Dual role for *Saccharomyces cerevisiae* Tel1 in the checkpoint response to double-strand breaks

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The main responder to DNA double-strand breaks (DSBs) in mammals is ataxia telangiectasia mutated (ATM), whereas DSB-induced checkpoint activation in budding yeast seems to depend primarily on the ATM and Rad-3-related (ATR) orthologue Mec1. Here, we show that *Saccharomyces cerevisiae* Tel1, the ATM orthologue, has two functions in checkpoint response to DSBs. First, Tel1 participates, together with the MRX complex, in Mec1-dependent DSB-induced checkpoint activation by increasing the efficiency of single-stranded DNA accumulation at the ends of DSBs, and this checkpoint function can be overcome by overproducing the exonuclease Exo1. Second, Tel1 can activate the checkpoint response to DSBs independently of Mec1, although its signalling activity only becomes apparent when several DSBs are generated. Furthermore, we provide evidence that the kinetics of DSB resection can influence Tel1 activation, indicating that processing of the DSB termini might influence the transition from Tel1/ATM- to Mec1/ATR-dependent checkpoint.

Keywords: Tel1; checkpoint; DSB; Exo1; MRX

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INTRODUCTION

Eukaryotic cells respond to DNA double-strand breaks (DSBs) by activating signal-transduction pathways known as DNA damage checkpoints, which temporarily halt the cell cycle while the damage is being repaired. At the center of the DNA damage checkpoint are two related protein kinases, which are called ataxia telangiectasia mutated (ATM) and ATM and Rad-3-related (ATR) in mammals, and Tel1 and Mec1 in *Saccharomyces cerevisiae* (Longhese *et al*, 2006; Shiloh, 2006).

Several studies in *S. cerevisiae* have shown that the ends of DSBs are subjected to nucleolytic degradation to generate 3'-ended single-stranded DNA (ssDNA) tails. Human ATM is primarily activated by DNA DSBs caused by ionizing radiation, and is required for the generation of ionizing radiation-induced

Replication Protein A (RPA) foci that lead to ATR recruitment and subsequent ATR-dependent checkpoint activation (Zou & Elledge, 2003; Myers & Cortez, 2006; Adams *et al*, 2006; Jazayeri *et al*, 2006). Conversely, in *S. cerevisiae* checkpoint activation in response to DSBs seems to depend primarily on the ATR orthologue Mec1 (Ira *et al*, 2004; Clerici *et al*, 2006), whereas the contribution of Tel1 to this process has not been clearly assessed.

To understand the possible role(s) of Tel1 in the checkpoint response to DSBs, we examined the ability of Tel1 to activate this response in either the presence or absence of Mec1. We now provide evidence that Tel1 not only influences DSB resection and subsequent Mec1 activation but it is also able to sense and signal DSBs independently of Mec1, although its signalling activity becomes apparent only after the generation of multiple DSBs.

RESULTS AND DISCUSSION

Tel1 contributes to 3'-ended ssDNA accumulation

Cells carrying a single irreparable DSB undergo a checkpoint-mediated cell-cycle block that is primarily dependent on Mec1, which recognizes RPA-coated ssDNA regions arising from DSB processing (Pellicioli *et al*, 2001; Zou & Elledge, 2003).

To analyse whether Tel1 has a role in activating this DSB-induced checkpoint, we examined checkpoint activation after the induction of a single unrepaired DSB in *tel1Δ* cells. A single DSB can be generated at the MAT locus of JKM139 derivative strains by expressing the site-specific HO endonuclease gene from a galactose-inducible promoter. The HO cut cannot be repaired by homologous recombination because the homologous donor sequences *HML* and *HMR* are deleted in JKM139 (Lee *et al*, 1998). When galactose was added to cell cultures that were blocked in mitosis with nocodazole, to avoid specific cell-cycle effects on DSB processing, phosphorylation of the Rad53 checkpoint kinase, which is required for its activation, was detected as an electrophoretic mobility shift about 45 min after HO induction in both wild-type and *tel1Δ* cells (Fig 1A). However, its amount was slightly lower in *tel1Δ* than in wild-type cells (Fig 1A), indicating that Tel1 might participate in activating the checkpoint in response to DSBs.

In human cells, both ATM and the Mre11–Rad50–Nbs1 (MRN) complex have been shown to be necessary for the generation of ionizing radiation-induced RPA foci required for ATR recruitment (Zou & Elledge, 2003; Myers & Cortez, 2006; Adams *et al*, 2006;

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Fig 1 | The lack of Tel1 impairs Rad53 phosphorylation and single-stranded DNA formation in response to a single DNA double-strand break. (A) YEP + raf nocodazole-arrested cell cultures of wild-type (WT) JKM139 and isogenic *tel1Δ*, *exo1Δ* and *tel1Δ exo1Δ* strains (time 0) were transferred to YEP + raf + gal to induce HO expression in the presence of nocodazole. Western blots of protein extracts prepared at the indicated time points were probed with Rad53 antibodies. (B) Schematic representation of the region immediately centromere-distal to the MAT HO site (bottom), and of the DSB and 5'-to-3' resection products (top) detectable with the indicated ssRNA probe after alkaline gel electrophoresis of *SspI* (S)-digested DNA. The probe is specific for the MAT locus and reveals a 1.1-kb fragment from the uncut MAT locus. When HO cuts the MAT locus, a smaller 0.9-kb HO-cut fragment is produced. 5'-to-3' resection progressively eliminates *SspI* sites, generating larger ssDNA *SspI* fragments (r1–r7) detected by the probe. (C) Genomic DNA prepared from samples taken at the indicated time points during the experiment in (A) was digested with *SspI* and run on alkaline agarose gel, followed by gel blotting and hybridization with the ssRNA probe shown in (B). DSB, DNA double-strand break; ssDNA, single-stranded DNA; ssRNA, single-stranded RNA; YEP + raf, yeast extract peptone and raffinose; YEP + raf + gal, YEP + raf and galactose.

Jazayeri et al, 2006). This prompted us to investigate whether the lack of Tel1 might impair Mec1-dependent signalling activity by altering ssDNA accumulation. When we monitored the kinetics of both HO-cut and 3'-ended ssDNA formation at the MAT locus in the galactose-induced cell cultures, *tel1Δ* JKM139 derivative cells showed some delay in the accumulation of 3'-ended resection products (r1–r7 in Fig 1B) compared with wild type (Fig 1C). Furthermore, the accumulation of resection products in *tel1Δ* cells lacking the exonuclease Exo1 was very slow compared with both *tel1Δ* and *exo1Δ* single-mutant cells (Fig 1C), indicating that Tel1 indeed has a role in DSB end processing. The synergistic effect on DSB processing observed in the *tel1Δ exo1Δ* double mutant

indicates that Tel1 and Exo1 participate in DSB resection independently of each other, suggesting that they act in two different pathways. Strikingly, as shown in Fig 1A, Rad53 phosphorylation was also markedly reduced in nocodazole-arrested galactose-induced *tel1Δ exo1Δ* double-mutant cells compared with *tel1Δ* and *exo1Δ* single mutants (Fig 1A). Thus, the reduced efficiency of ssDNA generation in *tel1Δ* cells might account for the Rad53 phosphorylation defects of the same cells.

A crucial implication of the above data is that Tel1 contributes to DSB resection by activating an exonuclease that resects DSB ends. A good target candidate is the Mre11–Rad50–Xrs2 (MRX) complex, which acts in DSB resection independently of Exo1, and

Fig 2 | *EXO1* overexpression overcomes the defective response of *tel1Δ* and *mre11Δ* cells to a single DNA double-strand break. (A,B) YEP + raf nocodazole-arrested cell cultures of wild-type (WT) JKM139 and isogenic *mre11Δ* and *tel1Δ* strains containing 2μ plasmids, either empty or carrying the *EXO1* gene (time 0), were transferred to YEP + raf + gal in the presence of nocodazole to induce HO expression. Genomic DNA from samples collected at the indicated time points was analysed as described in Fig 1B. (C) Western blots of protein extracts prepared at the indicated times were probed with Rad53 antibodies. DSB, DNA double-strand break; Exp, exponentially growing cells; YEP + raf, yeast extract peptone and raffinose; YEP + raf + gal, YEP + raf and galactose.

is required for the recruitment of both Tel1 and Mec1 to DSB ends (Nakada *et al*, 2003, 2004; Ira *et al*, 2004; Clerici *et al*, 2006). Furthermore, it is necessary to activate the checkpoint in response to a single HO-induced DSB, as phosphorylated Rad53 did not accumulate in galactose-induced JKM139 *mre11Δ* derivative cells, which markedly delayed resection of the HO-induced DSB ends (Fig 2A,C).

If the only function of both Tel1 and MRX in activating the Mec1-dependent checkpoint was to enhance the efficiency of ssDNA accumulation, their requirement might be overcome by artificially restoring wild-type levels of 3'-ended ssDNA accumulation. We therefore investigated whether an excess of Exo1, which has been shown to suppress the hypersensitivity of *mre11Δ* cells to genotoxic agents (Tsubouchi & Ogawa, 2000; Moreau *et al*, 2001; Lewis *et al*, 2002), could restore DSB resection and Rad53 phosphorylation in *mre11Δ* and *tel1Δ* cells (Fig 2). Indeed, when galactose was added to nocodazole-arrested cell cultures,

both *tel1Δ* and *mre11Δ* cells carrying the *EXO1* gene on 2μ plasmids accumulated 3'-ended resection products (Fig 2A,B) and phosphorylated Rad53 (Fig 2C) more efficiently than isogenic cells carrying the empty vector. Therefore, in a manner similar to their mammalian orthologues, both Tel1 and the MRX complex seem to contribute to Mec1-dependent checkpoint activation by allowing the generation of 3'-ended ssDNA that in turn leads to Mec1 recruitment to DSB ends. Interestingly, Rad53 phosphorylation and 3'-ended ssDNA accumulation occurred less efficiently in *mre11Δ* than in isogenic *tel1Δ* cells (Fig 2), indicating that MRX has additional functions in DSB resection compared with Tel1.

Tel1-dependent checkpoint activation by DSBs

Although the above findings indicate that Tel1 contributes to the activation of the Mec1-dependent checkpoint in response to a single DSB, they did not exclude the possibility that Tel1 could sense and transduce the DSB signal independently of Mec1. As

Fig 3 | Response to a single DNA double-strand break in *mec1Δ* cells. (A,B) Cell cultures of wild-type (WT) JKM139 and isogenic *exo1Δ*, *mec1Δ* and *mec1Δ exo1Δ* strains, exponentially growing in YEP + raf (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples withdrawn at the indicated times were used for FACS analysis of DNA contents (A) and western blot analysis of protein extracts with Rad53 antibodies (B). (C,D) Cell cultures of wild-type JKM139 and isogenic *GAL-TEL1*, *GAL-TEL1 mec1Δ* and *mec1Δ* strains, exponentially growing in YEP + raf (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples were collected at the indicated times for western blot analysis with Rad53 antibodies (C) and to determine the percentage of mononucleate large budded cells (D). DSB, DNA double-strand break; FACS, fluorescence-activated cell sorting; YEP + raf, yeast extract peptone and raffinose; YEP + raf + gal, YEP + raf and galactose.

shown in Fig 3, Tel1 could not trigger Rad53 phosphorylation and cell-cycle arrest in *mec1Δ* cells after generation of a single HO-induced DSB. When galactose was added to exponentially growing cell cultures, *mec1Δ* cells neither arrested the cell cycle (Fig 3A) nor underwent detectable Rad53 phosphorylation (Fig 3B), whereas most wild-type cells arrested with 2C DNA contents and heavily phosphorylated Rad53 2 h after galactose addition.

Tel1 and the MRX complex are among the first proteins recruited to DSB ends (Lisby *et al*, 2004); therefore Tel1 might recognize and signal unprocessed DSBs. Thus, we investigated whether delaying nucleolytic degradation of the DSB ends by eliminating Exo1 could enhance its signalling activity. However, Rad53 phosphorylation was still below detection level in *exo1Δ mec1Δ* double-mutant cells (Fig 3B), which did not arrest the cell cycle after HO induction (Fig 3A). Therefore, generation of a single DSB is not sufficient to activate a Tel1-dependent checkpoint even if DSB resection is delayed.

Conversely, the ability of Tel1 to sense and transduce the DSB signal became apparent by increasing the amount of Tel1. When galactose was added to *GAL-TEL1* cell cultures to induce the expression of both HO and *TEL1* at high levels, *GAL-TEL1 mec1Δ* cells slightly phosphorylated Rad53 (Fig 3C) and a subset of them

transiently accumulated as mononucleate large budded cells (Fig 3D). However, both Rad53 phosphorylation and cell-cycle arrest in *GAL-TEL1 mec1Δ* cells occurred less efficiently than in wild-type or *GAL-TEL1* cells, indicating that Tel1 is not rate limiting for checkpoint activation after the generation of a single DSB.

These results prompted us to investigate whether increasing the number of DSBs could uncover the contribution of physiological amounts of Tel1 in sensing and transducing DSB signals. To generate a fixed number of DSBs, we used yeast strains containing one, six or ten HO-recognition sites embedded within the randomly dispersed Ty1 elements, in addition to the endogenous HO-cut site present at the *MAT* locus (Llorente & Symington, 2004). As shown in Fig 4A,B, the generation of 2, 7 or 11 HO-induced DSBs caused most wild-type cells to arrest, as expected, as mononucleate large budded cells with heavily phosphorylated Rad53. Conversely, *mec1Δ* cells with two HO-cut sites neither arrested the cell cycle nor underwent significant Rad53 phosphorylation after HO induction (Fig 4A,B). Strikingly, seven HO-induced DSBs triggered Rad53 phosphorylation in *mec1Δ* cells that also transiently accumulated as mononucleate large budded cells, and induction of 11 HO cuts further increased both Rad53 phosphorylation and the fraction of G2-arrested *mec1Δ* cells (Fig 4A,B). Both HO-induced cell-cycle arrest and Rad53

Fig 4 | Multiple DNA double-strand breaks trigger Tel1-dependent checkpoint activation. (**A,B**) Cell cultures of wild-type (WT) LSY1170, LSY1223, LSY1259 and isogenic *mec1Δ* strains, exponentially growing in raffinose-containing selective medium (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples collected at the indicated times were used to determine the percentage of mononucleate large budded cells (**A**) and for western blot analysis with Rad53 antibodies (**B**). (**C**) LSY1259 *mec1Δ* and isogenic *mec1Δ tel1Δ* cell cultures, exponentially growing in raffinose-containing selective medium (time 0), were transferred to YEP + raf + gal to induce HO expression, and protein extracts prepared at the indicated times were used for western blot analysis with Rad53 antibodies. (**D**) Cell cultures of wild-type LSY1170, LSY1259 and isogenic *mec1Δ* strains, all expressing the *MRE11-HA3*-tagged allele from the corresponding endogenous promoter and exponentially growing in raffinose-containing selective medium (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples withdrawn at the indicated times were used for western blot analysis with HA antibodies. (**E**) Nocodazole-arrested (noc) cell cultures of wild-type JKM139 and isogenic *exo1Δ*, *mec1Δ*, *mec1Δ exo1Δ*, *tel1Δ*, *tel1Δ exo1Δ* and *tel1Δ mec1Δ* strains were transferred to YEPD containing 10 μg/ml phleomycin and 15 μg/ml nocodazole (+ phleo + noc). Western blot analysis with Rad53 antibodies was carried out on protein extracts prepared at the indicated times. (**F**) α-factor-arrested wild-type JKM139 and isogenic *mec1Δ*, *tel1Δ* and *tel1Δ mec1Δ* cell cultures were transferred at time zero (αf) in YEPD containing 5 μg/ml phleomycin and 5 μg/ml α-factor (+ phleo + α-factor), followed by western blot analysis with Rad53 antibodies on protein extracts prepared at the indicated times. Exp, exponentially growing cells; YEP + raf, yeast extract peptone and raffinose; YEP + raf + gal, YEP + raf and galactose.

phosphorylation in these *mec1Δ* cells were dependent on Tel1. Rad53 phosphorylation was not detectable when 11 HO-induced DSBs were generated in *mec1Δ tel1Δ* double-mutant cells (Fig 4C), which did not accumulate as large budded cells after the addition of galactose (data not shown). Therefore, Tel1 can, in part, substitute for Mec1 in activating the checkpoint in response

to multiple DSBs, and its signalling activity increases by increasing the number of DSBs.

We also found that both wild-type and *mec1Δ* cells phosphorylated Mre11 at comparable levels after the generation of 11 HO-induced DSBs, whereas Mre11 phosphorylation was not detectable in either wild-type or *mec1Δ* cells after induction of

Fig 5 | Kinetics of DNA double-strand break resection and Tel1-dependent checkpoint activation. (A–C) Cell cultures of LSY1259 (WT) and isogenic *mec1Δ*, *exo1Δ* and *mec1Δ exo1Δ* strains, exponentially growing in raffinose-containing selective medium (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples were collected at the indicated times to prepare genomic DNA for the analysis of DSB resection at the *MATα* locus (A), protein extracts for western blot analysis with Rad53 antibodies (B) and to determine the percentage of mononucleate large budded cells (C). To detect DSB formation and 5'-to-3' resection products, genomic DNA was digested with *Bam*HI and *Sty*I and separated on alkaline agarose gel. As indicated on the right part of (A), gel blot hybridization with the indicated single-stranded RNA probe specific for the *MAT* locus reveals HO-cut and uncut fragments of 0.7 and 1.9-kb, respectively. 5'-to-3' resection progressively eliminates *Bam*HI (B) and *Sty*I (S) sites, generating larger ssDNA fragments (r1–r6) detected by the probe. (D, E) Cell cultures of LSY1259 *mec1Δ* strains transformed with 2μ plasmids, either empty or carrying the *EXO1* gene, exponentially growing in raffinose-containing selective medium (time 0), were transferred to YEP + raf + gal to induce HO expression. Samples were collected at the indicated times to prepare genomic DNA (D) for the analysis of DSB resection as in (A) and protein extracts for western analysis with Rad53 antibodies (E). DSB, DNA double-strand break; YEP + raf, yeast extract peptone and raffinose; YEP + raf + gal, YEP + raf and galactose.

two DSBs (Fig 4D). Mre11 phosphorylation specifically depends on Tel1; therefore this indicates that the presence of Mec1 does not impair DSB-induced Tel1 signalling activity.

The Mec1-independent function of Tel1 in checkpoint activation in response to multiple DSBs was not limited to the checkpoint triggered by HO cuts. In fact, Rad53 phosphorylation was also induced by treatment with the radiomimetic drug

phleomycin of α -factor- or nocodazole-arrested *mec1Δ* cell cultures, whereas it was undetectable in similarly treated *mec1Δ tel1Δ* double-mutant cells (Fig 4E,F).

DSB resection kinetics influence Tel1 signalling activity

Although the above data indicated that Tel1 could trigger checkpoint activation by itself in response to multiple DSBs,

Tel1 signalling activity was transient. Rad53 phosphorylation decreased in *mec1Δ* cells 180 and 210 min after the generation of 7 or 11 HO cuts, respectively, and most cells concomitantly resumed cell-cycle progression (Fig 4A,B). To gain insights into this, we investigated whether Tel1 signalling activity was disrupted when the DSB ends were subjected to 5′–3′ nucleolytic degradation. Consistent with this hypothesis, we found that *EXO1* deletion increased the amount of Tel1-dependent Rad53 phosphorylation when G2-arrested *mec1Δ* cells were treated with phleomycin (Fig 4E). Furthermore, phleomycin treatment could induce Tel1-dependent Rad53 phosphorylation in G1-arrested *mec1Δ* cells (Fig 4F), where resection of an HO-induced DSB was shown to be impaired due to low Cyclin B/Cyclin-dependent-kinase 1 (Clb/Cdk1) activity (Ira et al, 2004). However, nucleolytic degradation of HO-induced DSBs in G1-arrested cells was not abolished (Shroff et al, 2004); therefore the lack of Mec1 still had a more marked effect on impairing phleomycin-induced Rad53 phosphorylation than the lack of Tel1 (Fig 4F).

Our strains were not suitable for measuring the effects of *EXO1* deletion on resection of phleomycin-induced for DSBs; therefore we monitored both DSB resection at the *MATα* HO-cut site and checkpoint activation in *exo1Δ mec1Δ* cells carrying 11 HO-recognition sites. When galactose was added to cell cultures growing exponentially in raffinose, the accumulation of 3′-ended resection products (r1 to r6, in Fig 5A, right) was delayed (Fig 5A), and both metaphase arrest and Rad53 phosphorylation persisted longer in *mec1Δ exo1Δ* double-mutant cells compared with otherwise isogenic *mec1Δ* cells (Fig 5B,C). Thus, delaying DSB resection increased the persistence of the Tel1-dependent checkpoint, indicating that Tel1 signalling activity is disrupted when the DSB ends are subjected to 5′–3′ nucleolytic degradation.

If this were the case, accelerating DSB resection should limit the ability of Tel1 to sense and signal DSBs. Thus, we monitored Rad53 phosphorylation and the kinetics of 3′-ended ssDNA formation at the *MATα* HO-cut site in galactose-induced *mec1Δ* cells carrying either *EXO1* on 2μ plasmid or the empty vector (Fig 5D,E). When galactose was added to cell cultures carrying 11 HO-cut sites, Rad53 phosphorylation, which was detectable in *mec1Δ* cells carrying the empty vector 90 min after HO induction, was markedly reduced in *mec1Δ* cells carrying *EXO1* on the 2μ plasmid (Fig 5E), which accumulated 3′-ended resection products faster than *mec1Δ* cells carrying the empty vector (Fig 5D).

These data might also indicate that the switch from MRX to Exo1 at DSBs might cause the transition from Tel1-dependent to Mec1-dependent checkpoint, prompting us to evaluate whether high levels of Mre11 as well as *EXO1* deletion could limit Mec1 checkpoint functions. However, we found that overexpression of *MRE11* from the *GAL* promoter did not impair HO-induced Rad53 phosphorylation in either wild-type or *tel1Δ* cells (data not shown). Furthermore, Mec1-dependent Rad53 phosphorylation after a single HO cut was only slightly impaired in *exo1Δ* cells and paralleled their resection defect (Fig 1A,C). Therefore, our data support the hypothesis that the ability of Tel1 to signal multiple DSBs is disrupted when the DSB ends are exposed to 5′–3′ nucleolytic degradation.

Altogether, our data indicate that Tel1 contributes to the generation of 3′-ended ssDNA that in turn leads to

Mec1-dependent checkpoint activation. Furthermore, Tel1 is able to sense and signal the presence of DSB independently of Mec1, but its signalling activity only becomes apparent after the generation of multiple DSBs and is disrupted when DSB termini are resected. This suggests that the kinetics of DSB resection could influence Tel1/ATM activation as well as the transition from Tel1/ATM- to Mec1/ATR-dependent checkpoint.

METHODS

Yeast strains. Yeast strains were generated by disrupting the *MEC1*, *TEL1*, *SML1*, *EXO1* or *MRE11* genes in strains JKM139 (*MATa hmlΔ hmrΔ ade1 lys5 leu2-3,112 trp1::hisG ura3-52 ho ade3::GAL-HO*; Lee et al, 1998), kindly provided by J. Haber (Brandeis University, Waltham, MA, USA), or LSY1170 (1 Ty1-HOcs-*HIS3*), LSY1223 (6 Ty1-HOcs-*HIS3*) and LSY1259 (10 Ty1-HOcs-*HIS3*), kindly provided by L. Symington (Columbia University, New York, USA; Llorente & Symington, 2004). Strain names and additional genotypes are listed in the supplementary Table S1 online. All the *mec1Δ* strains were kept viable by also deleting the *SML1* gene. The 2μ plasmid carrying the *EXO1* gene was kindly provided by E. Alani (Cornell University, New York, NY, USA). To induce HO expression in JKM139 and its derivative strains, cells were grown in Yeast extract peptone (YEP) and raffinose and then transferred to YEP and raffinose and galactose. To induce HO expression in LSY1170, LSY1223 LSY1259 and their derivative strains, cells were transformed using a centromeric plasmid carrying the *GAL-HO* fusion (pFH800) (Llorente & Symington, 2004). Transformants were then grown in raffinose-containing synthetic medium and then transferred to YEP + raf + gal.

Other techniques. DSB end resection at the *MAT* locus in JKM139 and LSY1259 (10 Ty1-HOcs-*HIS3*) derivative strains was analysed on alkaline agarose gels as described by Shroff et al (2004), using a single-stranded probe complementary to the unresected DSB strand. For western blot analysis, Rad53 was detected by using Rad53 polyclonal antibodies kindly provided by J. Diffley (Clare Hall, London, UK) and C. Santocanale (Nerviano Medical Sciences-Oncology, Milano, Italy).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

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