻*) using homologous recombination guided by the 1.4-kb *Hind*III fragment of p21-5. Integration into the appropriate locus was confirmed by Southern blotting. Mapping by recombination showed that the *LEU2* gene had integrated in the *rad21* region.

The location of the *rad21* gene on the *S. pombe* genome had been previously investigated by recombination (33). Hybridization of the p21-5 insert against a chromosomal Southern blot of *S. pombe* confirmed that *rad21* is situated on the *Not*I fragment A (25) on chromosome III.

Comparison of *rad21* cDNAs, isolated from a *S. pombe* cDNA library to the genomic clone p21-5 showed that the 3' end of the *rad21* transcript was not contained on p21-5. The mRNA extended about 600 bp past the 3' end of p21-5. By probing the genomic library with *rad21* cDNA we isolated the missing part of the gene. The two genomic clones were linked at their common *Bam*HI site and inserted into pFL20. The resulting clone (p21F, Figure 5) completely complemented the *rad21-45* mutation (Figure 1 and Figure 4).

Sequence analysis of the *rad21* gene

4.7 kb of *rad21* genomic sequence, from the *Hind*III (kb 1.8 on the kb-scale of Figure 5) to the *Bgl*III site (kb 6.5), was determined. The internal 3.3-kb *Eco*RI (kb 3.0) — *Eco*RV (kb 6.3) fragment was sequenced on both strands. Also the cDNA sequence was determined. The results are summarized in Figure 6.

Three of the four cDNAs homologous to the *rad21* gene contain polyA tails, which all start at the same site (bp 2447), and so define the 3'-end of the *rad21* transcript. The cDNA reaching the furthest in the 5'-direction starts at bp -156. The cDNA sequence contains an 1884-bp-long open reading frame (ORF) which lacks an 80-bp intron (bp 34–113). The genomic sequence in this region perfectly fits the consensus for introns in *S. pombe* (34). A potential TATA box is well positioned (34) 33 bp upstream of the start of the cDNA sequence (bp -189).

The A/T content of the 1.6-kb region upstream (72%) and 0.8-kb region downstream (68%) of the coding region is significantly higher than that of the coding region itself (57%). Therefore, the sequenced regions upstream and downstream of the deduced ORF are unlikely to contain any coding sequence. The ORF encodes a 628-amino acid peptide with a deduced molecular mass of 67,854 D. The peptide is very acidic and has a polarity index of 51. The isoelectric point is at pH 4.4.

Screening the data bases (GenBank, 71.0; EMBL, 30.0) for sequence homologies at the nucleotide and peptide level (35) revealed no significant similarity to known genes or proteins. The screening of a motif library (36) also failed to provide any information about functional domains or structural peculiarities.

Despite the relatively low codon-bias index of 0.21, we were able to detect transcripts of *rad21*. Total RNA isolated from exponentially growing wild-type (SPR11) and *rad21-45* mutant cells (SPR7) were examined by Northern blot analysis. Hybridization with cDNA showed the expected 2.5 kb band for wild-type as well as for the *rad21-45* mutant with the same intensity, indicating that the *rad21-45* mutation does not affect the transcription efficiency.

The nature of the *rad21-45* mutation

To investigate the genetic basis of the UV and γ -sensitivity of the *rad21-45* mutation, we cloned and sequenced the mutant gene. The only difference between the mutant and the wild-type gene is a T to C transition at position bp 280 (Figure 6) in the mutant gene. For functional confirmation we introduced the mutation into the complementing clone p21F. When transformed with this plasmid, the *rad21-45* mutant was still radiation-sensitive at its original level, proving that the indicated single nucleotide exchange causes the *Rad⁻* phenotype of the *rad21-45* mutant. This substitution changes isoleucine⁶⁷ to threonine. Computer peptide-structure analysis showed a higher probability for a turn in the polypeptide backbone of the mutant protein at this position.

The *rad21* gene is essential

Since attempts to create a viable *rad21* gene disruption in a haploid *S. pombe* strain failed, we used the stable diploid strain SPR55 (*ura4-D6/ura4-D6 leu1-32/leu1-32 his3/his3 h⁻/h⁻*) as a parental strain because *h⁻/h⁺* diploids of *S. pombe* are unstable (34). We made the following linear construct (Figure 4) to transform the diploid strain: In the 4.2-kb *Cla*I fragment of p21F, the internal 2.0-kb *Spe*I–*Bam*HI fragment was replaced by the 1.8-kb *Hind*III fragment containing the *S. pombe ura4* gene (37). Stable *Ura⁺* transformants were screened by Southern blotting for the appearance of the specific band pattern, which would indicate the haploid insertion of the *ura4* gene into the *rad21* gene.

One clone with the correct band pattern was detected (SPR56). This strain was crossed with the stable *h⁺* diploid strain SPR54 carrying the same auxotrophic markers as SPR55. Asci were dissected; each of them resulted in two *Ura⁺* and two *Ura⁻* colonies. Southern blot analysis showed that these colonies were still diploid (the *Ura⁺* colonies heterozygous for the *ura4* insertion). They were very unstable and sporulated spontaneously even on rich medium as expected for *h⁻/h⁺* diploids. Numerous asci derived from the *Ura⁺* colonies were dissected and resulted in two (in rare cases in one) *Ura⁻* colonies per ascus. No *Ura⁺* colonies grew. The same result was obtained by random-spore analysis of more than 400 spores. A closer look at the development of the *Ura⁺* spores of the dissections revealed that in most cases the spores germinated and divided once or twice before they died. This shows that the *rad21* gene product is essential for the mitotic growth of *S. pombe*. In other experiments we replaced in an haploid strain 1.5 kb of DNA upstream from the *Hind*III site (bp -8) with a different promoter and the *ura4* gene. That strain was fully viable, indicating that the removal of 272 bp upstream of the ORF in the disruption construct did not affect any other important gene.

Figure 6. Sequence of the *rad21* region. The nucleotide sequence (bp -653 to 2719, translation start is 1) and predicted amino acid sequence (aa 1 to 628, one letter code written below the first nt of each codon) are shown. The major restriction sites, beginnings of the cDNA clones (>) and polyadenylation site (bp 2447) are indicated above the relevant nucleotide. The potential TATA box (bp -189) is double underlined. The genomic sequence contains an intron (bp 34 to 113, dotted line).

DISCUSSION

The *rad21* gene was cloned by complementation of the radiation sensitivity of the *rad21-45* mutant. Evidence for the identity of the *rad21* gene was obtained from three experiments: First, recombinational mapping revealed that insertion of the *S. cerevisiae* *LEU2* gene via homologous recombination, into the genomic region from which the complementing clone p21-5 was derived, took place adjacent to the *rad21* gene. Second, mapping of the isolated gene to chromosome III of *S. pombe* by hybridization

confirmed the previous localization of the *rad21* gene, determined by meiotic recombination (33). Finally, isolation of the *rad21-45* mutation, and subsequent cloning of the mutated DNA into the wild-type gene, destroyed the complementing activity of the plasmid p21F.

The isolation of complete cDNAs of *rad21* strongly supported the characterization of the *rad21* gene structure. Comparison of the genomic to the cDNA sequence uncovered an 80-bp intron. Splicing removes an in-frame stop codon (Figure 6: bp 46) and thereby connects the first exon, which uses the first ATG in the

cDNA sequence, to the major ORF starting at bp 234. The full-length cDNAs as well as the Northern hybridization data show that the *rad21* transcript is about 2500 bp long. The 3' end is defined by the start point of the polyA tail (bp 2447) present in three cDNAs. The polyadenylation signal is probably located in an A/T-stretch (bp 2422–2433, AATTAATAATAT) situated 25 bp upstream of the polyA tail and 457 bp downstream of the stop codon (bp 1965). The 5' ends of 3 cDNAs (pcl-12, pcl-5, pcl-3) start within a 30-bp region 156 bp upstream of the start codon and 33 bp downstream of the most feasible TATA box (bp –189, TATATTCAT). This spacing is consistent with findings from other *S.pombe* genes (34).

Highly expressed *S.pombe* genes have a high codon-bias index (CBI; the scale reads from 0 to 1) which favors G/C nucleotides at the third position of the degenerate codon set (34). Therefore the A/T content of highly expressed coding sequences is usually low. For the *rad21* ORF the CBI is 0.21 and the A/T content drops from over 70% outside the ORF to 57% inside the ORF. Both values suggest a moderate level of expression for the *rad21* gene. This is consistent with our Northern blot analysis, in which a clear signal was obtained using 12 µg total RNA.

The *rad21-45* mutation consists of a T to C transition, which changes a nonpolar isoleucine at position 67 to a polar threonine. It is not clear yet whether a functional motif is destroyed or the structural conformation of the protein is altered by this mutation which causes the Rad[–] phenotype.

By CHEF electrophoresis we demonstrated that the *rad21-45* mutant has a deficiency in DNA dsb repair (Figure 1). This is consistent with the sensitivity of this mutant to ionizing radiation (Figure 4). While about 60% of the wild-type cells after irradiation reassembled the 40–50 DNA fragments to restore their three chromosomes, which appear with the correct sizes on the gel, only 6% of the mutant cells survived. The fact that caffeine treatment after irradiation with 60 krad results in an additional ten-fold drop in the mutant's survival (12) suggests that its 6% survival is based on the recombinational DNA repair pathway which is still intact. On the CHEF-gel no intact chromosomes can be detected in the *rad21-45* strain after 4 hours recovery time, perhaps because the resolution is too low or the residual repair activity responsible for the 6% survival needs more time than 4 hours.

In addition to other *S.pombe* *rad* mutants with a very pronounced G2-arrest defect, *rad21-45* has been reported to exhibit a partial defect, consisting of premature entry into mitosis after UV irradiation (13). We have been unable to detect any appreciable deficiency in G2 arrest in synchronous populations of *rad21-45* cells irradiated with the same doses of UV radiation used by Al-Khodairy and Carr (13). Our results presented here show that after γ-irradiation the *rad21-45* strain arrests even longer in G2 than wild-type cells (Figure 3). The *rad21-45* mutant is unusual, however, in the sense that it starts to septate after a cell cycle delay, despite the fact that its DNA damage is unrepaired. This entry of cells, containing damaged chromosomes, into mitosis results in aberrant segregation of chromosomes (or parts of chromosomes) and cell death. Unlike the *rad* mutants affecting G2-arrest such as *rad1* (15), *rad3* (14), *rad9*, and *rad17* (13), the *rad21-45* mutant is still as resistant to the DNA synthesis inhibitor hydroxyurea (HU) as wild-type cells, indicating that initiation of mitosis in this mutant is still dependent on completion of replication. These data show that the failure of the *rad21-45* mutant to repair dsb after γ-irradiation is not a consequence of a deficiency in recognition of DNA

damage or in the induction of cell cycle arrest in G2 to provide time for DNA repair. The *rad21* gene seems to be involved more directly in DNA repair.

Comparison to the databases did not result in any significant sequence homology of *rad21* to other genes or peptides. This includes the already well characterized genes of the *RAD52* epistasis group of *S.cerevisiae* which are also involved in DNA dsb repair (for references see 38 and 39). The only phenotype which these mutants (*rad51*, *rad52*, *rad54*) share with the *rad21-45* mutant is the pronounced sensitivity to γ-rays and DNA-damaging agents like MMS. Unlike the mutants of the *RAD52* group, the homothallic *rad21-45* mutant has no obvious deficiency in mating-type switching. Spore formation and germination is intact, and instead of being deficient in spontaneous and radiation-induced mitotic recombination like the *S.cerevisiae* mutants, *rad21-45* has been described to be elevated in spontaneous but not radiation-induced recombination (40). However it has to be considered that the *rad21-45* mutant shows only an incomplete phenotype.

The first plasmid p21-5, isolated by its ability to complement the radiation sensitivity of *rad21-45*, contained a truncated gene, missing the C-terminal 63 amino acids. Therefore the *rad21* gene may have more than the one function related to DNA repair which can be complemented by the truncated gene. This is strongly supported by the fact that, unlike the *S.cerevisiae* mutants, the *rad21* gene disruption is lethal even in the absence of DNA damage. Haploid cells carrying the *rad21* null allele die two or three cell divisions after sporulation of diploids heterozygous at this locus.

The experimental data collected thus far for the *S.pombe* *rad21* gene emphasize that at least one of the functions of this gene is DNA repair. It is probably not involved in the major recombinational repair pathway (involving the *rad1* group), as the mutant is still sensitive to caffeine treatment after irradiation. With further experiments we hope to elucidate whether the phenotype of the *rad21-45* point mutation is only a much weaker expression of the *rad21* null allele phenotype or if the lethality of the *rad21* gene disruption results from the loss of other functions required in the mitotic growth of *S.pombe*.

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