Rqh1 blocks recombination between sister chromatids during double strand break repair, independent of its helicase activity

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Many questions remain about the process of DNA double strand break (DSB) repair by homologous recombination (HR), particularly concerning the exact function played by individual proteins and the details of specific steps in this process. Some recent studies have shown that RecQ DNA helicases have a function in HR. We studied the role of the RecQ helicase Rqh1 with HR proteins in the repair of a DSB created at a unique site within the *Schizosaccharomyces pombe* genome. We found that DSBs in *rqh1*$^+$ cells, are predominantly repaired by interchromosomal gene conversion, with HR between sister chromatids [sister-chromatid conversion (SCC)], occurring less frequently. In Δ*rqh1* cells, repair by SCC is favored, and gene conversion rates slow significantly. When we limited the potential for SCC in Δ*rqh1* cells by reducing the length of the G2 phase of the cell cycle, DSB repair continued to be predominated by SCC, whereas it was essentially eliminated in wild-type cells. These data indicate that Rqh1 acts to regulate DSB repair by blocking SCC. Interestingly, we found that this role for Rqh1 is independent of its helicase activity. In the course of these studies, we also found nonhomologous end joining to be largely faithful in *S. pombe*, contrary to current belief. These findings provide insight into the regulation of DSB repair by RecQ helicases.

homologous recombination | nonhomologous end joining | *rqh1* | gene conversion

Double strand breaks (DSBs) pose a major problem for genomic instability and cell survival, because a single unrepaired DSB is, presumably, sufficient to cause cell death (1). The sources of DSBs can be either endogenous, such as those induced during the reshuffling of DNA in Ig gene diversification, or exogenous, such as those induced by exposure to ionizing radiation (2–4). The cell has two major mechanisms for the repair of DSBs: homologous recombination (HR) and nonhomologous end joining (NHEJ), each used to varying degrees in different organisms (2, 3, 5, 6). HR is characterized as an error-free process using homologous sequences as the template to repair the DSB. Repair by HR begins with the formation of 3′ single strand ends at the break that can then invade homologous duplex DNA. The 3′ end of the invading strand is extended by DNA polymerase. At this point, the DSB can be repaired by either DSB repair, which involves formation of a double Holliday junction (HJ) or synthesis-dependent strand annealing (3, 7).

In NHEJ, the DNA ends are resealed by rejoining the broken ends; however, this process can lead to loss of information at the break and is, thus, referred to as error-prone repair (5, 8). Whereas mammalian cells preferentially use NHEJ over HR, budding yeast, for the most part, use HR over NHEJ. In *Saccharomyces cerevisiae*, the MRX (MRN in *Schizosaccharomyces pombe*) complex has been shown to play a role in NHEJ (9, 10). In *S. pombe*, only pKu70/80 and Ligase IV have been identified as functioning in NHEJ, and MRN does not appear to be active in this process (11).

RecQ DNA helicases are found in virtually every organism from bacteria to humans. First described in *Escherichia coli* as a suppressor of cell death by thymine starvation, RecQ was later found to be a helicase that unwinds DNA in the 3′-to-5′ direction (12, 13). RecQ was first recognized in eukaryotes in *S. cerevisiae* as a slow growth suppressor (*SGS1*) of *top3* (14). The fission yeast *recQ*, originally known as *rad12*$^+$, was renamed *rqh1*$^+$ for *recQ* homologue (15–17). There are five *recQ* homologues in humans. Mutations in three of them (*BLM*, which leads to Bloom Syndrome (BS); *WRN*, which leads to Werner's Syndrome; and *RECQL4*, which causes Rothman Thomson Syndrome) all present with genomic instability and predisposition to cancer (18–20). How RecQ helicases function in maintaining genomic stability is only beginning to be understood, but several connections between RecQ function and HR have been identified. RecQ mutants show increased rates of HR (21–25). BS patients show very high levels of sister-chromatid exchanges, whereas, in yeast cells, *sgs1* and *rqh1* mutants have increased levels of HR, based on studies using various reporter systems (17, 26–28). HR is responsible for the synthetic lethality seen between *sgs1*/*rqh1* and *srs2*, another DNA helicase gene (29, 30). Loss of HR genes suppresses the synthetic interaction between *sgs1* and *mus81* (31, 32). When the *E. coli* HJ resolvase RusA was expressed in Δ*rqh1* cells, their UV and hydroxyurea (HU) sensitivities were partially suppressed, suggesting that in the absence of Rqh1, HJs accumulate (33). Finally, we recently reported that the UV and HU sensitivities of Δ*rqh1* are suppressed by loss of a subset of HR genes (34). Together, these findings strongly imply that RecQ helicases function through HR to provide genomic stability during both DNA damage and replication arrest.

In this study, we have used a system in which a DSB is induced at a unique site on a nonessential minichromosome (Ch16), which contains the pericentric regions of ChIII. The unique DSB is created by HO endonucleolytic cleavage of *MATa*, inserted into Ch16 (35). This system allows us to score the events that occur downstream of DSB formation in different genetic backgrounds, enabling us to assess the contributions of various proteins to this process. By using a partial diploid and generating the DSB in a nonessential chromosome, repair is not required for cell survival. We found that, in wild-type cells, DSBs are repaired preferentially through gene conversion (GC) and that this choice is regulated by the action of Rqh1 by suppressing sister-chromatid conversion (SCC) leading to increased GC. Interestingly, the DNA helicase activity of Rqh1 is apparently not required in the suppression of SCC.

Results

Repair of Site-Specific DSB in the Wild-Type Background. A unique system was used to analyze the repair of a site-specific DSB at

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Abbreviations: ChL, chromosome loss; DSB, double strand break; GC, gene conversion; HJ, Holliday junction; HR, homologous recombination; NHEJ, nonhomologous end joining; SCC, sister-chromatid conversion; YFP, yellow fluorescent protein.

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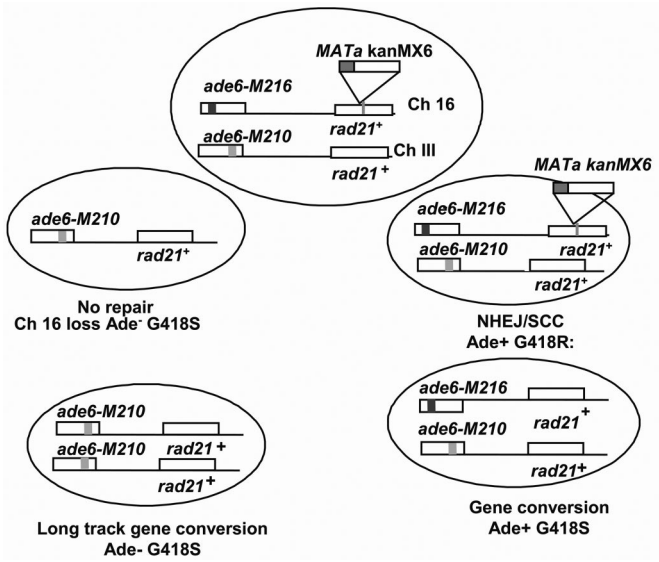


Fig. 1. A schematic depiction of the Th805 system and the predicted products that can form after repair of a DSB induced at *MATa*.

an ectopic *MATa* site through the expression of the HO endonuclease, under control of the thiamine-repressible promoter *nmt* (Fig. 1) (35). Strains carrying the minichromosome with the *MATa* site are referred to as TH805. After a period of HO induction, cells were spread onto yeast extract plates and incubated until colonies grew up. Colonies were analyzed for chromosome loss (ChL), GC, SCC, or NHEJ, as described in *Methods* and in supporting information, which is published on the PNAS web site. The results from the various strains analyzed are shown in Table 1 and Fig. 2*a*. In wild-type cells after 48 h of induction, 44 ± 5% of colonies remained Ade$^+$ but became G418-sensitive, indicative of repair by GC, whereas 45 ± 4% of colonies remained both Ade$^+$ and G418^R, consistent with repair by SCC or NHEJ. At 72 h postinduction, 60 ± 7% of colonies had repaired the DSB by GC, whereas 17 ± 7% remained Ade$^+$ G418^R.

A shortcoming of this system is that this marker analysis does not allow us to differentiate among uncut substrate, repair by SCC, or repair by NHEJ, because all three result in the retention of both the Ade6$^+$ and G418^R phenotype. Other authors have used Southern blot analysis to observe the efficiency of HO cleavage in this system (35, 36). These experiments showed that the cleavage product begins to appear 20 h after induction, with peak cleavage seen at 24 h. However, only a minor amount of cleavage product was visible, suggesting that repair of the DSB is very efficient and/or that cleavage occurs slowly. Because it is critical to know what portion of cells actually experienced an HO-induced DSB, we measured the number of cells that received a DSB by an indirect method.

The appearance of Rad22-yellow fluorescent protein (YFP) nuclear foci in *S. pombe* has been correlated with DSB formation (37–39). We created a Th805 strain containing *rad22-YFP-KanMX6* and looked for the appearance of Rad22-YFP nuclear foci as a measure of HO cleavage. We induced HO endonuclease in Th805-*rad22-YFP*, collected cells at 2-h intervals, and examined them by fluorescence microscopy. For the first 12 h, the number of cells containing nuclear foci was <5%. This number began to increase at 14 h, and, thereafter, the percentage of cells with visible nuclear foci increased rapidly (Fig. 3). By 28 h after HO induction, we observed foci in >75% of cells, and the level

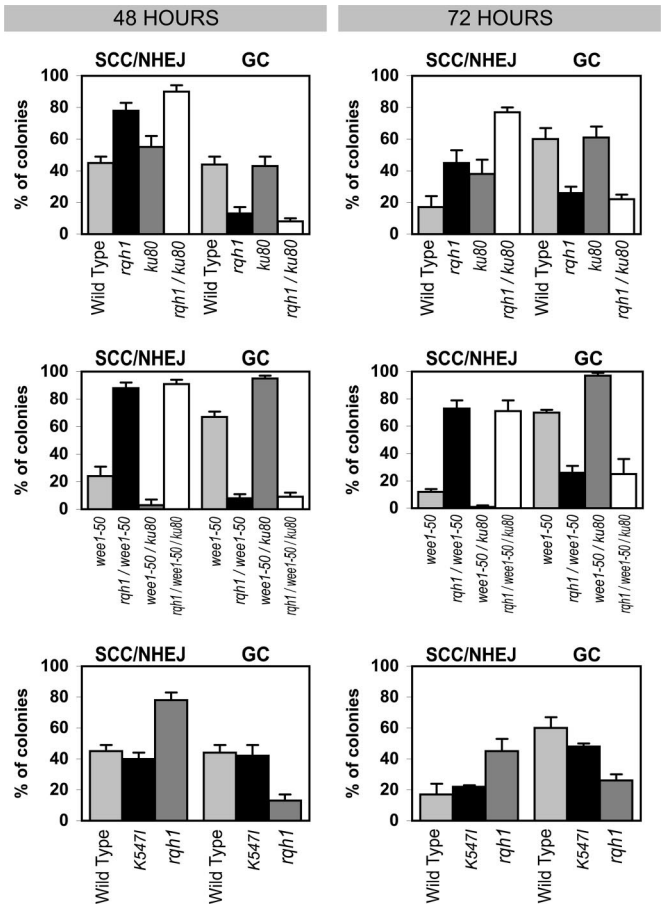
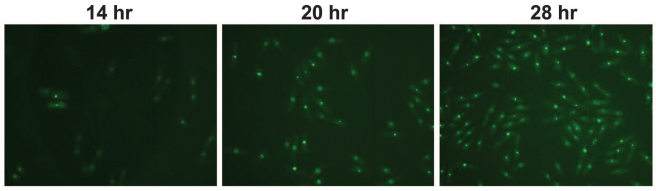


Fig. 2. Bar graphs showing comparisons in GC and SCC/NHEJ frequencies in various genetic backgrounds. Each bar represents an average of a minimum of three individual experiments. (*a*) Comparison of SCC/NHEJ and GC rates among Th805 (wild type), Th805 Δ*rqh1* (*rqh1*), Th805 Δ*pku80* (*pku80*), and Th805 Δ*rqh1* Δ*pku80* (*rqh1 pku80*). (*b*) Comparison of Th805 *wee1-50* (*wee1-50*), Th805 Δ*rqh1 wee1-50* (*rqh1 wee1-50*), Th805 Δ*pku80 wee1-50* (*rqh1 pku80*), and Th805 Δ*rqh1* Δ*pku80 wee1-50* (*rqh1 pku80 wee1-50*). (*c*) Comparison of Th805 (wild type), Th805 Δ*rqh1* (*rqh1*), and Th805 *rqh1-K547I* (*rqh1 K547I*).

Table 1. DSB repair results

Strain	Hours	ChL, %	SCC/NHEJ, %	GC, %	Total colonies
Wild type (Th805)	48	11 ± 2	45 ± 4	44 ± 5	3,615
	72	23 ± 8	17 ± 7	60 ± 7	2,358
K5471	48	18 ± 4	40 ± 4	42 ± 7	2,425
	72	29 ± 3	22 ± 1	48 ± 2	740
rqh1	48	9 ± 2	78 ± 5	13 ± 4	4,226
	72	27 ± 4	45 ± 8	26 ± 4	570
ku80	48	2 ± 2	55 ± 4	43 ± 2	973
	72	1 ± 1	38 ± 3	61 ± 3	758
wee1-50	48	9 ± 2	24 ± 7	67 ± 4	2,322
	72	18 ± 3	12 ± 2	70 ± 2	1,524
rqh1/ku80	48	2 ± 1	90 ± 7	8 ± 6	921
	72	2 ± 2	77 ± 9	22 ± 7	883
rqh1/wee1-50	48	3 ± 4	88 ± 4	8 ± 3	3,412
	72	1 ± 2	73 ± 6	26 ± 5	5,127
wee1-50/ku80	48	2 ± 2	3 ± 4	95 ± 2	2,178
	72	2 ± 2	1 ± 1	97 ± 2	2,460
rqh1/ku80/wee1-50	48	0 ± 1	91 ± 3	9 ± 3	4,938
	72	4 ± 4	71 ± 8	25 ± 1	3,739

These data represent the results of a minimum of three experiments.



Time (hr)	14	16	18	20	22	24	26	28	30
% with Rad22 YFP foci	26%	30%	36%	51%	41%	47%	66%	77%	75%

Fig. 3. Demonstration of cleavage by HO endonuclease *rad22-YFP* was introduced into Th805 in a wild-type background. HO endonuclease was induced by growth in media lacking thiamine and the cells followed for 30 h. Before induction, only a few cells ≈2–4% showed Rad22-YFP foci. By ≈16 h postinduction, foci were visible in many more cells (≈30%). By 28 h, nearly all cells (>77%) contained Rad22-YFP foci, suggesting that essentially every cell had received at least one HO-induced break by this time.

remained constant for the next 6 h. The persistence of Rad22-YFP foci in this study is consistent with previous studies where Rad22 foci were shown to persist for 8 h or longer after damage (37). It is important to note that the products of DSB repair by SCC or faithful NHEJ are substrates for another round of cutting by HO. This ongoing repair of the HO-induced DSB indicates that the fraction of cells with Rad22 foci is, likely, an underestimation of cells that have experienced a DSB, suggesting that, by 48 h postinduction, essentially every cell has experienced at least one DSB.

To further characterize the repair events that generate Ade$^+$ G418^R colonies, we asked whether these repair events depend on Ku70/Ku80 function. Experimental studies of NHEJ using linearized plasmids showed that repair of DSBs by NHEJ is essentially eliminated in the Δ*ku80* background (11). Although rare, Ku-independent repair was detected, resulting in large deletions at the repair junction (11). If the Ade$^+$ G418^R colonies represent repair by NHEJ, they should be greatly reduced in the Δ*ku80* background, whereas, if they represent repair by SCC, they should persist in this background. We found that deletion of Ku80 did not eliminate or reduce the Ade$^+$ G418^R colonies recovered after HO induction, suggesting that these colonies represent repair by SCC (Fig. 2*a* and Table 1). In fact, the level of Ade$^+$ G418^R colonies increased compared with wild type, likely because of an overall decrease in chromosome loss.

Repair of Site-Specific DSB in the Δ*rqh1* Background. To examine the role or Rqh1 in DSB repair, we created Th805-Δ*rqh1* by crossing *rqh1*::*ura4*$^+$ with Th805 (15, 35). ChL, GC, and SCC/NHEJ frequencies were determined for Th805-Δ*rqh1* at 48 and 72 h after induction of the HO endonuclease. The results are summarized in Table 1 and Fig. 2*a*. We found significant differences in the mechanism of DNA repair in the Δ*rqh1* background compared with wild type. In an *rqh1*$^+$ background, the majority of cells repaired the DSB by GC (60 ± 7% by 72 h after HO induction). By contrast, GC frequencies at 72 h after HO induction in a Δ*rqh1* background were only 26 ± 4% ($P < 0.001$), with high levels of SCC/NHEJ (45 ± 8% ($P < 0.001$) (Fig. 2*a* and Table 1). These data indicate that, in the absence of Rqh1, DSBs are less likely to be repaired by GC.

One simple explanation for this result is that the HO endonuclease might cleave inefficiently in Δ*rqh1* cells, accounting for the high levels of Ade$^+$ G418^R colonies seen in this background. We were unable to quantify DSBs by measuring Rad22-YFP foci in the Δ*rqh1* background because of a high level of spontaneous Rad22-YFP foci observed in Δ*rqh1* cells (J.C.H. and G.A.F., unpublished data). To verify that the level of DSBs after HO induction in a Δ*rqh1* background is the same as in wild-type cells,

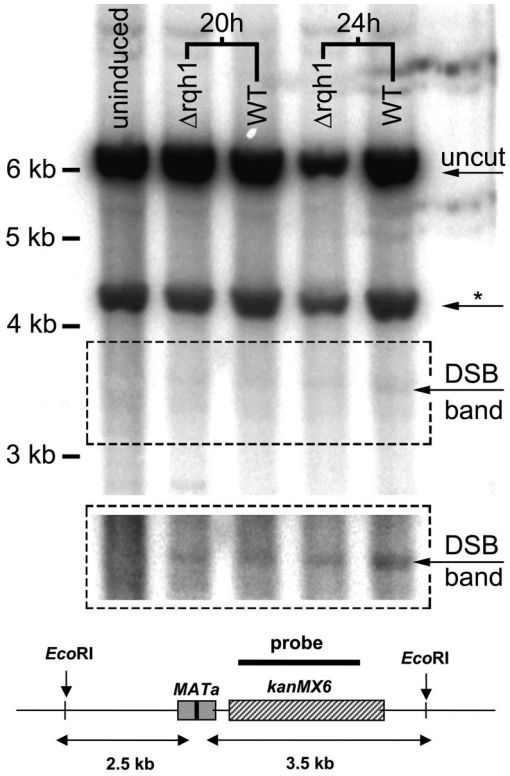


Fig. 4. HO cleavage is equivalent in wild-type and Δ*rqh1* backgrounds. Cultures of Th805 and Th805 Δ*rqh1* were grown for 20 or 24 h in media lacking thiamine to induce the HO endonuclease to cleave at the *MATa* locus. EcoRI-digested genomic DNA was isolated and used for Southern blot analysis. The blot was probed with a ^{32}P-labeled kanMX6 probe (black bar shown in diagram). The autoradiograph shows that equivalent levels of the 3.5-kb band generated by a combination of EcoRI and HO endonuclease cutting are seen at equivalent levels in both wild-type (WT) and Δ*rqh1* backgrounds.

we compared the efficiency of HO cutting in Δ*rqh1* and *rqh1*$^+$ by Southern blot analysis. Fig. 4 shows the result of the Southern blot, where the 3.5-kb fragment, indicative of HO cutting, is visible in equivalent intensities in both backgrounds, showing that the HO endonuclease cleaves with similar efficiency in both strains. We conclude that the increased number of Ade$^+$ G418^R colonies recovered in the Δ*rqh1* background represent increased repair by SCC or NHEJ and not a decreased frequency of substrate cleavage.

In the Absence of Rqh1, DSB Are Preferentially Repaired by SCC. Our data show that in a Δ*rqh1* background, Ade$^+$ G418^R colonies predominate, but this could be due to repair by either SCC or NHEJ. If the HO break is repaired by NHEJ, this should rely wholly, or in large part, on pKu70/80 (11). We created a Th805 Δ*rqh1* Δ*pku80* strain and examined it in the DSB repair assay. We found that, in the absence of both Rqh1 and pKu80, the number of Ade$^+$ G418^R colonies actually increased to 90 ± 7% and 77 ± 9% after 48 or 72 h of induction, respectively (Fig. 2*a* and Table 1). This increase over either single mutant can be accounted for by the apparent suppression of ChL in Δ*pk80* cells and increased SCC due to loss of Rqh1 activity. These results support the argument that DSBs in Δ*rqh1* cells are not repaired by NHEJ, leaving SCC as the likely process of repair.

Suppressing SCC Does Not Increase Repair by GC. Preferential repair by SCC in a Δ*rqh1* background can be explained in one of two ways; Rqh1 promotes DSB repair by GC, so, in its absence, GC levels decrease, leading to an increase in SCC or Rqh1 blocks

DSB repair by SCC, so, in its absence, SCCs simply rise because they are not blocked from forming. We reasoned that, if we could significantly reduce the length of the G2, we would reduce the availability of the sister chromatid as a repair template, limiting the opportunity for SCC. Under these conditions, if Rqh1 promotes GC, then limiting SCC in the absence of Rqh1 should result in increased levels of DSB repair by NHEJ and/or ChL. On the other hand, if Rqh1 blocks SCC, then, under these conditions, GC levels should return to levels comparable with those of wild-type cells. To test this hypothesis, we crossed the conditional mutant *wee1-50* into Th805, Th805-Δ*pku80*, Th805-Δ*rqh1*, and Th805-Δ*rqh1* Δ*pku80* cells. Cells containing the *wee1-50* allele, grown at a semipermissive temperature (33.5°C), have a very short G2 phase, essentially exiting S phase and directly entering mitosis (40, 41).

We found that, in Th805-*wee1-50* cells, the majority of colonies recovered represented repair of the DSBs by GC; 66 ± 7% and 68 ± 10% of cells, at 48 and 72 h postinduction, respectively, similar to Th805-wt (Fig. 2*b* and Table 1). In the TH805-*wee1-50* mutant, fewer colonies resulted from repair by SCC/NHEJ (24 ± 5% and 12 ± 2%, at 48 and 72 h postinduction), compared with 45 ± 4% and 17 ± 7%, at 48 and 72 h postinduction for wild type. If SCC is, indeed, impaired in *wee1-50*, then these Ade^+ $G418^R$ colonies should represent NHEJ events. Indeed, we found that, in Th805-*pku80 wee1-50* cells, where NHEJ is largely blocked, Ade^+ $G418^R$ colonies were virtually nonexistent (3 ± 3% and 1 ± 1% at 48 and 72 h after HO induction, respectively) (Fig. 2*b* and Table 1). Thus, the Ade^+ $G418^R$ colonies observed in *wee1-50* were likely the result of NHEJ, confirming that SCC is extremely rare in a *wee1-50* background.

When we analyzed Th805 Δ*rqh1 wee1-50* cells, we found that the majority of colonies were Ade^+ $G418^R$ (70 ± 14% and 63 ± 10% at 48 and 72 h after HO induction, respectively) (Fig. 2*b* and Table 1), and GC events were observed in only 8 ± 3% (48 h) and 26 ± 5% (72 h) of colonies. This degree of repair by GC is very similar to that observed in Δ*rqh1*, indicating that limiting SCC does not restore GCs to wild-type levels.

To test whether the Ade^+ $G418^R$ colonies resulted from repair of the DSB by NHEJ, we created Th805 Δ*rqh1* Δ*pku80 wee1-50*, where repair by SCC, NHEJ, and GC should all be limited. Surprisingly, the frequency of Ade^+ $G418^R$ colonies after 72 h was 71 ± 8% and GC frequencies were only 25 ± 1% (Fig. 2*b* and Table 1), very similar to the levels seen in Th805 Δ*rqh1 wee1-50*, suggesting that repair in this background is largely accomplished by SCC. One possible explanation for these data is that the loss of Rqh1 increases the length of G2 in a *wee1-50* background, increasing the possibility of SCC to occur. Two observations argue against this possibility. First, Th805-Δ*rqh1*-Δ*wee1-50* cells had the same small cell size as the Th805-*wee1-50* cells, indicative of a shortened cell cycle. Second, a longer G2 will lead to an overall increase in the length of the cell cycle. Thus, we compared the length of the cell cycle from G1 to cytokinesis of Th805-*wee1-50* with Th805-Δ*rqh1-wee1-50*. To accomplish this, both cell lines were arrested in G1 by overnight incubation in media lacking nitrogen. The cells were released from this block by resuspension into yeast extract adenine media. Cells were collected at 20-min intervals, and their septa were stained with Calcofluor. We found that in both backgrounds the number of septa began to increase after 200 min, indicating that the absence of Rqh1 did not significantly affect the length of the cell cycle and, by extrapolation, the length of G2.

We also tested the formal possibility that the cells were still able to repair by NHEJ in this background. Previous studies have shown that, when DSBs are repaired by end joining in the absence of Ku, large deletions at the junctions are observed (11). We isolated several Ade^+ $G418^R$ colonies, PCR amplified the *MATa* site, and separated the products on agarose gels. The results demonstrate that there was no apparent change in the size of the PCR products from repaired junctions compared with uncut substrate (see Supporting Information). Sequence analysis of these PCR products showed the HO cut site to be intact in all clones that were tested. Based on these results, we conclude that the Ade^+ $G418^R$ colonies formed in a TH805 Δ*rqh1* Δ*pku80 wee1-50* background arose through repair by SCC. Our interpretation of these results is that DSB repair in the Δ*rqh1* background occurs primarily by SCC, suggesting that the normal role of Rqh1 is to block repair by SCC. The high rates of SCC, even in a *wee1-50* background, demonstrate how significant a role Rqh1 plays in this process.

The Suppression of SCC by Rqh1 Is Independent of Its Helicase Activity. Previous studies of helicase-dead mutants of RecQ have generally demonstrated that helicase activity is important for most, but not all, of its associated functions. We had shown that suppression of Δ*top3* lethality was suppressed more efficiently when $rqh1^+$ was deleted compared with when its helicase activity was inactivated (42). Ahmad *et al.* (43) showed that recovery from S-phase arrest by Rqh1 only partially depended on its helicase activity. In *S. cerevisiae*, the ability of Sgs1 to suppress crossovers and reduce GC tract lengths is reportedly independent of its helicase activity (J. Nickloff, personal communications). We tested whether the helicase activity of Rqh1 was important in its role of suppressing repair by SCC, using a helicase-dead mutant *rqh1-K547I* (42). We found that the mechanism of repair in the helicase-dead mutant was nearly identical to $rqh1^+$ cells, as seen in Fig. 2*c* and Table 1; GC rates in Th805-wt and Th805-*rqh1-K547I* at 48 h after HO induction were 44 ± 5% and 42 ± 7% ($P = 0.44$), respectively, whereas SCC/NHEJ rates were 45 ± 4% and 40 ± 4% ($P = 0.14$). At 72 h after HO induction, the levels of GCs in Th805-wt compared with Th805-rqh1-K547I were 60 ± 7% and 48 ± 2% ($P = 0.04$), whereas SCC/NHEJ rates were 17 ± 7% and 22 ± 1% ($P = 0.149$), respectively, 48 and 72 h after HO induction (Fig. 2*c* and Table 1). The *P* values for two independent sample *t* tests confirm that cells in a *rqh1-K547I* background do not repair DSBs differently from cells in a wild-type background. These data suggest that the ability of Rqh1 to suppress SCC is largely independent of its helicase activity.

NHEJ of Linearized Plasmids Differs from Repair of an HO Cleavage in the Chromosome. Previous studies of NHEJ events in *S. pombe* using linearized plasmids found that varying amounts of DNA sequence was usually lost at the repair junction and concluded that NHEJ in *S. pombe* is generally not faithful (11, 44, 45). We examined the sequences at the cleavage site of *MATa* in multiple colonies after HO endonuclease induction and looked for evidence of sequence loss consistent with repair by NHEJ. Primers flanking the HO site were used to amplify a 315-nt fragment from 66 Ade^+ $G418^R$ colonies that formed after a 72-h induction in wild-type (12 colonies), Δ*rqh1* (34 colonies) and Δ*pku80* (20 colonies) backgrounds. In every case, 66 of 66 colonies, the *MATa* locus had been perfectly restored. We initially used these data as evidence that the Ade^+ $G418^R$ colonies recovered after HO induction represent SCC repair events. However, we analyzed Th805-w*ee1-50* cells in the DSB repair assay at semipermissive temperatures, where SCC should be largely blocked, a significant number of Ade^+ $G418^R$ colonies formed, suggestive of repair by NHEJ (Table 1). We confirmed this finding by demonstrating that Ade^+ $G418^R$ colonies in a *wee1-50* background were pKu80-dependent (Fig. 2*b* and Table 1). We isolated 10 Ade^+ $G418^R$ Th805-*wee1-50* colonies after a 72-h and 96-h HO induction (5 from each), PCR amplified the sequences flanking the HO junction, and analyzed the sequence. In all 10 PCR products, the repair was error-free, suggesting faithful end joining. This finding is in conflict with the conclusion of previous data using linearized plasmid DNA (11, 44, 45).

Discussion

The RecQ helicases are recognized as partners in HR, likely having a late function, possibly in processing HJs (46–48). This is supported by studies showing that sister-chromatid exchanges, which are a visualization of crossovers, increase dramatically in *BLM*$^{-/-}$ cells (49, 50). Also, biochemical data demonstrated that the BLM protein can catalyze the branch migration of HJs as well as participate in the resolution of a double-HJ-like four-way junction through a process called dissolution (51, 52). In *S. pombe*, the Δ*rqh1* phenotype is suppressed significantly when the HJ resolvase RusA is expressed, indicating that Rqh1 functions to prevent the accumulation of HJs (33). We have provided evidence that Rqh1 acts downstream of Rhp55/57 in HR (34). There is also data that support an early role in HR for RecQ helicases. Both BLM and Sgs1 associate with Rad51 and colocalize on ssDNA after exposure to ionizing radiation (53, 54). Similarly, Rqh1 foci form earlier than Rhp51 foci after UV irradiation of *S. pombe* cells (55). Together, these studies suggest that RecQ helicases act both early and late during HR.

Repair of DSBs by GC Is Reduced in a Δ*rqh1* Background. The power of using a specific site-of-damage system is in the ability to follow the products of the repair reaction and determine events that occur downstream of damage in various genetic backgrounds. This particular system has two advantages over those described in *S. cerevisiae*: first, by placing the DSB on a nonessential chromosome, lack of repair (which would be lethal if the DSB were on an essential chromosome) can be followed; second, the inefficient cleavage by HO endonuclease and/or efficient repair, largely limits DSBs to one sister chromatid at a time, allowing SCC to take place. Our initial observation that repair of the DSB in a Δ*rqh1* background resulted in reduced GC frequencies as compared with *rqh1*$^{+}$ cells seemed to conflict with previous studies that showed RecQ mutants typically have a hyperrecombination phenotype. However, our further studies strongly suggest that this decrease in GC frequencies is actually the result of an increase in the frequency of SCC repair. This conclusion was drawn from several results. First, using Southern blot analysis, we demonstrated that comparable levels of DSBs were being formed in Δ*rqh1* and *rqh1*$^{+}$ cells. Next, we demonstrated that the Ade^{+} G418^{R} colonies that formed in the Δ*rqh1* background represented SCC repair events. In Δ*rqh1* Δ*pku80* cells, where NHEJ should be largely blocked, the level of Ade^{+} G418^{R} colonies actually increased over that seen in the Δ*rqh1* single mutant, indicating that these Ade^{+} G418^{R} colonies are not the result of NHEJ and, therefore, must arise by SCC. This result also supports previous data suggesting that NHEJ is not commonly used for DSB repair in fission yeast cells (56). Studies of NHEJ in both *S. pombe* and *S. cerevisiae* have concluded that, in the absence of Ku proteins, NHEJ is diminished and results in terminal deletions of DNA sequence, often of a substantial length (11, 57). If Δ*rqh1* Δ*pku80* or Δ*pku80* mutants were able to repair DSBs by NHEJ, sequence loss at the HO cut site should be observed. Sequence analysis of the HO junction from multiple Δ*rqh1* Δ*pku80* and Δ*pku80* colonies that were Ade^{+} G418^{R} failed to reveal any loss of sequence at the junction, supporting the argument that the DSBs were not repaired by pKu80-independent end joining. Together, these data support the argument that it is SCC levels that are elevated in Δ*rqh1* cells and not NHEJ.

There are two possible explanations for increased levels of SCC in the Δ*rqh1* background: Rqh1 promotes GCs, and, in the absence of Rqh1, DSB repair shifts to SCC or Rqh1 blocks SCC, so, in its absence, SCC levels increase. We attempted to distinguish between these two possibilities by reducing the length of G2, thus limiting the ability of cells to undergo SCC. The temperature-sensitive *wee1-50* mutant was used for the purpose of reducing the length of G2. Our rationale for these studies was that, if Rqh1 blocked SCC, then reducing G2 should greatly limit SCC, returning GC rates to wild-type levels. Alternatively, if Rqh1 promoted GC, then the frequency of GCs should be reduced relative to that of wild-type cells, with concomitant increases in NHEJ, ChL, or both. We first found that in a *wee1-50* background, at semipermissive temperatures, the level of Ade^{+} G418^{R} colonies decreased, whereas the number of colonies that repaired by GC increased, relative to *wee1-50*$^{+}$ cells. Furthermore, we showed that the Ade^{+} G418^{R} colonies that did arise under these conditions resulted from NHEJ, because they were eliminated when *pku80*$^{+}$ was deleted. When we measured the level of Ade^{+} G418^{R} colonies that arose in Th805 Δ*rqh1 wee1-50* cells after HO induction, they did not decrease but remained at levels comparable with that of Th805 Δ*rqh1* cells. This result seemed to suggest that repair had switched to NHEJ. However, when we analyzed Th805 Δ*rqh1* Δ*pku80 wee1-50* strains in this assay, the level of Ade^{+} G418^{R} colonies was not diminished, leaving us with two possible explanations: NHEJ occurs in the absence of Ku80, or SCC was still being carried out despite the shortened G2 phase. When we analyzed the HO junctions of multiple Ade^{+} G418^{R} colonies from this background, all were intact. Thus, repair was almost certainly by SCC. The efficient repair by SCC in the absence of Rqh1, even with a reduced G2, supports the argument that Rqh1 is a potent inhibitor of SCC.

It should be noted that the sequences directly adjacent the HO cut site are not homologous to ChIII, and this could help favor SCC over GC. However, this is true for all backgrounds tested in our study and does not change our interpretation of the data.

The Helicase Activity Defines Two Functions for Rqh1. Our finding that the GC and SCC frequencies in the helicase-dead *rqh1-K547I* mutant were very close to wild-type levels supports the argument that the helicase activity is not required in this early role of Rqh1. These results indicate that the helicase activity of Rqh1 activity is not needed for Rqh1 to regulate the pathway of DSB repair. It will be interesting to determine which domains of Rqh1 are responsible for this regulation. Interestingly, we have shown that *rqh1-K547I* is as sensitive to DNA damage as is Δ*rqh1* (42). Because *rqh1-K547I* retains the ability to block SCC, the role of Rqh1 in determining the pathway of DSB repair is not critical for cell viability.

NHEJ in *S. pombe* Is Faithful. At the semipermissive temperature, in a *wee1-50* background, significant numbers of Ade^{+} G418^{R} colonies formed that likely arose by NHEJ, yet contained intact sequences at the HO junction. This faithful repair of DSBs by NHEJ differs from DSB repair assays based on linearized plasmids, in which sequence loss was consistently observed (11, 45). We suggest that this difference may be due to the chromosomal context of the DSB in our system, as opposed to the plasmid context of other studies. The HO system more faithfully represents cellular repair of DSBs, where, in the chromosomal context, the DNA is properly packaged into chromatin. We recognize that the universality of faithful repair by NHEJ in fission yeast awaits the study of DSBs with different terminal structures.

Methods

Genetic Manipulations and Creation of Strains. Standard protocols were used for the creation of strains. A table of strains used in these studies is provided in Supporting Information. The *rad22-YFP* strain was created by inserting the PCR-amplified YFP-kanMX6 sequences of pDH5 at the 3′ end of *rad22*$^{+}$. The resulting strain was shown to have wild-type levels of resistance to ionizing radiation.

HO-Induced DSB Repair Assay. The protocol followed for HO induction has been described in ref. 35. A detailed description is given in Supporting Information and in *Results*. Overnight cultures were begun in the presence of thiamine to suppress HO expression. The thiamine was removed and the cells plated at 48 and 72 h onto yeast extract plates and incubated 3–5 days, depending on growth rates. Red and white colonies were counted, and white colonies were picked and analyzed further for G418 sensitivity. Red colonies were scored as ChL.

Analysis of the HO-Recognition Sequence in Cells That Repaired Their HO Cut Site by Either NHEJ or SCC. Multiple colonies that were Ade^+ $G418^R$ were isolated after a 72-h induction of HO. Primers adjacent to the HO site (3′ to 5′: CAAGGAGGGTATTCTGGGCC; 5′ to 3′: TCGGTATCTGAGGCCCTTCC) were used to amplify this region. The PCR products were separated on 1.4% agarose gels, and the DNA was isolated and sequenced.

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