RESEARCH COMMUNICATION

Two modes of DNA double-strand break repair are reciprocally regulated through the fission yeast cell cycle

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Several considerations suggest that levels of the two major modes of double-strand break (DSB) repair, homologous recombination (HR), and nonhomologous end joining (NHEJ), are regulated through the cell cycle. However, this idea has not been explicitly tested. In the absence of the telomere-binding protein Taz1, fission yeast undergo lethal telomere fusions via NHEJ. These fusions occur only during periods of nitrogen starvation and fail to accumulate during logarithmic growth, when the majority of cells are in G2. We show that G1 arrest is the specific nitrogen starvation-induced event that promotes NHEJ between *taz1*⁻ telomeres. Furthermore, the general levels of NHEJ and HR are reciprocally regulated through the cell cycle, so that NHEJ is 10-fold higher in early G1 than in other cell cycle stages; the reverse is true for HR. Whereas NHEJ is known to be dispensable for survival of DSBs in cycling cells, we find that it is critical for repair and survival of DSBs arising during G1.

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DNA double-strand breaks (DSBs) are among the most deleterious types of damage with which cellular DNA repair systems must contend. If a DSB is left unrepaired in a dividing cell, the portion of the chromosome that is left unconnected to the centromere is unable to segregate to the daughter cell, giving rise to chromosome deletions. If incorrectly repaired, DSBs may lead to chromosome translocations and other aberrations (Pierce et al. 2001b).

Two major pathways repair DSBs, nonhomologous end joining (NHEJ), and homologous recombination (HR; van Gent et al. 2001). NHEJ joins two DNA ends irrespective of their sequence, thereby generating errors if the two ends are unrelated or inaccurately processed. Alternatively, DSBs can be joined via HR processes that use homologous DNA sequences (usually in the sister chromatid) as templates for repairing broken ends, thus providing error-free repair. HR is the pathway of choice in budding and fission yeast, as NHEJ mutants are resistant

to γ -radiation, whereas HR mutations severely compromise survival of γ -radiation (Siede et al. 1996; Manolis et al. 2001).

Remarkably, although cells can detect and respond to a single DSB generated by DNA damage (Sandell and Zakian 1993), they are perfectly capable of recognizing the numerous ends of chromosomes (e.g., 184 in a G2 human somatic cell) as nondeleterious structures. To conserve genome stability, telomeres have developed the property of being refractory to DNA repair processes. Recent studies have uncovered some of the components underlying this property (Ferreira et al. 2004). In humans, the TRF2 protein binds telomeres and protects them from inappropriate repair reactions; interfering with TRF2 function leads to chromosome end fusions, cell cycle arrest, and apoptosis (Karlseder et al. 1999).

Taz1 is the fission yeast ortholog of both TRF2 and the other human telomeric DNA-binding protein, TRF1 (Cooper et al. 1997; Li et al. 2000; Ferreira and Cooper 2001). In the absence of Taz1, several telomere functions are disrupted, but cells are still viable in unperturbed cell cycles (Cooper et al. 1997, 1998). We have shown that Taz1 loss renders telomeres vulnerable to the two DSB repair pathways, but that end fusions, which form via NHEJ, only occur as cells arrest in G1 during nitrogen starvation (Ferreira and Cooper 2001). During logarithmic growth, fission yeast are mainly in G2 and *taz1*⁻ telomere fusions are absent. However, NHEJ-mediated *taz1*⁻ telomere fusions arise during logarithmic growth in *rad22*⁻ HR-deficient cells, suggesting that HR protects dysfunctional (*taz1*⁻) telomeres from NHEJ. These results led us to propose that NHEJ becomes prominent in G1-arrested cells, whereas HR dominates during logarithmic (mainly postreplicative) growth.

Several other lines of evidence support the idea that the two major modes of DSB repair are cell cycle regulated. From a teleological standpoint, cells might prefer error-free HR whenever possible, that is, when template copies, preferably sister chromatids, are available. Conversely, during G1, error-prone NHEJ would be necessary, as sister chromatid templates are unavailable. Although diploid cells possess homologous chromosomes during G1, mechanisms exist to disfavor mitotic recombination between homologous chromosomes and the potential ensuing loss of heterozygosity in somatic mammalian cells (Moynahan and Jasin 1997). In chicken DT40 lymphocytes, NHEJ mutants (*ku70*^{-/-} and *DNA-PK*^{-/-}) are particularly sensitive to ionizing radiation during G1, whereas *Rad54*^{-/-} HR mutants are more sensitive during S and G2 phases (Takata et al. 1998). Furthermore, levels of plasmid recircularization via NHEJ increase during G1 in budding yeast (Karathanasis and Wilson 2002), whereas the formation of DSB-induced Rad52 HR foci is reduced in G1 cells (Lisby et al. 2001).

Here, we demonstrate that HR and NHEJ are reciprocally regulated through the cell cycle. By changing cell cycle profiles, we can modulate the appearance of NHEJ-mediated telomere fusions during both vegetative growth and nitrogen starvation. Furthermore, by directly measuring repair, we show that levels of the two modes of repair vary through the cell cycle by a factor of 10, with NHEJ being higher in G1 and HR being higher in G2.

[Keywords: Cell cycle; DNA repair; HR; NHEJ; Taz1; telomere]

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Results and Discussion

Occurrence of pre-START G1 during logarithmic growth induces *taz1*⁻ telomere fusions

Logarithmically growing fission yeast increase in size during the G2 phase of the cell cycle, and upon completion of mitosis, cells generally possess sufficient mass to proceed directly to S phase. Thus, the vegetative cell cycle lacks a pre-START G1 phase. However, nitrogen-starved cells arrest in G1. As fission yeast prefers haploidy, these G1 cells contain only one copy of each chromosome.

taz1⁻ cells accumulate telomere fusions when subjected to nitrogen starvation, yet these same cells lack telomere fusions, and indeed show virtually wild-type levels of viability, when grown in rich medium (Ferreira and Cooper 2001). To determine whether the *taz1*⁻ telomere fusions are a consequence of the G1 arrest induced by nitrogen starvation, we investigated cells in which a pre-START G1 phase occurs during vegetative growth. *wee1-50* cells enter mitosis prematurely, forming smaller cells than wild type. These cells cannot initiate replication until sufficient cell mass is attained, and remain in G1 for extended periods with low levels of Cdc2 kinase activity (Russell and Nurse 1987). *wee1-50 taz1*⁻ cultures were grown at the semipermissive temperature of 32°C and divided into halves. One half was starved for nitrogen for 24 h at 32°C, whereas the other was grown in nitrogen-rich medium; aliquots of both were taken for FACS and PFGE analyses. Southern blotting with telomeric probes revealed that telomere fusions were present not only in the nitrogen-starved culture, but also in vegetatively growing *wee1-50 taz1*⁻ cells (Fig. 1B). Thus, by

recreating pre-START G1 during the vegetative cell cycle, we induce telomere fusions in *taz1*⁻ cells growing in nitrogen-rich medium.

The telomere fusions seen in vegetatively growing *wee1-50 taz1*⁻ cells are primarily intramolecular (i.e., resulting in self-circularization), whereas both inter- and intramolecular fusions are seen in nitrogen starved *taz1*⁻ cells (Fig. 1B). Intrachromosomal fusions also predominate in logarithmically growing *taz1*⁻ cells impaired in HR (Ferreira and Cooper 2001). *Schizosaccharomyces pombe* cells possessing circular chromosomes are viable (Naito et al. 1998; Nakamura et al. 1998), whereas interchromosomal fusions generate lethal dicentric chromosomes and are only maintained in nondividing (i.e., G1-arrested) cultures. Therefore, the predominance of intrachromosomal fusions in *wee1-50 taz1*⁻ cells supports the idea that the fusions occur while the cells are actively dividing in rich medium.

Absence of G1 arrest during nitrogen starvation prevents telomere fusions

If prolonging the G1 period during vegetative growth can induce *taz1*⁻ telomere fusions, then the absence of G1 arrest during nitrogen starvation might prevent telomere fusions. To test this, we generated *taz1*⁻ strains lacking the CDK inhibitor Rum1. Upon nitrogen starvation, *rum1*⁻ cells are unable to restrain S phase and arrest with replicated chromosomes and high levels of Cdc2 kinase activity. Nonetheless, *rum1*⁻ mutants do activate the Ste11 transcription factor and its targets in response to nitrogen starvation (Stern and Nurse 1998). As expected, neither *rum1*⁻ nor *rum1*⁻ *taz1*⁻ strains were able to arrest in G1, even after prolonged nitrogen starvation and maintained 2C DNA content (Fig. 1C). Remarkably, *rum1*⁻ *taz1*⁻ cells exhibit no telomere fusions during nitrogen starvation, (Fig. 1C) indicating that the Rum1-dependent G1 arrest, and not some event downstream of Ste11 activation, activates the pathway that results in *taz1*⁻ telomere fusions. Thus, by indirectly manipulating the activity of the Cdc2 kinase, we can manipulate the susceptibility of *taz1*⁻ telomeres to undergo NHEJ-induced fusions in a manner independent of nutritional status, with low Cdc2 activity inducing telomere fusions and high Cdc2 activity preventing them.

Telomeric 3' overhangs persist in G1 cells

taz1⁻ telomeres possess extensive 3' G-strand overhangs during logarithmic growth (Tomita et al. 2003). In principle, such overhangs should promote the strand invasions that initiate HR, whereas NHEJ would require overhang removal. Indeed, upon inhibition of TRF2 function in human cells, NHEJ-dependent telomere fusions are accompanied by a reduction in 3'-overhang signal (van Steensel et al. 1998; Smogorzewska et al. 2002). To investigate whether elevated NHEJ stems from a global loss of 3' overhangs in G1 *taz1*⁻ cells, we used native in-gel hybridization analysis. Telomeric restriction fragments from asynchronous and nitrogen-starved cultures were electrophoresed under nondenaturing conditions and hybridized to a G-strand telomere probe. Both cycling and G1-blocked *taz1*⁻ cells show intense hybridization (Fig. 2A), whereas no signal is detectable using a complementary C-rich strand probe (data not shown). The overhang signal also persists in *taz1*⁻ cells lacking

Figure 1. The stage of the cell cycle determines whether *taz1*⁻ cells sustain telomere fusions. (A) Diagram of telomeric NotI restriction fragments on chromosome I and II (C, I, L, and M); chromosome III lacks NotI restriction sites. (B,C) Southern blot analysis of genomic DNA digested with NotI, separated by PFGE, and probed with a telomeric oligonucleotide. Telomeric restriction fragments are indicated. C* comprises the C restriction fragment as well as the C + L, C + M, and C + I fusions that cannot be resolved under these conditions. (B) Inactivation of *wee1*^{ts} extends early G1 and induces fusions of *taz1*⁻ telomeres during logarithmic growth. The most prominent fusion band (top arrow) corresponds to circularized chromosome I. Prolonged growth of *wee1*^{ts} cells at the semipermissive temperature typically distorts the FACS profile due to delayed cytokinesis in small cells. (C) *rum1*⁻ deletion prevents G1 arrest during nitrogen starvation, and prevents starvation from inducing fusion between *taz1*⁻ telomeres.

Figure 2. *taz1⁻* telomeres exhibit extensive ssDNA overhangs in G1. (A) Telomere 3' overhangs are detected in nitrogen-starved *taz1⁻*, *pku70⁻ taz1⁻*, *lig4⁻ taz1⁻* cells. In-gel hybridization of EcoRI-digested genomic DNA to a G-strand telomere oligonucleotide in nondenaturing and denaturing conditions. (B) Quantitation of telomeric 3' overhangs. Hybridization in each lane was quantified, and the ratio of nondenatured/denatured signal for each sample was normalized against the ratio derived from logarithmically growing *taz1⁻* cells. Error bars represent standard deviation for a minimum of three independent experiments.

telomere fusions (via *pku70⁻* and *lig4⁻* deletion). A caveat of the native hybridization method is that it can only address global changes in the abundance of overhanging DNA and does not address individual telomeres. Hence, some telomeres may lose overhangs, whereas others gain them, generating no net change in signal strength. Nevertheless, we speculate that the NHEJ machinery can engage *taz1⁻* overhang-containing telomeres as substrates, and that overhang removal is concomitant with the end-joining reaction. Furthermore, our observation that NHEJ-dependent telomere fusions do occur in logarithmically growing *taz1⁻* cells that lack Rad22 (Ferreira and Cooper 2001) argues against the idea that G2 *taz1⁻* telomeres are incompetent for NHEJ.

DSB repair pathways are cell cycle regulated

The appearance of telomere fusions in log-phase *taz1⁻rad22⁻* cells suggested that HR precludes NHEJ at dysfunctional telomeres during vegetative growth (Ferreira and Cooper 2000). Thus, unprotected telomeres may be recipients of whatever DNA repair pathway predominates generally in the cell at a given cell cycle stage. This idea implies that cell cycle regulation of DNA repair would be seen not only at telomeres, but also throughout the genome. To test this, we directly measured the two main modes of DSB repair in different stages of the cell cycle. We used nitrogen-starved cells as representative of the G1 phase and logarithmically growing cells as representative of the S, G2, and M phases. FACS analysis indicated that upon nitrogen starvation, 60%–70% of cells arrest in G1, whereas ~100% of the

cells in logarithmically growing cultures exhibit only a 2C DNA peak.

Plasmid-based assays have been used in both fission and budding yeasts to investigate the genetic requirements for NHEJ and HR (Orr-Weaver et al. 1981; Keeney and Boeke 1994; Boulton and Jackson 1996; Muris et al. 1997; Baumann and Cech 2000; Manolis et al. 2001). To assess NHEJ, a plasmid containing a replication origin is linearized within sequences that lack homology to the yeast genome, and then transformed into yeast cells. The uncut plasmid is transformed in parallel to normalize for differences in transformation efficiencies between strains. Logarithmically growing *S. pombe* cells display a low plasmid end-joining efficiency (Fig. 3A) and, as observed previously, this end-joining is almost entirely dependent on *pku70⁺* (Baumann and Cech 2000; Manolis et al. 2001). Strikingly, end-joining levels were seven- to 10-fold higher in nitrogen-starved G1 cells (Fig. 3A). However, this elevation of NHEJ was absent in nitrogen-starved *rum1⁻* cells that are unable to undergo G1 arrest (Fig. 3A). Thus, Ku-dependent DSB repair is up-regulated in G1-arrested cells, consistent with our hypothesis that *taz1⁻* telomeres fuse during G1 arrest, because NHEJ is generally elevated under these conditions. Moreover, the genetic requirement of *rum1⁺* for elevated NHEJ suggests that Rum1-dependent down-regulation of Cdc2 kinase is a key event in the switch between the two modes of repair.

A straightforward model of direct competition between NHEJ and HR pathways would predict that, in the absence of HR, NHEJ levels would rise during logarithmic growth. However, we did not detect an increase in plasmid end-joining efficiency in logarithmically growing HR-deficient *rad22⁻* or *rhp51⁻* mutants (Fig. 3A). This suggests that the two pathways are regulated independently, such that in the absence of one, the other mode of repair is still subject to cell cycle control. Alternatively, DNA ends that attempt HR in the absence of *rad22⁺* or *rhp51⁺* may be reversed and channeled to NHEJ.

To measure HR during different stages of the cell cycle, we used a plasmid-integration assay in which *leu1⁻* strains are transformed with a plasmid that lacks a replication origin and is linearized within the *leu1⁺* auxotrophic marker. These strains must integrate the

Figure 3. NHEJ and HR are reciprocally regulated through the cell cycle. (A) NHEJ is up-regulated in G1 (–N) arrested cells. Levels of plasmid end joining are represented as the number of transformants obtained with linear vs. circular plasmid. Cells lacking Rum1 fail to arrest in G1 and NHEJ levels remain low. (B) HR is down-regulated in G1 nitrogen-starved cells. Integration of a linear *leu1⁺* pJK148 plasmid into the genome is expressed as the ratio of transformants for the linearized vector (which lacks an origin of replication) vs. supercoiled pJK148-ars1. Rum1 is required for down-regulation of HR during nitrogen starvation.

plasmid to survive in medium lacking leucine. A circular plasmid carrying an origin of replication and *leu1⁺* was transformed in parallel to normalize for transformation efficiencies. Levels of plasmid integration were high during logarithmic growth and dependent on *rhp51⁺* (Fig. 3B), as previously reported (Muris et al. 1997). Deletion of *rad22⁺*, the fission yeast *RAD52* homolog, also led to a severe reduction in HR, in agreement with van den Bosch et al. (2001) and in contrast to the modest effect on HR reported for a truncation of *rad22⁺* (Ostermann et al. 1993; Muris et al. 1997). Conversely to NHEJ, plasmid integration levels were reduced 10- to 15-fold in G1-arrested cells (Fig. 3B). Southern blotting demonstrated that ~90% of the integration events occurred at the chromosomal *leu1* locus (data not shown), confirming that HR had been the pathway for integration. *rum1⁻* cells failed to exhibit reduced HR during nitrogen starvation, suggesting that down-regulation of HR depends upon G1 arrest. In agreement with a gene-targeting transfection assay in mammalian cells (Pierce et al. 2001a), deletion of *pku70⁺* did not lead to an elevation of HR (Fig. 3B).

Sister chromatid cohesion is a candidate regulator of HR through the cell cycle (Jessberger 2002), as cohesion is established during S phase and is required for efficient DSB repair. Coupled with the observed preference for sister chromatids over homologs as substrates for HR (Kadyk and Hartwell 1992; Moynahan and Jasin 1997), these observations suggest that HR dominates in G2 through the availability of a cohesed sister chromatid. Hence, the prevalence of HR at *taz1⁻* telomeres during G2 could simply reflect this availability. However, we were unable to promote telomere fusions during logarithmic growth by disrupting the cohesion complex in a *rad21^{ts} taz1⁻* double mutant (data not shown). Further-

more, the plasmid recircularization and integration events that we used as measures of NHEJ and HR occur in the absence of cohesion between damaged and undamaged substrates.

Thus, the two major modes of DSB repair are regulated reciprocally, such that during logarithmic growth, when cells harbor two copies of each chromosome, HR dominates over NHEJ. Conversely, in G1-arrested cells that contain only one copy of each chromosome, the levels of these two modes of repair are inverted, with lower HR and higher NHEJ. These patterns of general repair levels mirror the activities that we observe at *taz1⁻* telomeres, and support the idea that cell cycle regulation of repair directs the consequences of telomere dysfunction. The telomere fusions that follow expression of dominant-negative TRF2 in human cells can arise in both G1 and G2 (Smogorzewska et al. 2002), although the latter may reflect a requirement for passage through S phase to allow displacement of wild-type TRF2 by the dominant-negative form. Nonetheless, a relative paucity of fusions between dysfunctional telomeres during G2 in fission yeast versus human cells is consistent with the idea that mammalian cells have generally higher levels of NHEJ than the yeast systems.

NHEJ is required for radiation resistance during G1

Previous studies in yeasts showed that survival following DSB induction depended entirely on HR, whereas NHEJ mutants were insensitive to DSBs (Siede et al. 1996; Manolis et al. 2001). Our observation that NHEJ levels increase during G1 prompted us to test whether NHEJ becomes important for survival of DSBs during G1 arrest. Using wild-type cells and mutants in the HR

Figure 4. NHEJ is required for survival and repair of damage induced by γ -radiation during G1. (A,B) Logarithmic (Log) and nitrogen starved (-N) cultures were treated with varying doses of γ -radiation. NHEJ mutants are only sensitive during the G1 arrest induced by nitrogen starvation. (A) Serial dilution assay. (B) Quantitative survival analysis. (C) Cells were exposed to 100 Gy of γ radiation and allowed to recover for varying amounts of time. G1-arrested wild-type (*wt*) cells undergo DSB repair faster than NHEJ mutants, whereas NHEJ is dispensable for repair during log growth. (D) Quantitation of the data in C. Ethidium bromide signals corresponding to broken DNA were normalized to the signal for intact chromosome II for each lane. The value obtained at time 0 was designated as 1.00.

(*rad22⁻* and *rhp51⁻*) and NHEJ (*pku70⁻* and *lig4⁻*) pathways, we analyzed the viability of logarithmic and nitrogen-starved cultures exposed to varying doses of γ -irradiation (Fig. 4A,B). Whereas logarithmically growing HR mutants were extremely sensitive to γ -irradiation, NHEJ mutants exhibited viabilities comparable to that of wild-type cells (Fig. 4A). During nitrogen starvation, however, NHEJ mutants were 10-fold more sensitive to γ -irradiation than wild-type cells, denoting a role for NHEJ in survival of radiation during G1 (Fig. 4A,B). Interestingly, wild-type cells lose viability more severely during G1 than during logarithmic growth (Fig. 4B), perhaps indicating that elevated use of potentially inaccurate NHEJ during G1 confers some lethality. HR genes are also important for survival of γ -irradiation during nitrogen starvation (Fig. 4A,B), although dependence on HR is less severe in nitrogen-starved than logarithmic cultures (Fig. 4B). This could reflect HR that occurs following release from nitrogen starvation, the ~30% of cells that do not arrest in G1 upon nitrogen starvation, or HR-related processes that occur during G1 arrest.

NHEJ is required for efficient DSB repair in G1

As assessment of survival can only address the success of DSB repair and not when it was undertaken, we directly monitored the recovery of whole chromosomes following breakage by ionizing radiation. G1-arrested cells were exposed to 100 Gy of ionizing radiation to induce DSBs, then kept in G1 and analyzed by PFGE to monitor DSB repair as a function of time. Whereas whole chromosomes from untreated cells are visible as three intact bands (Fig. 4C), the irradiated cells accumulate degraded DNA appearing as a smear of higher-mobility DNA fragments. Remarkably, G1-arrested wild-type cells not only sustain less DNA damage, but also recover chromosome integrity faster than *pku70⁻* and *lig4⁻* mutants (Fig. 4C,D). This result demonstrates that repair occurs while the cells are blocked in G1, and that efficient repair requires the NHEJ pathway. *pku70⁻* and *lig4⁻* mutants only started to regain intact chromosomes upon prolonged recovery periods, consistent with the moderate decline of viability of NHEJ mutants. In IR-treated logarithmic cultures, recovery rates were independent of *pku70⁻* and *lig4⁻* (Fig. 4D), reinforcing the idea that NHEJ is a prominent mode of DSB repair during G1 exclusively.

Perspectives

As manipulating Cdc2 kinase activity can alter the relative levels of NHEJ and HR, we infer that Cdc2 is the ultimate determinant of the choice of repair pathway, with low levels of Cdc2 activity dictating elevated NHEJ and high levels directing HR. Indeed, replication studies have established that levels of Cdc2 kinase activity distinguish cells that have or have not accomplished genome duplication. This same kinase may direct DSBs to error-free repair pathways when sister chromatids are present. These ideas complement a recent report (Caspari et al. 2002) describing a

mutation in the B-type cyclin Cdc13 that reduces the formation of Rhp51 foci and impairs a later step in HR in response to DSBs during G2. It will be of great interest to identify the targets of Cdc2 kinase that control the regulation of DNA repair.

Materials and methods

Strains and medium

Strains are listed in Table 1. Cultures were grown at 32°C in standard YES or EMM medium with or without NH₄Cl and any required supplements.

Gene disruption strains

Gene disruption was performed using the method of Bahler et al. (1998), and verified by PCR using primers for the integrating constructs and flanking genomic sequences.

Nitrogen starvation and FACS analysis

Cultures were grown to log phase (0.5×10^7 cells/mL), washed extensively with EMM-N, resuspended in EMM-N at a density of $1\text{--}5 \times 10^6$ cells/mL, and starved for 36–72 h. FACS was performed on ethanol-fixed cells on a Becton Dickinson FACSscan.

Pulse-field gel electrophoresis, in-gel hybridization, and Southern blotting

Pulsed-field gel electrophoresis was performed as previously described (Ferreira and Cooper 2001). Telomere overhangs were analyzed as in Tomita et al. (2003). Overhang signals were quantified using Molecular Dynamics ImageQuant software.

Plasmid assays

The NHEJ plasmid assay was performed essentially as described (Boulton and Jackson 1996). The plasmid pKan1 (Haering et al. 2000) was linearized with KpnI and cells transformed in triplicate with 1 μ g of linear or circular pKan1. Cells were spread on YES containing 100 μ g/mL G418 and colonies counted after 5 d at 32°C. The HR plasmid assay was similar to that previously described (Keeney and Boeke 1994). Strains auxotrophic for *leu1⁺* were transformed in triplicate with 1 μ g NdeI-linearized pJK148 as above. To assess the uptake of DNA, an aliquot of competent cells of each strain was transformed with 1 μ g of pJK148-ars1 (same as pJK148, but with the EcoRI fragment of *ars1* filled in and cloned into the SmaI site of pJK148). Transformants were selected on EMM lacking leucine, and integration frequencies calculated by dividing the number of pJK148 *leu1⁺* colonies by the number of pJK148-ars1 *leu1⁺* colonies. For each such ratio, the average and standard deviation of at least three replicates of each transformation is presented.

Radiation sensitivity and DSB repair assay

Cultures were either diluted in triplicate to yield 200 colonies per plate or serially diluted 1:5 in 96-well plates and spotted on YES medium. The cells were immediately exposed to γ -rays using an IBL 637 Irradiator

Table 1. *S. pombe* strains used in this work

Strain	Genotype	Source
Wild type	<i>h⁻ ade6-M210 leu1-32 ura4-D18</i>	
<i>taz1⁻</i>	<i>h⁻ taz1::ura4⁺ ade6-M210 leu1-32 ura4-D18</i>	J.P. Cooper
<i>wee1^{ts}</i>	<i>wee1-50 ura4-D18</i>	P. Nurse
<i>wee1^{ts} taz1⁻</i>	<i>wee1-50 taz1::ura4⁺ ura4-D18</i>	This study
<i>rum1⁻</i>	<i>rum1::ura4⁺ ura4-D18</i>	S. Moreno
<i>rum1⁻ taz1⁻</i>	<i>rum1::ura4⁺ ura4-D18 taz1::kan^r</i>	This study
<i>rum1⁻ leu1⁻</i>	<i>h⁻ rum1::ura4⁺ ade6-M216 leu1-32 ura4-D18</i>	P. Nurse
<i>pku70⁻ leu1⁻</i>	<i>pku70::kan^r his3-D1 leu1-32 ura4-D18 ade6⁻</i>	P. Baumann
<i>pku70⁻ kan^s</i>	<i>h⁻ pku70::ura4⁺ leu1-32 ura4-D18 ade6-M210</i>	This study
<i>rad22⁻ ura4⁻</i>	<i>h⁺ rad22::LEU2 his3-D1 leu1-32 ura4-D18</i>	T. Nakamura
<i>rad22⁻ leu1⁻</i>	<i>h⁻ rad22::ura4⁺ ade6-M210 leu1-32 ura4-D18</i>	This study
<i>rhp51⁻</i>	<i>h⁺ rhp51::ura4⁺ ade6-704 leu1-32 ura4-D18</i>	A.M. Carr

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[^{137}Cs source at a rate of 3.45 Gy/min]. Plates were then incubated for 3–5 d at 32°C. The results are presented as the percentage of survivors of a given dose relative to the viabilities of the same strains unirradiated. Each experiment was repeated three times.

DSB repair assay

Logarithmic and nitrogen-starved cultures were exposed to 100 Gy γ -radiation in liquid medium. Samples were collected at various times and processed for PFGE as described above. Whole chromosomes were separated on a CHEF-DRIII apparatus (Bio-Rad) according to the manufacturer's specifications. Gels were stained with ethidium bromide and digitized using a UVP VisiDoc-IT system without oversaturation. Broken DNA and intact chromosome signals were quantified using NIH Image version 1.63 software.

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