Brc1-Mediated DNA Repair and Damage Tolerance

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ABSTRACT

The structural maintenance of chromosome (SMC) proteins are key elements in controlling chromosome dynamics. In eukaryotic cells, three essential SMC complexes have been defined: cohesin, condensin, and the Smc5/6 complex. The latter is essential for DNA damage responses; in its absence both repair and checkpoint responses fail. In fission yeast, the UVC and ionizing radiation (IR) sensitivity of a specific hypomorphic allele encoding the Smc6 subunit, rad18-74 (renamed smc6-74), is suppressed by mild overexpression of a six-BRCT-domain protein, Brc1. Deletion of brc1 does not result in a hypersensitivity to UVC or IR, and thus the function of Brc1 relative to the Smc5/6 complex has remained unclear. Here we show that brc1Δ cells are hypersensitive to a range of radiomimetic drugs that share the feature of creating lesions that are an impediment to the completion of DNA replication. Through a genetic analysis of brc1Δ epistasis and by defining genes required for Brc1 to suppress smc6-74, we find that Brc1 functions to promote recombination through a novel postreplication repair pathway and the structure-specific nucleases Ssl1 and Mus81. Activation of this pathway through overproduction of Brc1 bypasses a repair defect in smc6-74, reestablishing resolution of lesions by recombination.

CHROMOSOMAL integrity is essential for cell viability. Not surprisingly therefore, cells have evolved a plethora of enzymes to control the chromosome dynamics required for transcription, DNA replication, mitosis, and the repair of a wide range of DNA lesions and stalled replication intermediates. Such events are coordinated with cell cycle progression, thus ensuring the fidelity of chromosomal inheritance.

The structural maintenance of chromosome (SMC) proteins were identified by genetic screens in Saccharomyces cerevisiae for chromosome instability mutants. These chromatin-associated proteins are characterized by N- and C-terminal globular domains containing, respectively, Walker A and B ATPase motifs. Two coiled-coil domains with a central flexible hinge separate these globular domains. Initially, two highly conserved protein complexes were characterized, termed condensin and cohesin. Both complexes contain heterodimers of SMC subunits: Smc2 and -4 in condensin and Smc1 and -3 in cohesin. Each Smc subunit folds at the hinge to form an intramolecular interaction and is then thought to associate intermolecurly through the hinge domain. Each complex contains additional unique non-Smc subunits, and additional proteins most likely involved in their loading onto chromatin have also been identified. As their names suggest, condensin functions in mitotic chromosome condensation and cohesin in sister chromatid cohesion (Harvey et al. 2002; Hirano 2002, 2005). However, mutants in subunits of both complexes have also uncovered DNA repair defects (Aono et al. 2002; Nagao et al. 2004; Strom et al. 2004; Unal et al. 2004; Lehmann 2005), most likely through an indirect effect based on a more fundamental defect in chromosomal dynamics rather than through a direct enzymatic role in reversal of a lesion.

A third SMC complex, termed the Smc5/6 complex, is also present in all eukaryotes (Corbe and Heck 2000; Harvey et al. 2004). This was originally defined by the cloning of the Schizosaccharomyces pombe rad18 gene (Lehmann et al. 1995), which encodes Smc6, and to clarify the nomenclature, rad18 is referred to as smc6. With Smc5 and four non-Smc subunits [Nse1–4; nse4 is identical to rad62 (Morikawa et al. 2004)], these proteins form an essential complex of which the precise molecular function remains unknown (Fujioka et al. 2002; Hazbun et al. 2003; McDonald et al. 2003; Harvey et al. 2004; Pebernard et al. 2004; Andrews et al. 2005; Sergeant et al. 2005; Zhao and Blobel 2005). The hypomorphic smc6 alleles, smc6-X and smc6-74, are defective in the repair of a diverse array of DNA
lesions, and, in the case of smc6-74, a further defect in
the maintenance of the Chk1-dependent checkpoint
arrest exists, despite normal activation of Chk1 activity
(Verkade and O’Connell 1998; Verkade et al. 1999;
Harvey et al. 2004). Spore germination experiments
with cells deleted for either smc6 or nse1 and the analysis
of additional smc6 alleles have confirmed that this
complex is indeed required to successfully respond to
DNA damage and pass through mitosis. Further, condi-
tional and hypomorphic alleles of nse1, nse2, nse3, and
nse4, with a conditional allele of rad60, which encodes a
protein involved in Smc5/6 function without being a
member of the complex per se (Morishita et al. 2002;
Boddy et al. 2003), also show defects in DNA damage
responses. Recent data from S. cerevisiae have demon-
strated a defect in the segregation of rDNA at mitosis in
temperature-sensitive smc5-6 and smc6-9 mutants, lead-
ing to DNA damage that can be partially suppressed in
rad52 mutants, suggesting inappropriate recombina-
tion at the repetitive rDNA (Torres-Rosell et al. 2003).
This may be related to observed epistasis between vari-
sous smc6 and nse1-nse4 alleles and deletion of the
Rad51 homolog, rhp51 in S. pombe (Lehmann et al.
1995; McDonald et al. 2003; Morikawa et al. 2004;
Perbernard et al. 2004). Similar epistasis has been seen
between smc6-56 and rad52Δ in S. cerevisiae (Torres-
Rosell et al. 2003), and here the smc6 mutation also
results in defects in methyl methanesulfonate (MMS)-
induced interchromosomal and sister chromatid re-
combination (Onoda et al. 2004). Presumably, as with
condensin and cohesin, the defective DNA damage
responses of mutants in the Smc5/6 complex are a con-
sequence of a more fundamental defect in chromosome
organization.

Brc1 encodes a six-BRCT (BRCA1 C terminal)-domain
protein that was identified as an allele-specific high-copy
suppressor of smc6-74 (Verkade et al. 1999). While brc1Δ
cells are not hypersensitive to IR or UV-C, and Brc1 is
not part of the Smc5/6 complex, several observations
point toward a DNA damage response function for this
protein. First, brc1 becomes essential in strains with com-
promised SMC6 or NSE4 function and is also syntheti-
cally lethal with conditional alleles of rad60 and top2
(Verkade et al. 1999; Morishita et al. 2002; Boddy et al.
2003; Morikawa et al. 2004), which encodes a type II
Topoisomerase. Second, as with smc6 mutants, brc1Δ cells
lose chromatids at 200-fold the rate of wild-type cells,
comparable to rhp51Δ, and brc1Δ cells frequently show
cytologically abnormal nuclei (Verkade et al. 1999).
Finally, a putative homolog in S. cerevisiae, ESC4, has
been shown to be required for DNA repair, particularly
during S phase, where by an unknown mechanism it
may facilitate resumption of DNA replication (Rouse
2004), and a more distant human relative, PTIP, has
been implicated in chromosome segregation and DNA
damage responses (Cho et al. 2003; Jowsey et al. 2004).
However, the mechanism by which Brc1 bypasses defects
in Smc6 function in S. pombe cells, including those in G2,
is unknown and important to elucidate.

Here we show that S. pombe brc1Δ mutants are indeed
sensitive to a range of DNA-damaging drugs, which
share the feature of generating lesions during S phase
or lesions that are an impediment to DNA replica-
tion. Genetic epistasis analysis suggests that Brc1 may
function in this response with the structure-specific
nuclease Mus81/Eme1 and with Rhp51. In addition,
we investigated which genes are required for Brc1 to
suppress smc6-74. From these experiments we can fur-
ther place Brc1 function on a novel arm of postreplica-
tion repair, utilizing translesion synthesis polymerases
to bypass alkylated bases during S phase, and repair
through recombination initiated largely by the Sxl1/
Sxl4 structure-specific nuclease. Similar Sxl1/Sxl4-
stimulated recombination is used for Brc1 to bypass
the UV-C hypersensitivity of smc6-74 in G2 cells, for
which it still requires the postreplication repair machin-
ery, but not translesion synthesis polymerases. More-
over, sxl1Δ, while not hypersensitive to either MMS or
UV-C, dramatically enhances the sensitivities of smc6-74
but not smc6-X. Together, the data suggest that smc6-74
cells are specifically defective in recombinational repair,
and that Brc1 can, through Sxl1/Sxl4, generate an
alternative structure that can then be repaired by Smc5/
6-independent recombination. Such a defect in smc6-74
may explain the checkpoint maintenance defects not
seen in smc6-X.

MATERIALS AND METHODS

Fission yeast genetic methods: All strains used were
derivatives of 972k– and 975h+. Standard procedures and
media were used for propagation and genetic manipula-
tion (Moreno et al. 1991). Methods for UV-C survival assays,
transformation, microscopy, and FACS have been described
previously (O’Connell et al. 1997; Verkade et al. 1999, 2001;
Den Elzen and O’Connell 2004; Harvey et al. 2004).
New null alleles of sxl1, mus81, and sdx8 were constructed using
targeting constructs in which the entire open reading frames
were replaced by ura4. Successful deletion of these genes was
certified by Southern blotting. All other strains have been
described or were kindly provided by A. M. Carr (Genome
Damage and Stability Center, University of Sussex, United
Kingdom) or H. Shinagawa (Osaka University, Japan).

Drug sensitivity assays: Assays on agar plates were carried
out in YES medium, with plates photographed after 4 days at
30°C, or, in the case of temperature-sensitive mutants, after 5
days at 25°C. Cultures were grown in appropriately supple-
mented minimal medium to a density of 4 × 10⁶ cells/ml, and
5 μl of 10-fold serial dilutions was plated. For liquid exposure
to MMS, cells were grown to 4 × 10⁵/ml, and MMS was added
to samples taken immediately before MMS addition.

Brc1 suppression of smc6-74 assays: Plasmid-borne expres-
sion of Brc1 from its own promoter or from the wild-type or
attenuated nmt1 promoter with or without thiamine results
in equal suppression of smc6-74, presumably by passing a threshold level required to bypass smc6-74. For these assays, expression from the attenuated (pRep41) promoter (referred to as pbrc1) under repressing conditions (5 μM thiamine) was used and compared to controls containing pRep41 only (referred to as vector).

**RESULTS**

**Brc1 is required for tolerance of radiomimetic drugs:** brc1 was originally identified as an allele-specific high-copy suppressor of the UV-C and IR hypersensitivity of smc6-74. Somewhat surprisingly, brc1Δ cells did not display a hypersensitivity to these treatments, although they did show a high frequency of chromosome loss and synthetic lethality with both smc6-74 and smc6-X, suggesting a role in genome integrity (Verkade et al. 1999). We considered that Brc1 might either be involved in repairing a particular type of lesion that is prevalent in smc6 mutants or be required at a specific point in the cell cycle. To examine this possibility, we tested the sensitivity of brc1Δ cells to a range of radiomimetic drugs: MMS, 4-nitroquinoline-N-oxide (4-NQO), hydroxyurea (HU), and camptothecin (CPT). In each case brc1Δ cells failed to form colonies, and in liquid culture the cells became elongated due to checkpoint activation (Figure 1), suggesting that the hypersensitivity is due to a defect in repairing lesions caused by these compounds. Although the lesions caused by these compounds are diverse, they share a commonality of stalling replication and/or of damaging DNA during replication. Moreover, a plasmid expressing Brc1 could also rescue the hypersensitivity of smc6-74 cells to these agents. This rescue was complete for MMS and CPT, but only partial for 4-NQO and HU. From these data we propose that Brc1 participates in the cellular response to replication damage and that in smc6-74, similar structures to those forming in cells treated with these drugs occur or accumulate following defective DNA repair in response to UV-C and IR, which can then be processed in a manner mediated by Brc1.

**Epistasis analysis of brc1Δ:** We next used epistasis analysis using the MMS hypersensitivity of brc1Δ. In these experiments, double mutants between brc1Δ and alleles of genes known to function in various DNA repair pathways were constructed, and their sensitivity to MMS was assayed relative to wild-type and parental controls. MMS sensitivity was assayed by chronic exposure to a range of concentrations in agar plates and also by acute exposure to 0.05% MMS in liquid culture, and viability was measured after inactivation of the MMS. In each case both assays gave the same qualitative result. The classic interpretation of such experiments is that if double mutants have an enhanced hypersensitivity, then these genes function in different, although potentially overlapping, pathways. Alternatively, if the double mutant is as sensitive as the most sensitive parent, then the genes function in the same pathway.

These assays employed null and conditional alleles of the following genes to represent different DNA damage responses: nucleotide excision repair, rad13 and rad16 (Carr et al. 1993, 1994); UV excision repair, rad2 and uvr1 (Bowman et al. 1994; Murray et al. 1994); homologous recombination, rhp51, rhp54, rhp57, and smm5 (Jang et al. 1994; Murris et al. 1996; Tsutsumi et al. 2000; Akamatsu et al. 2003); double-stranded break repair, intra-S-phase checkpoint, rad32 (Tavassoli et al. 1995); postreplication repair, rhp18, mms2, ube3c, eso1, rev3, and dinB (Tanaka et al. 2000; Verkade et al. 2001; Brown et al. 2002; Kai and Wang 2003); structure-specific nucleases—replication restart, mus81, slx1, cds1, rhp1, and srs2 (Murakami and Okayama 1995; Stewart et al. 1997; Boddy et al. 2000; Wang et al. 2001; Coulon et al. 2004); and checkpoint arrest, chk1, rad3, hus1, and rad1 (Walworth et al. 1993; Bentley et al. 1996; Kostrub et al. 1998; Gaspari et al. 2000). Epistatic interactions were found only with rhp51Δ and mus81Δ strains (Figure 2, A and B), suggesting that these genes function in the same pathway as brc1. However, it is notable that these are among the most MMS-hypersensitive
strains. Only brc1Δ rhp1Δ and brc1Δ rhp54Δ double mutants showed reduced growth compared to parental strains in the absence of MMS (Figure 2D and data not shown), and brc1Δ is synthetically lethal with smc6-X, smc6-74, top2-191, rad60-1, and rad62-1 (nse4-1) (Verkade et al. 1999; Morishita et al. 2002; Boddy et al. 2003; Morikawa et al. 2004). In all other cases the double mutants were substantially more sensitive to MMS than was either parent (for examples, see Figure 2, C and D).

**Brc1-mediated rescue of smc6-74—pathways required for MMS tolerance:** The above data suggest that Brc1 functions in a pathway that includes Rhp51 and Mus81, but do not rule out that other genes in these experiments are also involved in a Brc1-mediated response to DNA alkylation, but play additional roles that are independent of Brc1, and so epistasis is not observed. We therefore sought to identify the genes required for the Brc1-mediated suppression of the MMS hypersensitivity of smc6-74. In these experiments, double mutants were made between smc6-74 and mutants in defined DNA damage response pathways. In cases where these double mutants were more sensitive than either parent, a criterion that excluded rhp51Δ, rhp54Δ, and rad2Δ that are epistatic with smc6-74, cells were transformed with a Brc1-expressing plasmid or vector only controls, and chronic and acute MMS sensitivity was assayed. A summary of these experiments is presented in Table 1.

We first investigated the postreplication repair pathway, which enables tolerance of alkylated bases, allowing continued DNA synthesis. This is achieved either by lesion bypass by translesion synthesis polymerases or by mechanisms involving template switching by the recombination pathway. Under current models (Prakash et al. 2005), Rhp18 (homolog of S. cerevisiae RAD18, an E3 ubiquitin ligase), with Rhp6 (homolog of S. cerevisiae RAD6, an E2 ubiquitin-conjugating enzyme), catalyzes the monoubiquitination of proliferating cell nuclear antigen (PCNA). As a result, the translesion synthesis polymerases, including polymerases η, ζ, and κ (encoded by eso1, rev3, and dinB in S. pombe), replicate past the lesion for several nucleotides, and then replication continues with the replicative polymerases such as polymerase δ. However, the monoubiquitinated PCNA can be polyubiquitinated using Lysine-63 linkages by a complex of Ubc13 (an E2) and Mms2/Rad5 (an E3, the latter encoded by radδ8 in S. pombe). This signals for bypass by recombination-dependent template switching.

These assays clearly showed that rhp18, but not ubc13, is required for Brc1 to suppress smc6-74 (Figure 3). Under the model above, this result predicts that the translesion synthesis polymerases would also be required, and

![Figure 2](image-url)
Figure 4A shows that this is indeed the case. A lack of suppression was observed in cells lacking eso1, rev3, and dinB (denoted 3TLSΔ), and a relatively weak suppression was observed in dinB strains. All other combinations of single and double mutants corroborated these findings (data not shown), demonstrating that, at least in part, the suppression of the MMS hypersensitivity of smc6-74 by Brc1 involves this mechanism to bypass stalled replication.

Recent data suggest a role for two checkpoint complexes in the loading of dinB: the PCNA-related 9-1-1...
complex, composed of Rad9, Rad1, and Hus1, and its clamp loader, composed of Rad17 and the four small subunits of replication factor C (RFC) (Kai and Wang 2003). In keeping with this model, we found little suppression in rad9Δ or rad17Δ backgrounds (Figure 4B). This lack of suppression was not a consequence of checkpoint failure, however, as complete suppression was observed in a chk1Δ background (Figure 4C). The lack of suppression was greater than that seen for dinBΔ, suggesting that 9-1-1 and Rad17/Rfc2-5 may play additional roles in the Brc1-mediated suppression.

**Brc1-mediated rescue of smc6-74—pathways required for base alkylation repair:** Postreplication repair pathways enable tolerance of alkylated bases, that is, bypass but not repair of the actual lesion. In *S. pombe*, there is ample genetic evidence that alkylated bases are repaired by recombinational mechanisms (for examples, see Jang et al. 1994; Tsutsui et al. 2000; Kai and Wang 2003; Smeets et al. 2003; Morikawa et al. 2004). As stated above, we were not able to assay mutants in the homologous recombination pathway due to epistasis with smc6-74. However, two complexes function redundantly in the recombination pathway that aid in the loading of Rhp51 into the nucleoprotein filament on single-stranded DNA. One complex consists of Swi5 and Srf1, the other consists of Rhp55 and Rhp57 (homologs of *S. cerevisiae* RAD55 and RAD57), and only when both complexes are absent do cells phenocopy rhp51Δ (Akamatsu et al. 2003). As shown in Figure 5, suppression of smc6-74 by Brc1 clearly required Rhp57, but not Swi5. Interestingly given the epistasis between smc6-74 and rhp51Δ or rhp54Δ, both the rhp57Δ smc6-74 and swi5-39 smc6-74 double mutants were significantly more MMS sensitive than either parent. These data are consistent with the model that Brc1 promotes recombinational repair of alkylated bases in smc6-74 mutants in G2 phase, following bypass of the lesion during S phase by the postreplication repair pathway. It is also possible that the recombination pathway is processing stalled replication forks in S phase and promoting replication restart.

Stability of stalled replication forks and the successful resumption of DNA replication are reliant on two redundant groups of response enzymes. The predominant response is via the RecQ-type helicases, encoded by rqh1 in *S. pombe*, with Topoisomerase III (Khakhar et al. 2003). *rhp57* is not an essential gene, and MMS sensitivity assays showed suppression of an *rhp57*Δ smc6-74 by Brc1 back to the sensitivity seen for the *rhp57*Δ single mutant (data not shown). This is in keeping with our observed lack of epistasis between *brc1Δ* and *rhp1Δ* (Figure 2D). Three dimeric protein complexes have been identified by genetic studies in *S. cerevisiae* and *S. pombe* that act redundantly with Sgs1 and Rqh1, respectively (Mullen et al. 2001; Doe et al. 2002; Coulon et al. 2004). These complexes include two structure-specific nucleases, Mus81/Eme1 and Slx1/Slx4, which have a preference to cleave structures resembling stalled replication forks, and a third complex, Slx5/Slx8, of unknown function. We deleted the *S. pombe* slx8 gene, which resulted in extremely slow growing and poorly viable cells (data not shown), and so no further genetic analyses were attempted. However, we could assay the requirement for the other complexes using *mus81Δ* and *slx1Δ* strains. For *slx1Δ* smc6-74, we observed very little suppression by Brc1 in both chronic and acute exposure to MMS (Figure 6, A and C), a somewhat surprising result given that *slx1Δ* by itself shows wild-type levels of MMS sensitivity (Coulon et al. 2004). For *mus81Δ* smc6-74, nearly complete suppression was observed (Figure 6, B and C), although compared to the smc6-74 parent, we reproducibly observed an increased MMS sensitivity in the double mutant overexpressing Brc1 in several independent experiments, assayed by both chronic and acute MMS exposure. These data show a strong requirement for Slx1 and a more modest requirement for Mus81 for Brc1 overexpression to suppress smc6-74.

In the course of these experiments we noted that smc6-74 slx1Δ double mutants were substantially more MMS sensitive than either smc6-74 alone or smc6-X slx1Δ double mutants that were identical to smc6-X single mutants (Figure 7). Again, this is particularly notable as slx1Δ cells are not hypersensitive to MMS (Coulon et al. 2004), and smc6-X is not suppressible by Brc1 overexpression (Verkade et al. 1999). Combined, these data
suggest that structures that are efficiently processed by Slx1/Slx4 are prevalent in \textit{smc6-74} cells grown in the presence of MMS and that Brc1 may promote their processing via Slx1/Slx4 into the Rhp57-dependent arm of recombination.

\textbf{Brc1-mediated suppression of the UV-C hypersensitivity of \textit{smc6-74}:} The repair of alkylation damage, and its relationship to replication fork stalling, largely investigates DNA repair and tolerance mechanisms during DNA replication. However, Brc1 overexpression can also suppress the UV-C hypersensitivity of \textit{smc6-74}. In these experiments, an asynchronous culture is irradiated, but in \textit{S. pombe} this represents $\sim 75\%$ of cells in G2 phase and only 10% in S phase. Thus, the Brc1-mediated suppression of repair defects in \textit{smc6-74} is not confined to S-phase damage, even though \textit{brc1} cells are not hypersensitive to UV-C in G2. We therefore took a similar genetic approach to the above experiments, using MMS to investigate which genes are required to suppress defective UV-C lesion processing in \textit{smc6-74} cells, bearing in mind that the majority of these responses are occurring in G2. The data are summarized in Table 1, with example survival curves in Figure 8.

As seen with MMS exposure, the suppression of \textit{smc6-74} by Brc1 overexpression was wholly dependent on \textit{rhp18}, although independent of \textit{ubc13}. In the case of MMS, similar observation predicted a role for trans-lesion synthesis. However, these polymerases are largely not required in G2, and hence their absence does not result in a significant UV-C hypersensitivity in asynchronous cultures. Not surprisingly therefore, Brc1 overexpression still largely suppressed the UV-C hypersensitivity of \textit{smc6-74} in the absence of polymerases $\eta$, $\zeta$, and $\kappa$ (3TLS$\Delta$), with the residual sensitivity presumably accounted for by the G1- and S-phase cells in the culture. Moreover, we consistently observed that Brc1 overexpression increased the sensitivity of the 3TLS$\Delta$ strain to UV-C. Brc1-mediated channeling during G1 and S phase of the lesions into a pathway that is dependent on these polymerases, which becomes a toxic event in their absence, may explain this. Further, unlike the case for alkylation damage, we saw no effect of deleting \textit{rad9}. The checkpoint response itself was clearly not required, as we observed complete suppression of \textit{chk1}\textit{D smc6-74}.

We next assayed the Rhp57- and Swi5-dependent recombination pathways. Epistasis again excluded assays with downstream genes in the recombination pathway such as \textit{rhp51}. As with alkylation damage, Brc1-mediated suppression of \textit{smc6-74} UV-C hypersensitivity was dependent on \textit{rhp57}, but in this case \textit{swi5-39 smc6-74} mutants were partially defective in the suppression assays. Further, \textit{slx1}\textit{D smc6-74} cells showed little suppression, despite the observation that \textit{slx1}\textit{D} cells show wild-type sensitivity to UV-C, and \textit{mus81}\textit{D smc6-74} cells showed

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure6.png}
\caption{Structure-specific nucleases are required for Brc1-mediated suppression of the MMS hypersensitivity of \textit{smc6-74}. Brc1 suppression assays using the indicated strains were performed for both chronic (A and B) and acute (C) exposure to MMS. For chronic exposure, a range of MMS concentrations were assayed, and representative plates are shown. (A) \textit{slx1}\textit{D} almost completely abolished suppression by both assays. (B) \textit{mus81}\textit{D} results in an incomplete suppression.}
\end{figure}
considerable although incomplete suppression. In the UV-C irradiated S-phase cells (~10% of the population), the recombination genes may also process stalled replication forks and ensure restart of replication.

Together these data suggest that UV-C-irradiated smc6-74 cells accumulate aberrant DNA structures that can be resolved by recombination and that such a recombination pathway is promoted by the concerted action of Brc1, Slx1, Rhp18, and, to a lesser extent, Mus81. As neither brc1D nor slx1D cells are hypersensitive to UV-C, the structures forming in the background of smc6-74 either must not form in wild-type cells or are rapidly processed by another pathway, perhaps requiring wild-type smc6.

**DISCUSSION**

The Smc5/6 complex, although relatively poorly understood at this stage, is as fundamental to chromosome dynamics as its relatives condensin and cohesin. The genetic relationships between smc6 and brc1 in *S. pombe* described here provide a framework on which future molecular studies can elucidate molecular details to the precise function of the Smc5/6 complex. Previous studies have implicated a role for the Smc5/6 complex in recombinational repair, although how it could influence the core recombination machine is not yet clear.
by studying how overexpression of Brc1 can suppress the hypersensitivity of the checkpoint and repair-defective smc6 allele smc6-74, we can build a model of the defect in smc6-74 cells and thus dissect the function for the wild-type Smc5/6 complex.

First, considering the response to the alkylating agent MMS, cells need to contend with both the physical interference with the replication complexes and the mechanisms by which the lesions are repaired. There is considerable evidence in S. pombe that while translesion synthesis polymerases can bypass alkylated bases, on the basis of the sensitivity of various DNA repair mutants, their repair is largely dependent on recombination and, to a lesser extent, on nucleotide excision repair (NER). For Brc1 to suppress the MMS hypersensitivity of smc6-74, translesion synthesis polymerases are required, suggesting that the actual repair occurs in the subsequent G2 phase following lesion bypass during S phase. The requirement for Rhp57 for the suppression and the published observations that this protein, in complex with Rhp55, aids in the loading of Rhp51 in G2 (Akamatsu et al. 2003) are consistent with a repair in G2 following bypass of the initial lesions. However, there is a strong requirement for Slx1 for this suppression and for MMS resistance in smc6-74 but not in smc6-74 cells. Slx1 with its partner Slx4 cleaves Y-shaped structures characteristic of replication forks and recombination intermediates (Coulon et al. 2004). Upon Brc1 overexpression, Slx1/Slx4 may cleave such accumulating structures in smc6-74 cells to recruit the recombination machinery or to process an intermediate of recombination. It is tempting to speculate that in wild-type cells, the Smc5/6 complex normally functions in such recruitment of recombination factors to stalled replication forks or alkylated lesions. Notably, smc6-74 substantially increased the MMS sensitivity of rhp57Δ and swi5-39 strains, which indeed are defective in initiating recombination, suggesting that wild-type Smc6 function may be critical for recombination in these backgrounds. Mus81, which with its partner Eme1 also cleaves Y-shaped structures albeit with a different specificity (Kaliraman et al. 2001; Gaillard et al. 2003; Osman et al. 2003), played a minor role in the Brc1-mediated suppression of smc6-74. Thus, Mus81/Eme1 may be essential for the processing of a minority of lesions in smc6-74, perhaps explaining our observed epistasis between mus81Δ and brc1Δ. Mus81/Eme1 is essential for the viability in the hypomorphic nse1-1, nse2-1, nse3-1, and nse4-1 (rad62-1) mutants (Morikawa et al. 2004; Pebernard et al. 2004). We have not, however, observed this for smc6-74 or smc6-X, and so the differential requirement for a particular structure-specific nuclease likely reflects the accumulation of different lesions depending on the nature of the mutation in a particular Smc or non-Smc subunit.

In the case of UV-C irradiation in G2 cells, the requirements for suppression were similar to that for MMS with two exceptions. First, the translesion synthesis polymerases were not required, which is not surprising since they are thought to function primarily during S phase. Second, Swi5 was required for complete rescue, which it was not for MMS. Regardless, the observations for UV-C sensitivity also suggest that Slx1/Slx4 processes a structure in smc6-74 cells that promotes recombination repair. We assayed UV-C sensitivity in G2- and S-phase brc1Δ cells, but were unable to demonstrate any sensitivity. This suggests that Brc1 is required only to repair UV-C-induced lesions once inappropriately processed into a structure in smc6-74 cells that perhaps resembles those present in MMS-treated cells and that this uses Slx1-initiated recombination. Notably, we found no evidence to link Smc5/6 with Brc1 to the NER pathway.

The absolute dependence on Rqh1 is in keeping with our previous findings in synchronous populations of cells that this protein is required for UV-C tolerance throughout the cell cycle (Verkade et al. 2001). In the case of MMS treatment, the lack of requirement for ubc13 can easily be rationalized by the channeling of lesions into the tolerance pathway controlled by translesion synthesis polymerases. Importantly, however, the lack of requirement for both ubc13 and translesion synthesis for the UV-C response suggests a hitherto unseen pathway acting downstream of Rqh1 during G2 that awaits identification.

In wild-type cells, rqh1 plays key roles in rescuing stalled replication forks in S phase and in the repair of UV-C lesions in G2 (Murray et al. 1997; Khakhar et al. 2003). As the Brc1-mediated rescue of smc6-74 does not require rqh1, the defect in smc6-74 is not resolved using this pathway. It is therefore possible that wild-type Smc5/6 may contribute to Rqh1 function. Curiously, while rqh1Δ slx1Δ show a synthetic lethality (Coulon et al. 2004), rqh1Δ brc1Δ cells are viable although they show a high degree of mitotic abnormalities and grow significantly slower than wild-type cells. Thus, while Brc1 can stimulate Slx1-dependent repair in smc6-74, it is not absolutely required for Slx1 activity in rqh1Δ cells.

With its many BRCT domains, Brc1 is predicted to bind to one or more phosphoproteins (Manke et al. 2003). We have assayed for physical interaction between Brc1 and Slx1 and failed to detect it, even with over-expression. We have also attempted to assay interaction with Slx4, but have found that epitope tagging of Slx4 results in a nonfunctional and extremely unstable protein.

In summary, from these observations we propose a model (Figure 9) that in wild-type cells, the Smc5/6 complex functions in the recruitment of recombination complexes to lesions. Brc1 controls an alternative pathway to initiate recombination utilizing primarily Slx1 and a yet to be deciphered subbranch of the postreplication repair pathway. Clearly, it will be important to dissect molecular details to explain the genetic observations described here.
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**LITERATURE CITED**


Fujoka, Y., Y. Kimata, K. Nomaguchi, K. Watanabe and K. Kohn, 2002 Identification of a novel non-structural maintenance of


Verkade, H. M., S. J. Bugg, H. D. Lindsay, A. M. Carr and M. J. O’Connell, 1999 Rad18 is required for DNA repair and...


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