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Role of *Schizosaccharomyces pombe* RecQ Homolog, Recombination, and Checkpoint Genes in UV Damage Tolerance

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The cellular responses to DNA damage are complex and include direct DNA repair pathways that remove the damage and indirect damage responses which allow cells to survive DNA damage that has not been, or cannot be, removed. We have identified the gene mutated in the *rad12.502* strain as a *Schizosaccharomyces pombe recQ* homolog. The same gene (designated *rqh1*) is also mutated in the *hus2.22* mutant. We show that Rqh1 is involved in a DNA damage survival mechanism which prevents cell death when UV-induced DNA damage cannot be removed. This pathway also requires the correct functioning of the recombination machinery and the six checkpoint *rad* gene products plus the Cds1 kinase. Our data suggest that Rqh1 operates during S phase as part of a mechanism which prevents DNA damage causing cell lethality. This process may involve the bypass of DNA damage sites by the replication fork. Finally, in contrast with the reported literature, we do not find that *rqh1* (*rad12*) mutant cells are defective in UV dimer endonuclease activity.

Cellular responses to DNA damage can be defined as either direct DNA repair responses, which result in the removal and repair of the damage, or indirect responses (such as the G_2 damage checkpoint) which do not directly repair or remove damage but allow the cell to survive such damage (8). The relationships between the various DNA damage responses are complex and difficult to study in mammalian cells. Since there is good evidence that most of the damage response pathways are highly structurally and functionally conserved (5, 13, 28, 33, 51, 53), we are studying these pathways in the fission yeast model system.

In fission yeast *Schizosaccharomyces pombe*, UV-induced DNA damage is removed by one of two pathways, either the conserved nucleotide excision repair (NER) pathway (10) or a novel pathway initiated by the UV dimer endonuclease (UVDE) (63). In addition to DNA repair, *S. pombe* has several damage responses that do not result in the direct removal of photoproducts but allow the cells to survive DNA damage more effectively. These are controlled by the checkpoint pathway(s) and include G_2 delay (1, 49), transcriptional activation (16, 57), and the control of progress through S phase following DNA damage or exposure to hydroxyurea (2, 15).

In *S. pombe*, the DNA structure checkpoint responses all require a group of six proteins commonly referred to as the checkpoint Rad proteins (7). Mutations in any one of these result in very similar phenotypes, including sensitivity both to DNA damage and to S-phase arrest by hydroxyurea. It has been proposed that the checkpoint Rad proteins form a complex that is capable of monitoring various different aspects of DNA metabolism, including DNA damage/repair and DNA replication (7).

In response to DNA damage, the checkpoint Rad complex activates two downstream kinases, Chk1 and Cds1. Chk1 activation is required for G_2 arrest in response to DNA damage and can be monitored by a phosphorylation event (58). Cds1 activation is S-phase specific and does not occur in G_1 or G_2 (34). As anticipated, *cds1* deletion cells are sensitive to DNA damage but do not exhibit a defect in mitotic arrest following DNA damage. Cds1 defines a checkpoint-related response specific to S phase. An interesting allele of one of the checkpoint *rad* genes, *rad26.T12*, that is defective specifically in the Cds1 response but not in the Chk1 response has been described (2, 34). *rad26.T12* cells are sensitive to DNA damage during S phase, further indicating that the Cds1-dependent response is important specifically during S phase.

At the beginning of this work, we identify the *S. pombe* RecQ homolog, Rqh1, by virtue of its involvement in the DNA damage response. RecQ in *Escherichia coli* is a DNA helicase involved in the RecF recombination pathway (38). RecQ homologs have been identified in eukaryotes, but these have not previously been shown to be involved in the response to DNA damage. In *Saccharomyces cerevisiae*, a single RecQ homolog, Sgs1, has been identified. This protein appears to associate with DNA topoisomerases (20, 61), although the precise function is not known. There is no radiation sensitivity associated with loss of Sgs1 function (60). In human cells, three RecQ-related proteins, RecQL, WRN, and BLM, have been identified (14, 48, 64). Two of these are associated with genetic diseases: the *WRN* gene is defective in patients with the premature aging disorder Werner's syndrome (64), and the *BLM* gene is defective in those with Bloom's syndrome (14), who suffer from sun sensitivity and developmental abnormalities (21).

Cells from Bloom's syndrome patients are not particularly sensitive to DNA-damaging agents and have not been reported to manifest checkpoint deficiencies. However, these patients exhibit a 300-fold increase in neoplasia and have elevated in vivo mutant frequencies in their lymphocytes (31). The most dramatic cellular phenotypes associated with Bloom's syndrome are elevated levels of sister chromatid exchange (11) and a high frequency of chromosome abnormalities (22). These also appear to be associated with a slowdown of the progression of the replication fork (23, 27). While a defect in ligase

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function has been reported for Bloom's syndrome cells (12, 62), the genetic defect is not associated with a DNA ligase.

The work reported here links the phenotypes associated with mutation of the *S. pombe* RecQ homolog (Rqh1) to the correct operation of the checkpoint proteins and Cds1. Our work suggests that RecQ functions in a checkpoint protein-dependent DNA damage response during S phase. This response allows cells to survive damage through a process which also involves the recombination repair proteins. We discuss these results in the context of the Bloom's syndrome phenotypes and suggest that *S. pombe* may prove to be a useful organism with which to study the RecQ helicase family in eukaryotes and its involvement in DNA metabolism.

MATERIALS AND METHODS

Strains, media, and physiological tests. All strains except that with the null mutation at the *rqh1* locus, which was a gift from T. Enoch, have been described previously (see Table 2) or are derived from such strains by classical genetic methodologies (25). Media for cell culture have been described elsewhere (39). UV and gamma survival measurements were performed as previously described (2, 43).

Cosmid clones and P1 clones. Cosmid and P1 clones were supplied by the resource center/primary database of the German human genome project (Max Planck Institute for Molecular Genetics) and are described in reference 29. To tag the cosmids and P1 phage with a selectable marker, the transposon tags developed by Morgan et al. (40) were used. Briefly, the *ars1 ura4* transposon was introduced at random sites by transformation of the cosmid and P1 clones into the *E. coli* donor strain MH1831, followed by transfer by bacterial mating into the recipient strain HB101. DNA was prepared and transformed into *S. pombe* by using standard techniques (39). To avoid the possibility that the transposon inserted into the *rad12* locus and inactivated the gene, DNA was prepared from a mixed population of tagged DNAs for each cosmid or P1 phage. Survival was assayed by comparative strip tests (9).

Sequencing of *rqh1.r12* (*rad12.502*) and *rqh1.h2* (*hus2.22*). The entire open reading frame (ORF) of the *rqh1* gene was amplified in three overlapping PCRs and cloned into pGem plasmids for the preparation of single-stranded DNA. Two independent isolates from each mutant were sequenced side by side and compared for mutations. In each case, a single change was seen in both isolates. These changes were subsequently confirmed by cloning of a number of further isolates of the relevant fragments followed by sequencing with the relevant primer.

UVDE activity assays. UVDE (also known as *S. pombe* dimer endonuclease) activity was measured in extracts as described previously (54). Two hundred milliliters of culture at a cell density of 10^7/ml was split in two. Extracts were prepared directly from one sample, while the other was irradiated with UV light (100 J/m^2) in water in a Stratalinker. Following a 1-h recovery in fresh medium, cells were broken by dismembranation, using glass beads in 20 mM Tris-HCl (pH 7.9)–1 mM EDTA–10 mM $MgCl_2$–0.3 M ammonium sulfate, 0.5 mM dithiothreitol (DTT)–10% glycerol–1 mM phenylmethylsulfonyl fluoride. Supernatants were spun at 40,000 rpm for 1 h at 4°C, dialyzed three times against 20 mM HEPES (pH 7.6)–10 mM $MgSO_4$–10 mM EGTA–5 mM DTT–20% glycerol–1 mM phenylmethylsulfonyl fluoride, and frozen at −70°C in aliquots. Reactions were carried out with 0 to 30 μg of protein in 25 μl in 9 mM HEPES–14 mM KCl–1.5 mM $MgCl_2$–0.08 mM EDTA–0.7% glycerol (pH 7.8)–0.9 mM DTT–0.02 mM deoxynucleoside triphosphates with 100 ng of DNA (irradiated or unirradiated) for 1 h at 37°C. Samples were then treated with RNase and proteinase K (9) and analyzed by electrophoresis.

Cdc10 synchronization. Double mutants carrying *cdc10.V50* and *rqh1.d* or *cds1.d* were created. Logarithmic cultures of *cdc10.V50*, *cdc10.V50 cds1.d*, and *cdc10.V50 rqh1.d* cells were shifted to 37°C for 3 h to accumulate cells at the G_1/S boundary. Cells were then shifted to 26°C and irradiated with 250 Gy 35 min later. Samples were taken for analysis of Cds1 kinase activity following 40 min of recovery. At the time of irradiation, appropriately diluted samples (10^4 cells per ml) of *cdc10.V50* and *cdc10.V50 rqh1.d* cells were divided in two, and one half of each sample was irradiated with 100 Gy. Irradiated and mock-irradiated samples were plated for viability counts. Fluorescence-activated cell sorting analysis indicated that cells were in S phase at the time of irradiation. At 140 min, before the peak in septation and after S phase (G_2 cells), similar dilutions were divided in two, and one half was irradiated to assay survival in G_2.

Chk1 phosphorylation and Cds1 kinase assays. Chk1 phosphorylation in *rqh1* null cells was monitored by mobility shift after Western blotting of cell extracts made from a strain containing an integrated triple-hemagglutinin-tagged *chk1* locus (58). Cds1 activity was monitored by immunoprecipitation-kinase (IP-kinase) assays using anti-Cds1 serum (34). Extracts were prepared from cells washed in phosphate-buffered saline and then lysis buffer [50 mM Tris (pH 7.5), 80 mM β-glycerophosphate, 250 mM NaCl, 15 mM nitrophenylphosphate, 50 mM NaF, 5 mM EDTA, 1 mM DTT, 0.1% Nonidet P-40, 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF), leupeptin, aprotinin, pepstatin, bestatin, E-64 (the latter six at 10 μg/ml)]. Cells were disrupted with glass beads (BDH) in a Ribolyser (Hybaid) for 20 s. For Cds IP-kinase assays, 1 mg of total protein in 500 μl of lysis buffer was incubated with affinity-purified Cds1 antibody at a dilution of 1:250 at 4°C for 2 h. Immunocomplexes were collected with protein A-agarose (Sigma), washed three times in lysis buffer, and then washed three times in kinase buffer (10 mM HEPES [pH 7.5], 75 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT). Twenty microliters (50% slurry) of bead pellet was incubated with 10 μl of 2× kinase buffer–5 μCi of [γ-^{32}P]ATP (ICN)–1 μl of 2 mM ATP–5 μl of myelin basic protein (MBP; 1-mg/ml stock) at 30°C for 15 min. The reaction was stopped by the addition of 20 μl of 2× sodium dodecyl sulfate (SDS) sample buffer. After boiling for 3 min, samples were run on 15% polyacrylamide gels, fixed in 40% methanol–10% acetic acid, and dried before exposure to film (Hyperfilm; Amersham). For immunodetection of Chk1, 100 μg of total protein was boiled in SDS sample buffer and loaded onto an SDS–10% polyacrylamide gel. Proteins were transferred to nitrocellulose; nonspecific sites were blocked with BLOTTO (phosphate-buffered saline, 1% fat-free milk powder, 0.05% Tween 20) and incubated in BLOTTO plus antihemagglutinin monoclonal antibody (1:1,000 dilution; BaBco). After being washed in BLOTTO, filters were incubated with peroxidase-conjugated secondary antibody (1:5,000 dilution; DAKO). Chemiluminescence detection of horseradish peroxidase-conjugated secondary antibodies was carried out by mixing 10 ml of 100 mM Tris (pH 8.5)–5.4 mM H_2O_2 with 25 μl of 90 mM *p*-coumaric acid (Sigma) and 50 μl of 250 mM luminol (Fluka). Blots were incubated with this mixture for 60 s before excess solution was removed and the filter was exposed to Hyperfilm-MP (Amersham).

RESULTS

Identification of a candidate ORF for *rad12*. *rad12.502* strain is the only *S. pombe* radiation-sensitive mutant of 24 (each defining a separate complementation group) from which we and others have failed to clone a gene by complementation of the radiation sensitivity (4). We therefore mapped the genetic location of *rad12.502* relative to known markers along the chromosomes. *rad12.502* mapped to within 7 centimorgans of *rad4* on the top arm of chromosome I, between *rad15* and *rad4*. Correlation with the physical map (29) allowed us to identify cosmids 2g11, 26b6, 27a5, 16h3, and 11a10 and two P1 clones, 32e5 and 34c8, as potentially encoding the *rad12* gene. These cosmids and phage were marked with the selectable marker *ura4*$^+$ and with the autonomously replicating sequence *ars1* by transposon tagging (40). The tagged constructs were prepared and transformed into *rad12.502 ura4.D18* mutant cells. One cosmid, 2g11, was able to restore radiation resistance to *rad12.502* mutants. This cosmid has been sequenced by the *S. pombe* genome project, and the sequence data is available from the Sanger Center (http://www.sanger.ac.uk/pombe/pombe.htm1). Cosmid 2g11 contains an ORF with homology to genes encoding the RecQ family of helicases and to the human *BLM* gene (Fig. 1). This ORF has previously been identified by complementation of the *hus2.22* mutant, which is defective in the recovery from treatment with the DNA synthesis inhibitor hydroxyurea and exhibits radiation sensitivity (15, 55).

Identification of the mutations in *rad12.502* and *hus2.22*. As anticipated, crosses between *rad12.502* and *hus2.22* mutants yielded no wild-type segregants, confirming allelism. Since *rad12.502* and *hus2.22* mutants have distinct phenotypes, we cloned by PCR amplification the entire *recQ*-like ORF from both *rad12.502* and *hus2.22* cells in order to identify the causative mutations. Single base changes were found for both *rad12.502*- and *hus2.22*-derived DNAs, which were present in several independent PCR isolates.

The *hus2.22* mutation introduces a stop codon at amino acid position 790, effectively truncating the remaining region of 538 amino acids, which contains the last two conserved helicase motifs (Fig. 1). *hus2.22* is therefore likely to have a null phenotype. This has been confirmed by deletion of the gene (55). The *rad12.502* mutation changes a threonine to an isoleucine at amino acid position 543, within the highly conserved putative ATP binding site (Fig. 1). *rad12.502* clearly does not confer a null phenotype, since *rad12.502* cells are not ionizing radia-

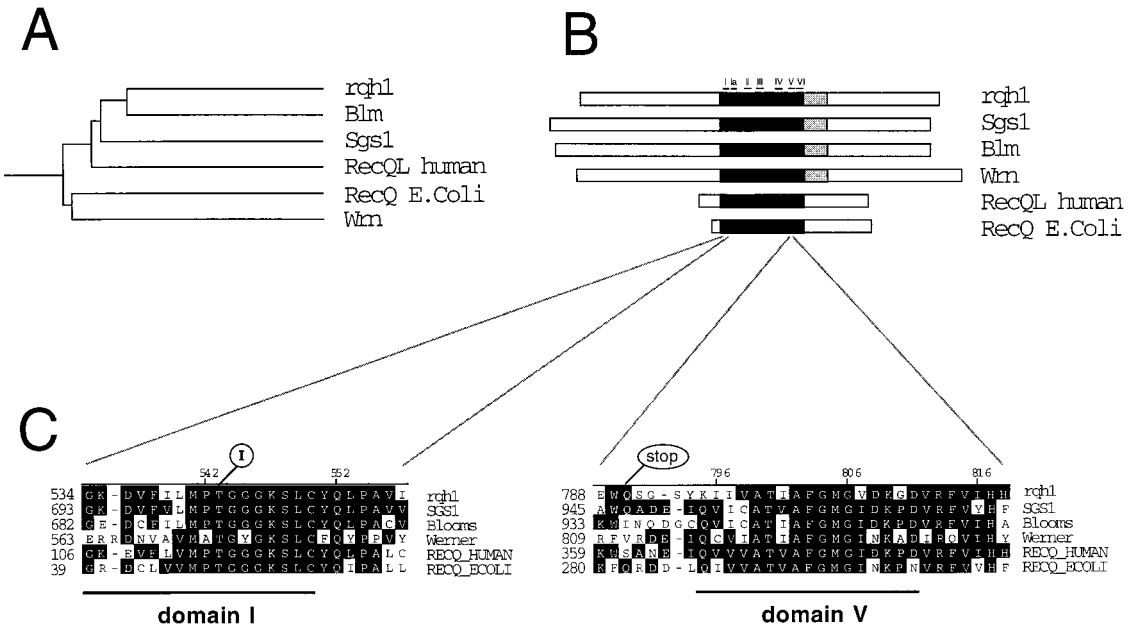

FIG. 1. Relationship between Rqh1, Sgs1, Blm, Wrn, RecQL, and RecQ. (A) Phylogenetic tree showing the relatedness of the six helicases. The region containing the helicase motifs (approximately 340 amino acids) was aligned by using DNAstar software. (B) Bar diagram indicating relatedness and structure of the six proteins. The black bar indicates the helicase region used to generate the phylogenetic tree. This region contains the six domains typical of many helicases. The shaded box indicates a short region with additional homology found in four of the six proteins. The unshaded regions show the extensions for each protein N and C terminal to the conserved regions. These show no homology. (C) Alignments of the two helicase domains containing mutations in *rqh1.r12* (*rad12.502*) and *rqh1.h2* (*hus2.22*), respectively. *rqh1.r12* cells contain a mutation that changes a conserved threonine to an isoleucine in helicase domain I, within the putative nucleotide binding site. *rqh1.h2* cells contain a mutation which introduces a stop codon at the start of helicase domain V. Alignments were generated by using DNAstar software. Numbers indicate amino acid position on the Rqh1 protein. Changes are indicated in circles above the sequence.

tion sensitive, a phenotype associated with *hus2.22* cells (15), and these cells are not as sensitive to UV as *hus2.22* or the deletion mutant cells.

To reduce confusion, we have renamed the *hus2* locus *rqh1* (*recQ* homolog), with the following allele designations: *rqh1.h2* (*hus2.22*) and *rqh1.r12* (*rad12.502*).

The *rqh1*-dependent UV response requires the recombination proteins. *rqh1* null mutants are sensitive to S-phase arrest by hydroxyurea, and the original *rqh1.h2* and *rqh1.r12* alleles have been reported to confer sensitivity to UV light. We have investigated in more detail the relationship between *rqh1* and the known DNA repair mechanisms. Two pathways that remove UV-induced DNA damage have been identified in *S. pombe* (8): NER pathway and the UVDE pathway, which removes dimers in a parallel pathway to NER. Interestingly, recombination repair mutations in *S. pombe*, such as *rhp51* and *rhp54* (the equivalents of *RAD51* and *RAD54*, respectively, of *S. cerevisiae*), also confer significant sensitivity to UV light, demonstrating a role for recombination repair in the response to this form of DNA damage (41, 42).

Double mutants carrying an *rqh1* mutation and either a NER mutation (such as *rad13* [Fig. 2A]) or a mutation in the structural gene for the UVDE enzyme (designated *uvde* [Fig. 2B]) show significantly more sensitivity to UV than the equivalent single mutants. This result indicates that Rqh1 acts in a pathway separate from either of these repair mechanisms. In contrast, double mutants between *rqh1* and representatives of the recombination repair pathway (such as *rhp51* [Fig. 2C]) did not show greater sensitivity to UV light than the respective single mutants, which suggests that Rqh1 and recombination repair proteins are involved in a common response to UV radiation damage.

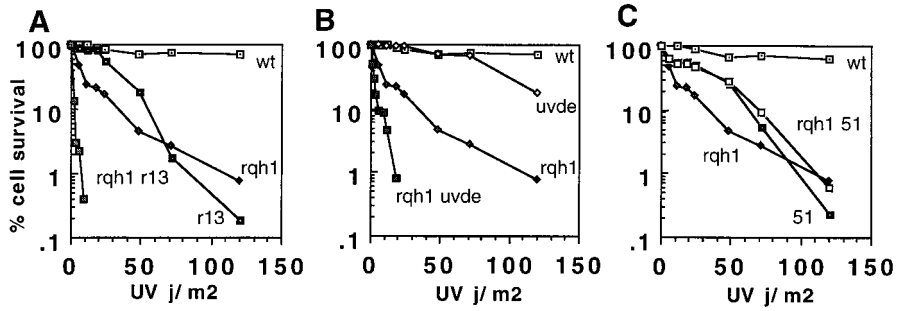

FIG. 2. Survival analysis after UV irradiation. (A) Rqh1 does not act in the NER pathway. wt, wild-type cells; r13, *rad13.d* mutant cells; rqh1, *rqh1.d* mutant cells; r13 rqh1, *rad13.d rqh1.d* double-null mutant cells. (B) Rqh1 acts independently of the UVDE pathway. wt, wild-type cells; uvde, *uvde* null mutant cells; rqh1, *rqh1.d* mutant cells; rqh1 uvde, *rqh1.d uvde* double-null mutant cells. (C) Rqh1 function requires recombination repair proteins. wt, wild-type cells; rqh1, *rqh1.d* mutant cells; 51, *rhp51.d* null mutant cells; rqh1 51, *rqh1.d rhp51.d* double-mutant cells.

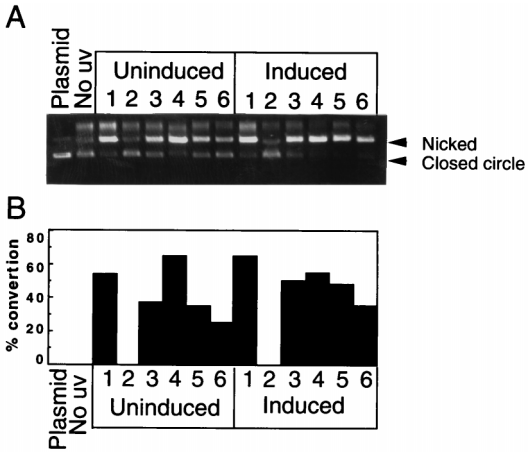

FIG. 3. Relationship between UVDE activity and *rqh1*. (A) UVDE activity was measured in wild-type cells, *uvde* null mutant cells, *rqh1.r12* (*rad12.502*) cells, *rqh1.d* null mutant cells, *rad3* null mutant cells, and *rad9* null mutant cells (lanes 1 to 6, respectively). The assay measures conversion of a UV damage-containing supercoiled plasmid to the nicked circular form (54). The responsible nuclease activity is dependent on the UVDE activity, since extracts prepared from *uvde* null mutant cells (lanes 2, induced and uninduced) do not show this activity. For the strains corresponding to lanes 1 to 6, extracts were prepared as described in Materials and Methods either with (induced) or without (uninduced) prior treatment of the cells with UV light. Following incubation of these extracts with equal amounts of UV-irradiated plasmid, DNA was assayed by electrophoresis for conversion into nicked circle form. Two control lanes, UV damage-containing plasmid without any extract (plasmid) and damage-free plasmid in wild-type cell extract (No uv), establish that the activity is dependent on cell extract and plasmid damage. (B) Quantification of the conversion from supercoiled to nicked plasmid compared to control.

UVDE and *rqh1* mutants. *rqh1* has previously been linked to the UVDE-dependent second excision repair pathway of *S. pombe* (19), and the *rqh1.r12* (*rad12.502*) mutant has been reported (19) to be deficient in UVDE activity. However, the structural gene for the UVDE enzyme has recently been identified and is independent of the *rqh1* loci (63). Therefore, the published data suggest that Rqh1 has a role in regulating the activity of the UVDE enzyme. To verify this proposition, and to determine the effect of a null mutation of *rqh1* on UVDE activity, we prepared cell extracts from logarithmically growing *rqh1.r12* and *rqh1.d* (null) cells. As controls, we also included *uvde* (null), *rad3.d*, *rad9.d*, and wild-type cells. Extracts were prepared both before and 1 h after UV irradiation. Examination of UVDE activity (Fig. 3) by using a plasmid nicking assay indicates that significant activity can be detected in all cells except *uvde* null mutants and that in all of these cases, significant induction is seen following irradiation. While this nicking assay is not quantitative, these results are reproducible and clearly show that, contrary to the reported literature, UVDE activity does not require Rqh1 function in logarithmically growing *S. pombe* cells.

Rqh1 is required to tolerate DNA damage that is not removed by NER or the UVDE pathway. When both the UVDE and NER pathways are abolished in *S. pombe*, photoproducts are not repaired at all (63). Rqh1 does not function in either of these pathways, which suggests that Rqh1 is involved in tolerating rather than removing DNA damage from cells. In support of this possibility, we find that *uvde rad13* (NER) *rqh1* triple mutants are more sensitive to UV than the *uvde rad13* double mutant (Fig. 4A). Since all excision activity is lost in the *uvde rad13* double mutant (63), the increased sensitivity of the triple mutant argues for an additional role for Rqh1 independent of direct damage removal.

Rqh1 functions in a pathway that also involves the checkpoint Rad proteins. The *S. pombe* checkpoint *rad* mutants *rad1*, *rad3*, *rad9*, *rad17*, *rad26*, and *hus1* are all very sensitive to UV and ionizing radiation and to exposure to hydroxyurea (8). We have verified (data not shown) that these six mutants define a single epistasis group. We have previously established that the radiation sensitivity of the checkpoint *rad* mutants results from at least two identifiable defects: the inability to prevent passage through mitosis when the DNA is damaged (dependent on the Chk1 kinase) and a second defect (in a pathway that requires activation of the Cds1 kinase) which results in cell death during S phase when DNA is damaged or S phase arrested with hydroxyurea (2, 15, 34). Within the checkpoint Rad protein-dependent damage response, these two defects can be genetically defined: mutants defective in the Chk1 kinase are defective specifically in the mitotic arrest pathway (2, 59). In contrast, mutants defective in Cds1 kinase or the specific checkpoint *rad* allele *rad26.T12* have normal mitotic arrest following activation of the checkpoint *rad* pathway (34) but are specifically sensitive to DNA damage during S phase (2).

To address the relationship between *rqh1* and the checkpoint genes within the DNA damage response, we constructed double mutants carrying *rqh1.r12* or *rqh1.d* and each of the checkpoint *rad* mutants. We have compared the UV sensitivities of the double mutants and the respective single mutants. We find that, for example, the *rqh1.d rad17.d* double mutant is no more sensitive than the *rad17.d* single mutant. This finding indicates that Rqh1 acts in a pathway that requires the correct operation of the checkpoint Rad group of proteins (Fig. 4B).

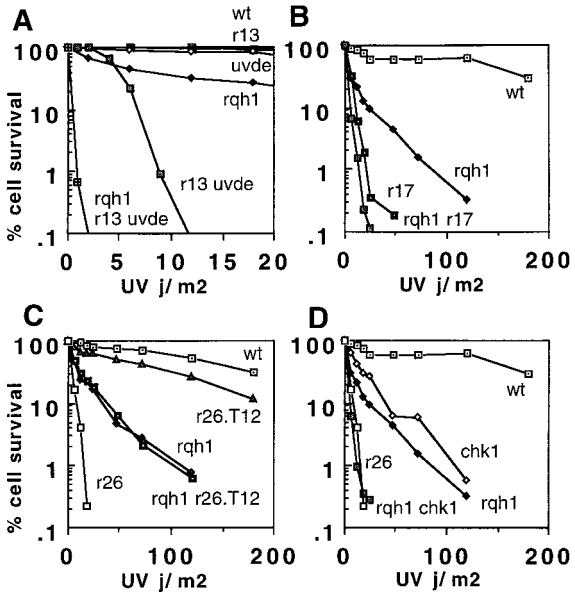

FIG. 4. Rqh1 and checkpoint proteins are required to survive unrepaired damage. (A) Rqh1 is required to survive damage in strains which cannot repair. wt, wild-type cells; uvde, *uvde* null mutant; r13, strain with null mutation of the *rad13* gene, required for NER; rqh1, *rqh1.d* null mutant cells. Double and triple mutants are also indicated (e.g., rqh1 r13 uvde is an *rqh1 rad13 uvde* triple-null mutant). (B) Epistasis between *rqh1* and checkpoint mutants. wt, wild-type cells; rqh1, *rqh1.d* mutant cells; r17, *rad17* null mutant cells; rqh1 r17, *rqh1.d rad17* double-mutant cells. (C) Rqh1 is specific to the checkpoint recovery pathway. wt, wild-type cells; rqh1, *rqh1.d* mutant cells; r26, *rad26* null mutant cells; r26.T12, *rad26.T12* mutant cells; rqh1 r26.T12, *rqh1.d rad26.T12* double-mutant cells. (D) Rqh1 is not in the same pathways as Chk1. wt, wild-type cells; rqh1, *rqh1.d* mutant cells; chk1, *chk1* null mutant cells; r26, *rad26* null mutant cells; rqh1 chk1, *rqh1.d chk1* double-mutant cells.

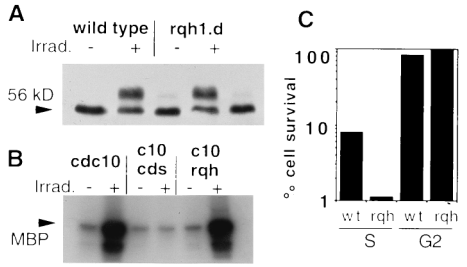

FIG. 5. Chk1 and Cds1 are activated by DNA damage in *rqh1* null cells. (A) The phosphorylation status of Chk1 in wild-type and *rqh1.d* mutant cells 1 h after irradiation (Irrad.) (+) or mock irradiation (−). Chk1 migrates as a single band in unirradiated cells of approximately 56 kDa. After irradiation it becomes phosphorylated, which causes an apparent increase in molecular weight. (B) Cds1 kinase activity is not dependent on Rqh1. Cells were synchronized by temperature shift in a *cdc10.V50* background for 3 h and released into S phase before irradiation (Irrad.) (+) or mock irradiation (−). Immunoprecipitation with anti-Cds1 antibodies followed by a kinase assay against MBP measures the activity of Cds1. *cdc10.V50* cells, irradiated 35 min after release from 37 to 26°C, activate Cds1 following irradiation. IP-kinase assays from *cdc10.V50 cds1.d* (c10 cds1) double-mutant cells do not show significant activity against MBP. Results of IP-kinase assays for *cdc10.V50 rqh1.d* (c10 rqh) double-mutant cells are identical to those for *cdc10.V50* single-mutant cells. (C) Clonogenic survival of the same cultures of *rqh1*$^+$ (wild-type [wt]) and *rqh.d* (rqh) cells following irradiation in S phase (35 min after release from *cdc10* arrest) or in G_2 (150 min after release from *cdc10* arrest).

Since the checkpoint Rad proteins are required for at least two distinct damage responses, Chk1-dependent G_2 arrest and the Cds1-dependent recovery pathway, we constructed *rqh1 chk1*, *rqh1 cds1*, and *rqh1 rad26.T12* double mutants and compared their sensitivities to those of the respective single mutants and the *rad26* null phenotype strain. Combining the *rqh1* mutation (which does not confer a mitotic arrest defect) with the *chk1.d* mutation yields a strain more sensitive than the *chk1.d* or *rqh1.d* single mutant. This sensitivity approaches that of the checkpoint *rad* null mutants such as *rad3.d* and *rad26.d* (Fig. 4D). Strains which combine the *rqh1* mutation with either the *rad26.T12* or *cds1* mutation did not show additional sensitivity, being no more sensitive than the most sensitive single mutants (Fig. 4C and data not shown). The classical interpretation of epistasis data in DNA repair studies is as follows: mutations which, when combined, do not lead to increased radiation sensitivity compared to the most sensitive single mutation act in the same pathway. Mutations which do increase radiation sensitivity when combined act in different pathways. Applying this interpretation, our data show that Rqh1 functions in the Cds1-mediated checkpoint *rad*-dependent recovery pathway but is not required for Chk1-mediated mitotic arrest.

To address whether Rqh1 functions upstream or downstream of Cds1, and to verify that the Chk1 response is not affected by *rqh1* deletion, we assayed the ability of the *rqh1* null mutant to activate Cds1 and Chk1 kinases in response to DNA damage. In *rqh1.d* cells, the Chk1 kinase is phosphorylated to an extent similar to that seen in *rqh1*$^+$ cells (Fig. 5A), which is consistent with our genetic analysis of *rqh1*. The activation of Cds1 by DNA damage is specific to S phase (34). Unfortunately, *rqh1.d* cells are not uniform in size at mitosis. Although this defect is subtle (data not shown), it prevents us from synchronizing *rqh1* cells by elutriation. To examine Cds1 activation during S phase, we therefore synchronized cells by a *cdc10* arrest-and-release strategy. Cds1 is activated to similar extents in *rqh1*$^+$ and *rqh1.d* cells after irradiation during S phase (Fig. 5B). Furthermore, while wild-type cells were approximately 10-fold more sensitive to irradiation in S phase than in G_2, *rqh1.d* mutant cells were approximately 50 times more sensitive in S phase than in G_2 (Fig. 5C). Together with the genetical analysis, these data indicate that Rqh1 is not required to generate or propagate the checkpoint-dependent signal and formally place Rqh1 downstream of the checkpoint Rad proteins and Cds1.

Rqh1 is required for viability in cells with defects in DNA synthesis. While studying the genetics of the *rqh1.r12* allele, we observed that correct Rqh1 function is required for viability in cells with defects in DNA synthesis that affected chain elongation. We had constructed double mutants containing *rqh1.r12* and a selection of *cdc* mutations. Table 1 shows the detailed results of these analyses. In brief, the *rqh1.r12* mutation did not reduce the growth rate or restrictive temperature for cell elongation and inviability when combined with mutations that are involved in the initiation of S phase, *cdc18*, *cdc19*, and *cdc21* (17, 30, 37). However, the *rqh1.r12* mutation did reduce both the growth rate and the restrictive temperature for cell elongation and inviability in mutants of DNA polymerase subunits, DNA polymerase-associated proteins and ligase, *cdc1*, *cdc6*, *cdc17*, *cdc20*, and *cdc27* and showed a synthetic interaction with *rad2* which affects DNA chain elongation (18, 36, 44–46). For the remaining *cdc* mutations tested, *cdc10* (G_1 arrest [3]) and *cdc25* (G_2 arrest [50]), *rqh1.r12* did not have any effect.

DISCUSSION

The *rqh1.r12* (*rad12.502*) mutant is sensitive to UV radiation but is not significantly sensitive to ionizing radiation (47). Efforts to clone the gene mutated in *rqh1.r12* cells by complementation of the radiation sensitivity have not been successful (4). By physical mapping, we have identified the mutation responsible for the *rqh1.r12* phenotype as a single base change that results in substitution of a threonine residue for isoleucine within the conserved nucleotide binding site of a RecQ homolog. This same ORF is mutated (by a stop codon within the conserved helicase region) in the *rqh1.h2* (*hus2.22*) mutant, which confers both UV and ionizing radiation sensitivity and is required for recovery from hydroxyurea treatment (15). *rqh1* null mutants show a phenotype indistinguishable from that of *rqh1.h2* mutants (55). The *rqh1.r12* mutation separates an aspect of the UV response from other functions of the Rqh1 protein. The helicase activity of the *S. cerevisiae* homolog of Rqh1, Sgs1, is not required for all of its functions (35), suggesting that biochemical helicase activity is not the only role for this class of proteins. In this context, it is interesting that the *rqh1.r12* mutation is within the putative ATP binding motif of

TABLE 1. Summary of *cdc-rqh1.r12* interactions

cdc mutation	Function	Interaction	Comment
cdc18	Initiation	No	
cdc19	Initiation (MCM[a])	No	
cdc21	Initiation (MCM)	No	
cdc1	Polymerase subunit	Yes[b]	Slow growing at 27°C
cdc6	Polymerase subunit	Yes[b]	Slow growing at 27°C
cdc20	Polymerase subunit	Yes[b]	
cdc27	Coprecipitates with Cdc6	Yes[b]	
cdc17	DNA ligase	Yes[b]	Slow growing at 27°C
cdc23	Late S	Yes[b]	
cdc25	G_2	No	
rad2.d	FEN1/MF-1	Yes	Slow growing at 27°C

[a] MCM, minichromosome maintenance.
[b] Mutant cannot form colonies at the semipermissive temperature, at which the *cdc* single mutant can form colonies. These double mutants also showed an increased level of cell death during short incubations at the restrictive temperature but do not show a cut (premature mitosis) phenotype.

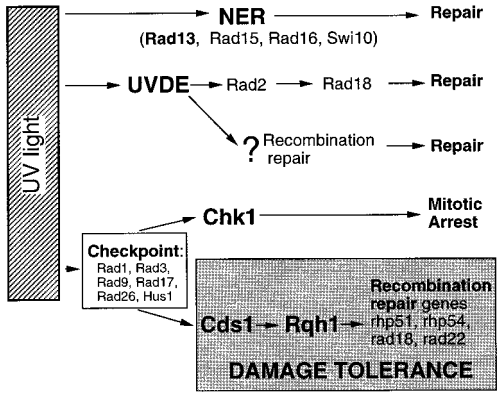

FIG. 6. A model for the UV response in *S. pombe*. UV light causes mainly 6-4 and cyclobutane dimers. This damage is repaired either by the classical NER pathway or by a novel repair pathway initiated by UVDE. The processing of sites incised by UVDE appears to follow one of two pathways. The first requires the *rad2* and *rad18* genes. The second is not characterized (?) but may require recombination functions. In addition to the removal of DNA damage, *S. pombe* responds to irradiation by activating DNA damage checkpoint genes. This results in several responses, including the activation of the Chk1 kinase (which in turn causes G_2 arrest) and activation of Cds1 during S phase. Rqh1 function in the DNA damage response is dependent on the activation of the Cds1-dependent DNA damage tolerance pathway. This pathway also involves the products of the *rad18* and recombination repair genes. However, unlike the direct repair pathways (top three arrows), this pathway does not result in the removal of DNA lesions. We speculate that the Rqh1-dependent pathway may involve bypass synthesis, allowing cells to replicate damaged templates. The fact that Rqh1 is required to prevent increased recombination in cells arrested in S phase by hydroxyurea is consistent with a role for Rqh1 in coordinating S phase and recombination at sites of DNA damage.

Rqh1, and it will be interesting to correlate the phenotypes with the biochemical properties of the mutant proteins.

***rqh1.r12* and *rqh1* deletion mutants have UVDE activity.** Freyer et al. have reported that *rad12* (*rqh1*) defines the second excision repair pathway in *S. pombe*, which is dependent on the activity of the UVDE enzyme (19). However, the *UVDE* gene is distinct from *rqh1* (63). The published data thus indicate that *rqh1* regulates UVDE activity. Our work does not agree with the published report, since we see essentially similar levels of UVDE activity in the *rqh1.r12 rqh1* null mutant and wild-type cell extracts. Furthermore, we do not see a requirement for other aspects of the checkpoint mechanism for UVDE activity since *rad3* and *rad9* mutant cells also exhibit UVDE-dependent nuclease activity. Since the UVDE enzyme has now been cloned (56), it should be possible to study any subtle relationship between Rqh1, checkpoint proteins, and UVDE activity in more detail.

A model for UV-induced damage repair and Rqh1-dependent damage tolerance in *S. pombe*. The radiation-sensitive phenotype associated with the *rqh1* mutants is a clear phenotypic marker that allows us to delineate the relationship between the different DNA damage responses and a homolog of the *BLM* and *recQ* genes. We have integrated all of our observations and those reported previously into a model that describes the response to UV-induced DNA damage in fission yeast (Fig. 6). The data on which the model is based are presented in Table 2.

UV light produces primarily cyclobutane pyrimidine dimers and 6-4 photoproducts. These can be repaired or tolerated by a number of defined pathways, as follows. The *rad16*, *rad13*, *rad15*, and *swi10* gene products act to excise photoproducts by the classical NER mechanism. Damage not repaired by NER can be repaired by the UVDE pathway. Once a nick is intro-

TABLE 2. Summary of UV sensitivity data used to construct model[a]

Strain genotype	Sensitivity compared to single mutants, comment	Reference
rqh1.d UVDE	More sensitive	
rqh1.r12 rad13.d	More sensitive	
rad13.d UVDE	More sensitive, no excision of UV photoproducts	
rqh1.r12 rad13.d UVDE	More sensitive than relevant double mutants	
rqh1.d UVDE	More sensitive	
rqh1.d rad13.d	More sensitive	
rqh1.d rad13.d UVDE	More sensitive than relevant double mutants	
rad1.d rad3.d	No additional sensitivity, epistatic	
rad1.d rad9.d	No additional sensitivity, epistatic	
rad1.d rad17.d	No additional sensitivity, epistatic	
rad1.d rad26.d	No additional sensitivity, epistatic	
rad1.d hus1.d	No additional sensitivity, epistatic	
rad3.d rad9.d	No additional sensitivity, epistatic	
rad3.d rad17.d	No additional sensitivity, epistatic	
rad3.d rad26.d	No additional sensitivity, epistatic	
rad3.d hus1.d	No additional sensitivity, epistatic	
rad9.d rad17.d	No additional sensitivity, epistatic	
rad9.d rad26.d	No additional sensitivity, epistatic	
rad9.d hus1.d	No additional sensitivity, epistatic	
rad17.d rad26.d	No additional sensitivity, epistatic	
rad17.d hus1.d	No additional sensitivity, epistatic	
rad26.d hus1.d	No additional sensitivity, epistatic	
rad1.d rqh1.r12	Very slightly more sensitive than *rad1.d* strain	
rad3.d rqh1.r12	No additional sensitivity, epistatic	
rad9.d rqh1.r12	No additional sensitivity, epistatic	
rad17.d rqh1.r12	Very slightly more sensitive than *rad17.d* strain	
rad26.d rqh1.r12	No additional sensitivity, epistatic	
hus1.d rqh1.r12	Very slightly more sensitive than *hus1.d* strain	
rad1.d rqh1.d	Viable, very slightly more sensitive than *rad1.d* strain	
rad3.d rqh1.d	Synthetic lethality	
rad9.d rqh1.d	Viable, no additional sensitivity	
rad17.d rqh1.d	Viable, very slightly more sensitive than *rad17.d* strain	
rad26.d rqh1.d	Synthetic lethality	
hus1.d rqh1.d	Viable, very slightly more sensitive than *hus1.d* strain	
rad26.T12 rqh1.r12	No additional sensitivity, epistatic	
chk1.d rqh1.r12	More sensitive, similar to *rad26.d* strain	
rad26.T12 rqh1.d	No additional sensitivity, epistatic	
chk1.d rqh1.d	More sensitive	
chk1.d rad26.T12	More sensitive, similar to *rad26.d* strain	2
rhp51.d rqh1.r12	No additional sensitivity, epistatic	
rhp54.d rqh1.r12	No additional sensitivity, epistatic	
rhp51.d rqh1.d	No additional sensitivity, epistatic	
rhp54.d rqh1.d	No additional sensitivity, epistatic	
rad18.X rqh1.r12	No additional sensitivity, epistatic	
rhp51.d UVDE	More sensitive	
rad18.X UVDE	More sensitive	
rad18.X rad2.d	No additional sensitivity, epistatic	32
rqh1.r12 rad2.d	More sensitive	
rhp51.d rad13.d	More sensitive	
rad9.d rad13.d	More sensitive	24
rad17.d rad13.d	More sensitive	24
rad18.X rad13.d	More sensitive	32
rad9.d UVDE	More sensitive	
rad17.d UVDE	More sensitive	

[a] Where data have been previously published, a reference is given; otherwise, data were generated during this study. In classical epistasis analysis, when double mutants are not more sensitive than the most sensitive single mutant, the relevant proteins are thought to operate in the same damage response. When sensitivity is increased, some aspect of one protein must act in a response that does not involve the second protein.

duced by the UVDE enzyme (6), it is processed in one of two ways. One is not defined (Fig. 6) but may involve recombination proteins, while the other involves further processing of the lesion by Rad2 (the FEN1 homolog) and subsequent repair by a Rad18-dependent mechanism.

In addition to repair, UV damage causes the activation of the checkpoint proteins which mediate cell cycle arrest. The checkpoint proteins activate kinases which define subpathways: a Chk1-dependent mitotic arrest pathway and a Cds1-dependent pathway that results in the survival of DNA damage during S phase. This Cds1-dependent response requires both Rqh1 and recombination repair functions. The ability to survive small amounts of DNA damage that cannot be or have not been removed is most clearly seen when one compares the effects of loss of Rqh1 function in cells that cannot remove UV photoproducts (Fig. 4A).

It is interesting to speculate that this S-phase-specific survival pathway may be related to the bypass of damaged sites by the replication apparatus, a process that is known to require recombination functions in *E. coli*. The relationship between Rqh1-dependent damage survival and recombination is intriguing, since it has recently been demonstrated that mitotic recombination is elevated in Rqh1 mutants (55). This increase may reflect a direct role for Rqh1 in suppressing recombination, or it could be due to an increased level of spontaneous DNA damage in *rqh1* mutants (perhaps associated with a role for Rqh1 during normal S phase) which provides appropriate substrates to initiate recombination. Further work will clearly be required to clarify this point.

Rqh1 as a model for RecQ-related helicases in eukaryotic replication and damage responses. The *E. coli* RecQ helicase is involved in both DNA recombination and repair (38) and has been proposed to regulate recombination (26). Human homologs of the *recQ* gene include the Bloom's syndrome gene *BLM* (14), the Werner's syndrome gene *WRN* (64), and a third potential helicase gene known as the RECQL (48) or helicase Q1 (52), which encodes the major DNA-dependent ATPase activity in human cells. Phylogenetic analysis indicates that the Rqh1 protein is most closely related to BLM and RECQL. A single *S. cerevisiae* member of the RecQ family of proteins, Sgs1p, has been identified as interacting with topoisomerases (20, 61) but does not apparently impart significant radiation or hydroxyurea sensitivity to cells (60). The association of a radiation-sensitive phenotype with mutation of the *S. pombe recQ* homolog *rqh1* provides an ideal phenotypic marker with which we can investigate the mechanism of action of this class of helicases in DNA replication and repair.

Our work demonstrates, for the first time, a link between Rqh1 function, DNA damage checkpoint proteins, and recombination functions. The requirement for Rqh1 when DNA synthesis is compromised by mutations affecting chain elongation (but not its initiation) further suggests a relationship between these Rqh1-dependent responses and DNA replication. Stewart et al. demonstrated enhanced mitotic recombination associated with loss of *rqh1*, a phenotypic link between the *rqh1* null mutant and Bloom's syndrome cells (55). Taken together, these data suggest that the RecQ-related helicase in fission yeast, and by extension the BLM function in human cells, acts to affect recombination functions during S-phase arrest. When S phase is arrested by hydroxyurea (which depletes nucleotide pools), recombination is repressed by the Rqh1 function. We suggest that in response to unrepaired DNA damage, recombination is channeled into a postreplication repair-like pathway that allows the bypass of otherwise toxic lesions during DNA synthesis. Insight into the relationship between BLM function, the checkpoint proteins, and the effects on genomic stability and cancer susceptibility should thus be gained by studying the RecQ homolog in fission yeast.

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