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Role of Blm and collaborating factors in recombination and survival following replication stress in *Ustilago maydis*

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Abstract

Inactivation of the structural gene for the RecQ family member, BLM in human, Sgs1 in budding yeast, or Rqh1 in fission yeast leads to inappropriate recombination, chromosome abnormalities, and disturbed replication fork progression. Studies with yeasts have demonstrated that auxiliary gene functions can contribute in overlapping ways with Sgs1 or Rqh1 to circumvent or overcome lesions in DNA caused by certain genotoxic agents. In the combined absence of these functions, recombination-mediated processes lead to severe loss of fitness. Here we performed a genetic study to determine the role of the *Ustilago maydis* Blm homolog in DNA repair and in alleviating replication stress. We characterized the single mutant as well as double mutants additionally deleted of genes encoding Srs2, Fbh1, Mus81, or Exo1. Unlike yeasts, neither the *blm srs2*, *blm exo1*, nor *blm mus81* double mutant exhibited extreme loss of fitness. Inactivation of Brh2, the BRCA2 homolog, suppressed toxicity to hydroxyurea caused by loss of Blm function. However, differential suppression by Brh2 derivatives lacking the canonical DNA-binding region suggests that the particular domain structure comprising this DNA-binding region may be instrumental in promoting the observed hydroxyurea toxicity.

Keywords

BLM; BRCA2; Rad51; Srs2; Fbh1; Exo1; Mus81 recombination; DNA repair

1. Introduction

During DNA replication, forks encounter a variety of obstacles that must be overcome or circumvented to complete the course. Transcription, topological impediments, inappropriately bound proteins, diminished nucleotide pool size, innate slowly traversed sequences, and DNA damage are all factors that present barriers to fork movement. Failure to surmount or neutralize such barriers can have dire consequences including chromosome breakage and rearrangements, elevated recombination, and loss of fitness. To cope with such an unpredictable terrain, cells have molecular tool kits equipped with a wide range of implements that can be deployed. Included among these are helicases and nucleases that can act to re-establish replication forks after stalling.

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As part of a set of factors, prokaryotes use the RecQ helicase and the 5′-3′-directed exonuclease RecJ in restarting stalled replication [1]. Likewise in eukaryotes, helicases and nucleases are also employed to re-establish forks after stalling. BLM, a RecQ homologue in human, has long been known for its role in promoting efficient replication fork progression [2], for suppressing inappropriate sister chromatid exchange, chromosomal abnormalities, and in predisposition to cancer of all types [3,4]. In budding yeast *Saccharomyces cerevisiae* and fission yeast *Schizosaccharomyces pombe*, the RecQ homologues Sgs1 and Rqh1, respectively, are required for efficient replication on damaged DNA templates [5,6], and for attenuating recombination and preventing aberrant chromosome rearrangements in mitotic cells [7–10]. The 5′-3′-directed exonuclease Exo1 has been shown to act on stalled replication forks in yeast [11,12] and loss of both Exo1 and Sgs1 function causes a synthetic loss of fitness [13].

Stalled replication forks also appear to be acted upon by the structure-specific endonuclease Mus81 [14]. Synthetic lethality resulting from the combined loss of Mus81 and Sgs1 (or Rqh1) is strong genetic evidence that these factors contribute in an overlapping manner to alleviate stalled replication [15–18]. Srs2 is a helicase related to bacterial UvrD that also overlaps functionally with Sgs1. As with the case of Mus81, there is synthetic lethality in the absence of both Sgs1 (or Rqh1) and Srs2 [19,20]. Inactivation of the gene for Rad51 suppresses the lethality caused by loss of Sgs1 and Srs2 or loss of Sgs1 and Mus81 [17,18,21]. This rescue has been interpreted to mean that toxic DNA structures accumulate in the absence of Sgs1 and Srs2 or Mus81, but that the formation is dependent on recombination function [5]. Or alternatively, these factors act at an earlier step to prevent formation of abnormal DNA structures that are then processed by the homologous recombination machinery to generate poisonous intermediates. Thus, in the absence of recombination, synthetic lethality is suppressed.

Besides the roles of BLM/Sgs1, Exo1, Mus81, and Srs2 proteins in surmounting obstacles to DNA replication and clearing fork blockage, there is mounting evidence linking all of these proteins to direct roles in homologous recombination and DNA-double-strand break (DSB) repair. Sgs1 and Exo1 act redundantly on DNA ends at the site of DSBs to resect the duplex and reveal 3′-single-stranded tails, the substrate for Rad51-promoted strand invasion [22,23]. BLM can promote branch migration [24], unwind D-loops [25], catalyze regression of replication forks [26], resolve double Holliday junctions with the combined action of topoisomerase III to reduce formation of crossover products [27,28] and disrupt Rad51-nucleoprotein filaments on single-stranded DNA [29]. Srs2 can strip Rad51 from nucleoprotein filaments and dissociate nascent Rad51-paired joint molecules [30–32], and in vivo is important in crossover control [33,34]. Loss of Srs2 function results in hyperrecombination and hypersensitivity to genotoxic agents [20,35,36]. Mus81 acts to resolve Holliday junction precursors in vitro and appears to provide a failsafe mechanism in crossover control [37]. Loss of Mus81 function in yeasts can result in a meiotic phenotype. In fission yeast Mus81 is responsible for nearly all meiotic crossovers, and in its absence spore viability is reduced to 1% or less with high levels of chromosome missegregation [38]. In budding yeast, depending on temperature and strain background, *mus81* mutants can arrest in meiotic prophase due to checkpoint activation stemming from unprocessed recombination intermediates [39–41]. These defects can be circumvented by mutations that prevent initiation of recombination [37, 38,41].

Our studies on mechanisms and control of recombination and genome stability make use of the fungus *Ustilago maydis* as an experimental system. In *U. maydis*, the constellation of proteins dedicated to recombinational repair and maintenance of genome integrity overlaps in many instances with the system in budding yeast, but as these organisms are evolutionarily extremely divergent, it is not surprising that there are numerous differences [42]. In budding yeast there is the single instance of a RecQ homolog, but in *U. maydis* there are two RecQ

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family members present. One denoted as Blm exhibits strong similarity to BLM and Sgs1 [42], while the gene for the other is highly reiterated and located at the subtelomeric region of the chromosomes [43], a feature in common with a number of other fungi [44,45] including fission yeast [46]. There is an Srs2 homolog in *U. maydis*, but in addition, there is another Srs2-related protein similar to Fbh1, the F-box helicase present in human and in fission yeast, but absent in budding yeast [47–49]. In fission yeast, slow growth suppressors with mutations in the Fbh1 structural gene appear frequently in the absence of Rad52 function [50], which is of primary importance in all aspects of homologous recombination in budding and fission yeasts and which serves as an important mediator of Rad51 in DSB repair [51]. In *U. maydis* Rad52 appears to play little to no role in recombination [52], leaving the job of mediating delivery of Rad51 to sites of DNA lesions to Brh2, a member of the Brca2 class of proteins [53]. In addition, homologs of Exo1 and Mus81 are present in *U. maydis*, but no investigation has been made as yet regarding their biological function. Little genetic information is available regarding how Blm functions in overcoming replication stress and homologous recombination in a Brca2-dependent organism, or how Brca2, Srs2, Fbh1, Exo1 and Mus81 might contribute to or overlap with Blm action. Therefore, we performed an analysis to investigate genetic interactions of these functions using *U. maydis* as a representative Brca2 model system.

2. Materials and Methods

2.1 U. maydis genetic methods

Manipulations with *U. maydis*, culture methods, gene disruption and gene transfer procedures, survival after DNA damage, synthesis of diploids on charcoal medium, and genetic crosses by mating *in planta* determinations have been described previously (see [54,55] and references therein). Allelic recombination at the *nar1* locus was measured by determining Nar^+ prototroph formation in diploids. Frequencies were determined based on the median value obtained after plating 11 to 15 independent cultures. The genes encoding Blm (um02874), Srs2 (um01691), Fbh1 (um03756), Exo1 (um03141), and Mus81 (um04630) were identified as entries (noted in parenthesis) in the manually annotated MIPS *U. maydis* database [see http://mips.gsf.de/genre/proj/ustilago/]. Null mutants were constructed by replacing the entire open reading frames with cassettes expressing resistance to hygromycin (Hyg^R), or nourseothricin (Nat^R) by standard methodology [56,57]. Briefly DNA fragments of approximately 1 kbp upstream (5′-flanking sequence) and downstream (3′flanking sequence) from the gene to be disrupted were amplified from genomic DNA with appropriate primers by polymerase chain reaction. These were fused to cassettes expressing Hyg^R, or Nat^R, then the construct was amplified by PCR, the DNA fragment was gel-purified, and used to transform *U. maydis* protoplasts. Drug resistant transformants were screened and confirmed for the gene deletion using PCR *U. maydis* strains (Δ*brh2*) were either described previously [58] or else constructed as part of this study. Self-replicating plasmids expressing the genes encoding Brh2, $Brh2^{NT}$, $Brh2^{CT}$, Brh2ΔBRC, Brh2ΔCRE, or Brh2-RPA70 fusion under control of the glyceraldehyde 3-phosphate dehydrogenase promoter all relied on selecting for hygromycin resistance in *U. maydis* and have been described before [55,59].

A *blm-K443R* mutant strain was constructed by allele replacement [60] modeled along the lines developed for “pop-in/pop-out” recombination in yeast. The *pyr6* gene encoding orotidine-5′-phosphate decarboxylase was deleted using a knockout vector consisting of a fusion of the upstream and downstream sequences flanking the *pyr6* open reading frame, but with no intervening marker, by selection on medium containing 5-fluoroorotic acid and cytidine. The *blm-K443R* allele was introduced into this strain on a plasmid containing the wild type *pyr6* gene. Single-crossover integration events at the *blm* locus were identified after selection for pyrimidine prototrophy. After counter selection on 5-fluoroorotic acid and cytidine, pop-outs in which the wild type allele had been replaced by the *blm-K443R* allele were identified by

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PCR screening using primers specific for the wild type and mutant alleles. The wild type *pyr6* gene was then re-introduced and integrated at the endogenous locus.

3. Results

3.1 Phenotype of DNA helicase mutants

U. maydis proteins in this study orthologous to yeast and/or human counterparts are shown schematically and domain structures are highlighted (Fig. 1). To assess the role of these proteins in DNA repair we measured survival following irradiation with ultraviolet light (UV). Similarly, to assess their role in overcoming replication stress we measured growth in the continuous presence of methylmethansulfonate (MMS), which is thought to cause lesions that interfere with fork progression [61], or in the presence of hydroxyurea (HU), which stalls DNA synthesis by depleting nucleotide pools [62].

In *U. maydis* genetic analysis involving multiple genes can be problematic due to the limited range of selectable markers available for gene disruption. To circumvent this difficulty we constructed an unmarked *blm* mutant by an allele swapping procedure that leaves behind no selectable marker, then used this strain as a primary source in disrupting other genes by replacement with drug resistance markers. Since it had been previously reported in studies with Sgs1 that mutation of a critical lysine residue in the ATP binding loop resulted in a helicase-defective, functionally compromised mutant [9,63,64], we made the equivalent change (K443R) in Blm, although the caveat should be kept in mind that not all Blm-related functions are necessarily abolished by inactivating the helicase. In the case of *S. cerevisiae sgs1* or *S. pombe rqh1* helicase-defective mutants it has been established that certain activities are still retained independent of the helicase activity [65–69]. This is not necessarily unexpected as these proteins operate in the context of multicomponent complexes [70,71]. For our purposes, however, the helicase-defective allele was suitable for the assays we employed.

As a reference the null mutant completely deleted of the Blm structural gene (*blm*) showed little sensitivity to UV, but was moderately sensitive to MMS, and markedly sensitive to HU. By comparison the *blm-K443R* mutant was marginally more sensitive to UV, and slightly more sensitive to MMS and HU than the deletion mutant (Fig. 2). These results indicate that the *blm-K443R* allele is crippled in functions with respect to the processes involved in resistance to MMS and HU, but shows a more pronounced phenotype than the deletion, probably through a dominant negative effect by the helicase-defective protein with interacting partners, reminiscent of the findings with the *sgs1*-helicase defective allele of *S. cerevisiae* [9,64]. This phenotype contrasts with that resulting from deletion of the gene for Brh2, which is directly involved in homologous recombination. In this situation there is extreme sensitivity to UV and MMS, but resistance to HU. In the case of the *blm-K443R* mutant, resistance to HU was partially restored when the gene for Brh2 was deleted suggesting that lethality caused by the HU-induced replication stress was mediated through homologous recombination activity.

Studies in *S. pombe* have shown that deletion of the gene for Srs2 results in moderate sensitivity to DNA damage by genotoxins although without much effect on growth in the absence of induced damage [20], while deletion of the gene for Fbh1 results in reduced growth rate as well as sensitivity to genotoxins [48]. The double mutant combinations *rqh1 srs2*, *rqh1 fbh1*, *srs2 fbh1* are either synthetically lethal or else severely debilitated [20,21,48]. Deletion of the genes for Srs2 or Fbh1 in *U. maydis* had little to no effect on growth or resistance to MMS, HU, or UV (Fig. 2). On the other hand, the *blm-K443R srs2* double mutant was more sensitive, and the *blm-K443R fbh1* double mutant was much more sensitive to MMS and HU than the *blm-K443R* single mutant. The *srs2 fbh1* double showed a synthetic response in loss of resistance to MMS and HU. We were unable to construct a *blm srs2 fbh1* triple mutant and presume this combination was synthetically lethal analogous to the situation reported for the

sgs1 srs2, rqh1 srs2, or *rqh1 fbh1* double mutant combinations in budding or fission yeast [13,19,21,48]. The viability in the case of all three double mutant combinations of *U. maydis* genes, but putative synthetic lethality in the triple mutant, could be interpreted to mean there is more shared overlap in function among these three genes in *U. maydis* than in budding and fission yeasts.

3.2 Functional interactions between Blm and Mus 81 or Exo1

As mentioned above accumulating genetic and biochemical evidence from yeasts has shown that the nucleases Mus81 and Exo1 can serve to salvage a replication fork or restore fork progression that is hindered by DNA lesions. In addition Mus81 and Exo1 have important roles in recombination that also appear to be redundant or overlapping with Blm/Sgs1[14,17,22, 23]. Therefore, we examined the role of the respective *U. maydis* genes for their contributions to DNA repair, recombination and replication stress (Fig. 3). The single mutant *exo1* exhibited a slight sensitivity to MMS but not to UV or HU, while the double mutant *blm-K443R exo1* showed a synthetic sensitive phenotype approaching that of the *brh2* mutant when cells were treated with UV or MMS suggesting a role in homologous recombination. On the other hand *mus81* showed slight sensitivity to HU, but not UV or MMS. This is in contrast to yeast, in which *mus81* is sensitive to MMS [39] as is *mms4* [72], the gene product of which partners with Mus81 to form a heteromeric complex [14]. The *blm-K443R mus81* double mutant, however, was extremely sensitive to UV, MMS, and HU, again suggesting a possible role in homologous recombination. In both double mutants, inactivation of homologous recombination function by deletion of the gene for Brh2 substantially restored ability to grow in the presence of HU. These results support the notion that toxic intermediates are processed by Exo1 and Mus81 and that Exo1 and Mus81 collaborate with Blm to provide some overlapping function to overcome replication stress, but they are not completely equivalent in terms of their response to different types of DNA lesions. It is remarkable that in yeasts the double mutant combinations *sgs1 exo1* and *sgs1/rqh1 mus81* lead to inviability [9,13,15–17], but there is little notable decrease in fitness of the corresponding double mutants in *U. maydis* under normal conditions of growth.

To determine if there is a recombination phenotype associated with the mutants, we measured mitotic allelic recombination in homozygous diploids. This was determined between heteroalleles at the *nar1* (nitrate reductase) locus and detected as nitrate protoptrophy. Recombination in the *blm-K443R exo1* double mutant was 1.7 times higher than wild type, and in *exo1* or *mus81* was about 3.5 times higher. In *exo1* or *mus81* allelic recombination was elevated about 14-fold over the wild type level (Table 1). This additive effect indicates some redundancy in function of Blm and Exo1.

Given the importance of Exo1 in double-strand-break end processing in *S. cerevisiae* [22,23] and the requirement for Mus81 in crossing over during meiosis in *S. pombe* [38], we were interested to determine the phenotype of these mutants in meiosis. In *U. maydis* successful meiosis is evinced teliospore germination followed by promycelia development and formation of viable asporidial haploid cells[73,74]. Homozygous crosses between *blm-K443R, exo1, mus81, and blm-K443R exo1* strains were performed, but in no case was there any obvious difference from the wild type control (Table 1). Therefore, loss of any of these gene functions individually does not confer a strong effect on meiosis.

3.3 Role of hyperactive Brh2 derivatives in HU-induced toxicity of the blm mutant

Eliminating homologous recombination proficiency can rescue the lethal combination of *sgs1* (*rqh1*) and *srs2* mutations in budding and fission yeasts [19,21] and can rescue the slow grow phenotype of *top3* [75,76], the product of which physically partners with Sgs1 [77,78]. It has been reported that in *S. cerevisiae* loss of both Sgs1 and Rad51 results in sensitivity to

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HU [76]. By contrast in *S. pombe* loss of Rqh1 function sensitizes cells to HU, but this sensitivity is suppressed by loss of homologous recombination proficiency [20]. In this same vein, we found that inactivation of any of the genes directly involved in homologous recombination in *U. maydis*, including the structural genes for Brh2, Rad51, and Rec2 could suppress the HU sensitivity of the *blm-K443R* mutant (Figs. 2,4B and data not shown). However, as functional domains of Brh2 have been mapped by deletion analysis and Brh2 derivatives altered in recombination proficiency have been constructed [59], we asked whether there were limits or conditions to the quality or degree of recombination proficiency that could overcome replication stress sensitivity in the absence of Blm action.

Inactivation by mutation or deletion of the N-terminal Rad51-interacting BRC element (see Fig. 4A) of Brh2 (Brh2 ΔBRC) similarly causes loss of biological activity [55]. Similarly, removal of 41 residues from the C-terminus of Brh2 (Brh2 ΔCRE) disrupts a different Rad51-interacting region, the CRE, which also results in complete loss of biological activity [55]. However, truncations terminating the protein within a narrow segment (Brh2^{NT}), such that a putative nuclear localization signal remains untouched but the entire C-terminal DNA-binding domain (Brh2^{CT}) is deleted, are partially active in complementing UV sensitivity, are restored in ability to support Rad51-focus formation, and are hyperactive in recombination [59]. A synthetic Brh2 (Brh2-RPA70) constructed by replacing the endogenous DNA-binding domain with a heterologous DNA-binding domain from the large subunit of the single-strand DNA binding protein replication protein A (RPA70) is even more active in complementing UV sensitivity, Rad51-focus formation and recombination, and can suppress the DNA repair deficiency and meiotic block caused by the loss of Rec2 function [54,59]. Thus, Brh2^{NT} and Brh2-RPA70 have an inherent capacity to confer recombination and repair activity but are missing some aspect of regulatory control.

Brh2 reintroduced into the *blm-K443R brh2* double mutant restored UV resistance and HU sensitivity indicating that recombination proficiency had been restored (Fig. 4B). Brh2 ΔCRE, Brh2 ΔBRC, and Brh2^{CT} were unable to do so as expected given the loss of function associated with these derivatives. Brh2-RPA70 was similar, but not quite as effective as native Brh2 in restoring function. However, Brh2^{NT} was unable to revert the HU resistance, but was capable of restoring resistance to UV indicative of a separation in function of recombination activity. These results suggest that Brh2^{NT} has the ability to direct cleansing of lesions resulting from UV irradiation, which is most likely through post-replicational repair of single-stranded gaps [79,80], but is not capable of processing DNA structures formed during HU-induced replication stress into toxic lesions.

4. Discussion

This genetic study was focused primarily on the function of Blm in *U. maydis*, but by approaching the question from the point of view of examining the *blm* phenotpe in double mutant combinations additional information on interacting genes was obtained. Three conclusions can be drawn from this study. First, Blm functions in an overlapping capacity with the helicases Srs2 and Fbh1 to alleviate recombination-mediated problems in replication arising from damage caused by MMS and stress caused by HU. Second, Blm also collaborates with two nucleases, Exo1 and Mus81, to process recombination-mediated toxic DNA structures that accumulate in response to MMS or HU treatment, and also to serve in repair of DNA damaged by UV. Third, the results with the separation-of-function Brh2^{NT} variant implicate Blm in the Brh2-mediated homologous recombination pathway, but at a level outside or beyond the basic machinery dedicated to repair of UV-damaged DNA. The action of Brh2 leading to intervention or involvement of Blm requires the OB-fold DNA-binding domain of Brh2.

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It is interesting to compare and contrast the properties of the *blm* mutant of *U. maydis* with *sgs1* or *rqh1* mutants of *S. cerevisiae* or *S. pombe* as these fungi are evolutionarily divergent. Similar to the situation with *rqh1* in *S. pombe* [20] but unlike *sgs1* of *S. cerevisiae* [76], the *blm* mutant is highly sensitive to HU and this sensitivity can be suppressed by loss of homologous recombination function. Furthermore, as in *S. pombe* [48,49], but unlike *S. cerevisiae*, there is an additional Srs2-like protein, the F-box helicase Fbh1, that appears to serve in an overlapping manner with Blm and Srs2 to alleviate stress caused by MMS or HU treatment.

The end-processing nuclease Exo1 and the structure-specific nuclease Mus81 also overlap functionally with Blm as witnessed by the synergistic sensitization of the double mutants to MMS and to UV. Given the important role attributed to these nucleases in preparing DNA or processing DNA intermediates in the homologous recombination systems of yeasts, we were curious to determine if there was a defect in recombination caused by their loss in *U. maydis*. Indeed in mitotic cells a hyperrecombination phenotype was observed in agreement with the findings from other systems [63,81]. The hyperrecombination phenotype could be due to re-channeling spontaneous lesions towards the recombination pathway. In the case of the *exo1 blm* double mutant the elevated recombination could be due to altered end processing at DNA breaks or gaps resulting in shorter heteroduplex DNA (hDNA) lengths such that only one of the two heteroalleles was covered. Mismatch repair over the shorter hDNA tract would lead to a recombinant. In meiosis, we would have expected to see teliospore germination abort if there were a failure to initiate homologous pairing resulting from lack of DNA end processing by Exo1 and/or Blm. Or if there were an inability to resolve Holliday junctions by Mus81 we would have expected to see arrest in promyclium development. That asporidial cells formed normally and viable progeny were produced with efficiency not noticeably different from wild type suggests redundancy with other functions. Likely candidates whose orthologs appear present in the *U. maydis* proteome might include Mre11 and/or Dna2 for end processing and Yen1 and/or Rad1 (human GEN1 and XPF, respectively) for Holliday junction resolution.

Addition of Brh2 or derivatives to reverse the HU resistance of the *blm-K443R brh2* double mutant led to a surprising association of the DNA-binding domain of Brh2 and RPA70 with toxic intermediate formation. It was established in previous studies that the Brh2 N-terminal region alone possessed an inherent capacity to complement the radiation sensitivity of the *brh2* mutant, to promote Rad51-focus formation in response to DNA damage, and to support homologous recombination to an even higher level than in the wild type [59]. Expression of $Brh2^{NT}$ in *blm-K443R brh2* effectively complemented the UV sensitivity but did not restore the sensitivity to HU, suggesting that the recombination function supplied by $Brh2^{NT}$ is sufficient to provide activity required for repair of DNA damaged by UV, but does not suffice to induce toxicity or reverse the HU phenotype of *blm-K443R brh2*. This failure to reverse the HU phenotype is in contrast to the full-length Brh2 protein or the Brh2-RPA70 fusion protein, in which the endogenous DNA-binding domain has been swapped for that of RPA70. Both of these can cause the reversal to HU sensitivity. As both DNA-binding domains contain tandem arrangements of OB-folds, it would appear that the presumed formation of toxic recombination intermediates is dependent upon the OB-fold domain of Brh2. One notion to explain these findings is that the OB-fold domain of Brh2 becomes inappropriately bound to DNA structures formed under HU-induced replication stress when Blm is missing and it is this protein bound structure that effectively poisons the replication machinery. Alternatively, recombination intermediates formed by $Brh2^{NT}$ do not become toxic. Experiments are underway to distinguish between these possibilities.

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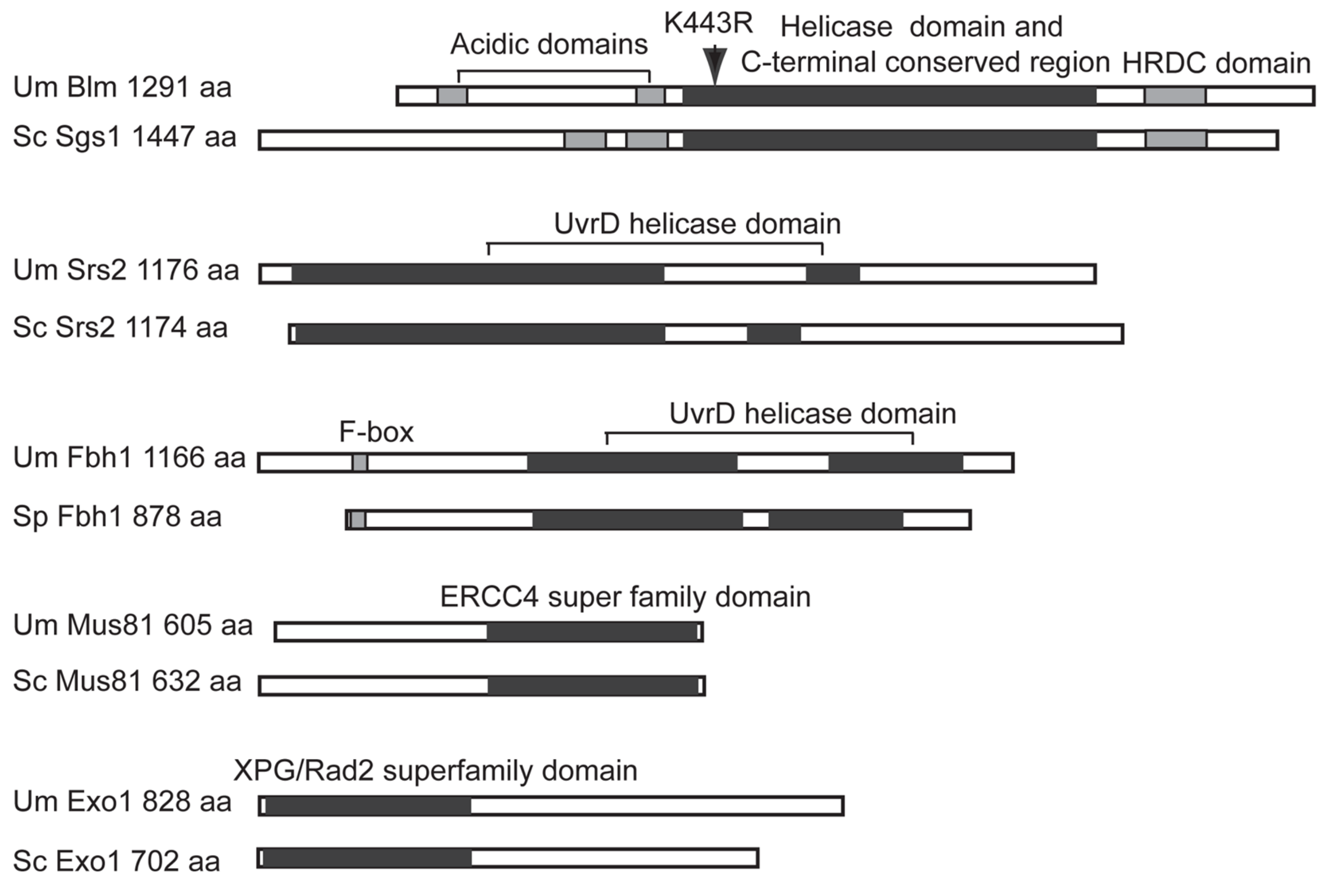

Figure 1.
Pairwise schematic representations of *U. maydis* (Um) proteins in comparison to homologues in *S. cerevisiae* (Sc) or *Schizosaccharomyces pombe* (Sp). Functional domains are in blocks of gray or black as indicated.

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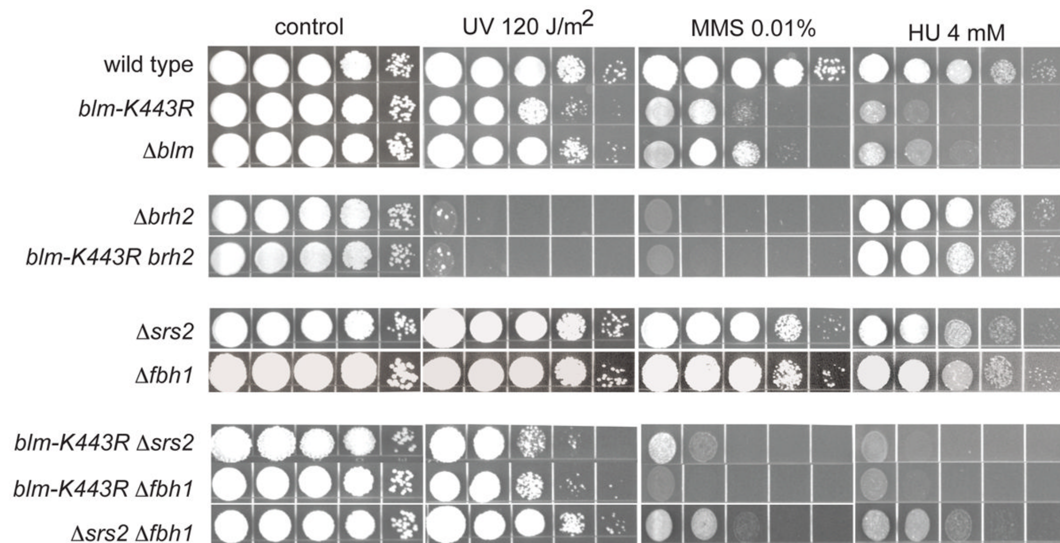

Figure 2.
Phenotype of helicase-defective single and double mutants. Survival of the indicated strains after treatment with UV or grown in medium containing MMS or HU. Cell suspensions were adjusted to ~4×10^7 per ml, diluted in 10-fold serial dilutions and aliquots (10 μl) of each were spotted from left to right as shown. Survival is indicated as growth of colonies 3 days.

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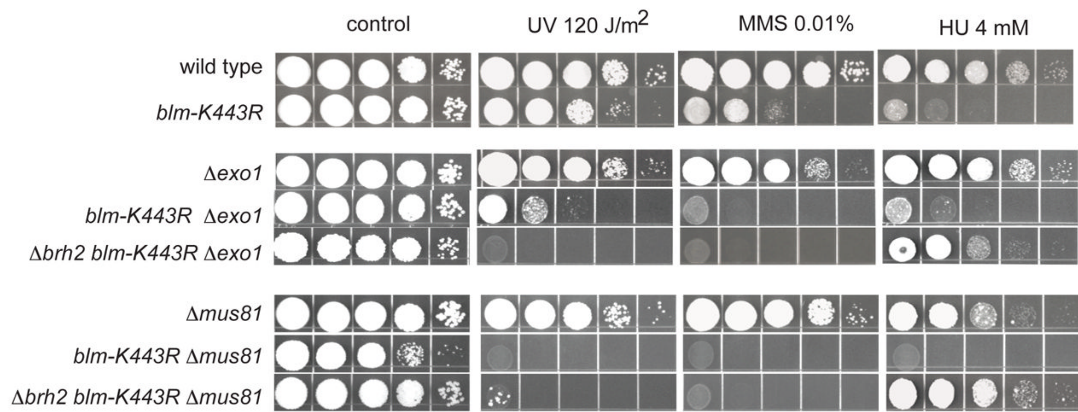

Figure 3.

Phenotype of nuclease deficient mutants. Survival of the indicated strains after treatment with UV or grown in medium containing MMS or HU was performed as in Figure 2.

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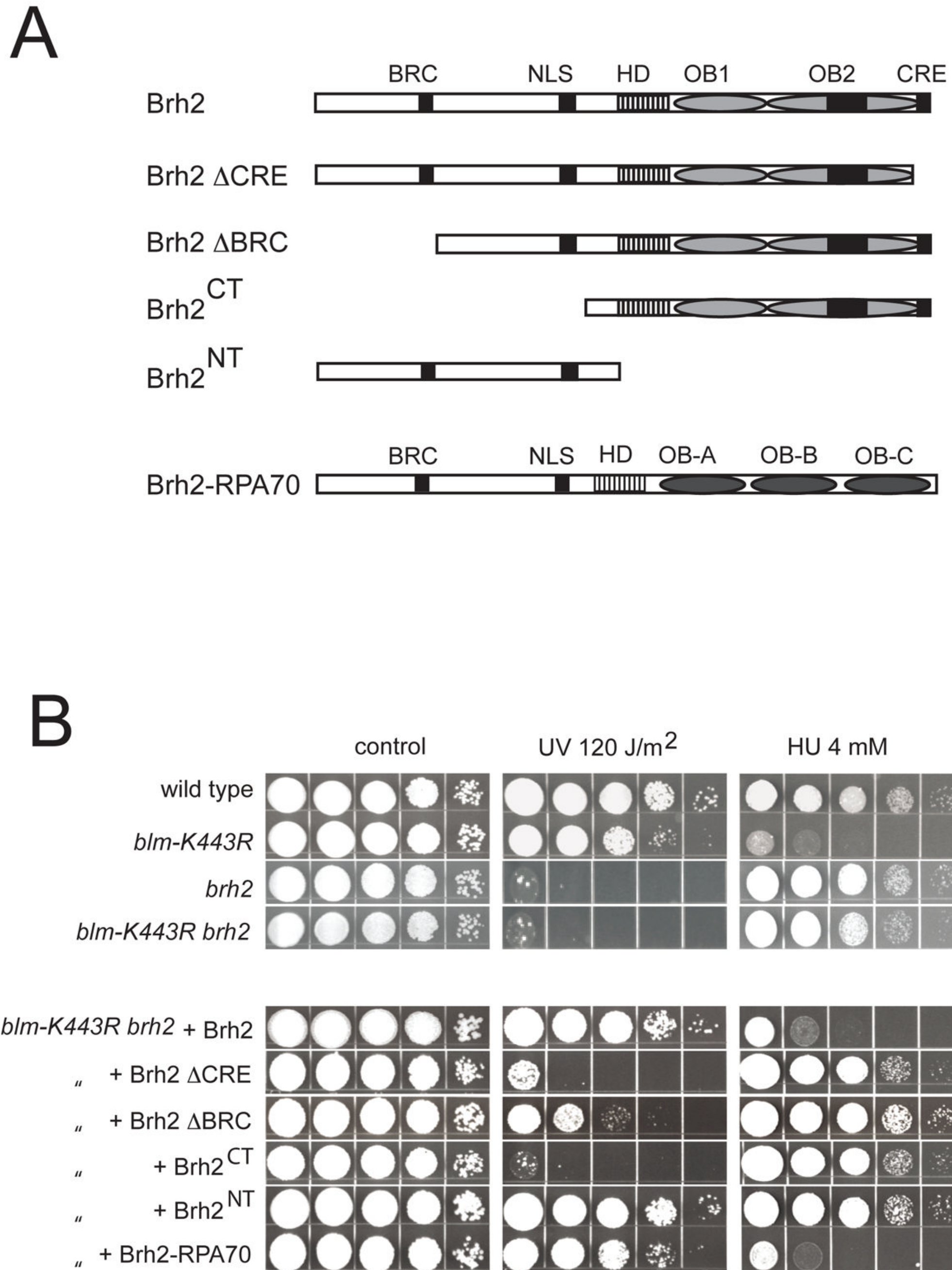

Figure 4.

Recombination proficiency and phenotype restoration. A. Schematic illustration of the domain maps of Brh2 (1–1075 aa), Brh2 ΔCRE (1–1034 aa), Brh2 ΔBRC (316–1075 aa), Brh2^{CT} (506–1075 aa), Brh2^{NT} (1–551 amino acids), and. Brh2-RPA70, (Brh2^{NT} fused with RPA70 DNA binding domain). Rad51 interacting elements BRC and CRE; putative nuclear localization signal NLS -- black bars. Helix-rich region HD-- vertical hatch. OB1 and OB2 (gray ovals). Tower insert in OB2 -- black bar. RPA70 OB-A, OB-B, OB-C (dark gray ovals). B. Survival of the indicated strains after treatment with UV or growth on HU. Strains were transformed as indicated with self-replicating plasmids expressing Brh2, or derivatives as indicated. Cell suspensions were adjusted to ~4 × 10^7 per ml, diluted in 10-fold serial dilutions and aliquots

(10 μl) of each were spotted from left to right as shown. Survival is indicated as growth of colonies 3 days after irradiation.

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TABLE 1

Summary of phenotypes

strain	Recombination Nar^{+} per cell ($\times 10^{-6}$)[a]	Teliospore germination[b]	Asporidia viability[c]
wild type	4.8 ± 1.3	+++	+++
Δbrh2	<0.05	−	n/a[d]
blm-K443R	8.2 ± 3.4	+++	+++
Δexo1	17 ± 2.7	+++	+++
Δmus81	16 ± 2.3	+++	+++
blm-K443R Δexo1	66 ± 6.6	+++	+++

[a]Nar^{+} mitotic recombinants after plating 10^{7} diploid cells.

[b]Promycelium formation as determined 18–20 hours after plating teliospores.

[c]Colony formation determined 48 hours post plating teliospores.

[d]Not applicable. Teliospores failed to produce asporidial products.