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The BRCA2-Interacting Protein DSS1 Is Vital for DNA Repair, Recombination, and Genome Stability in *Ustilago maydis*

Short Article

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Summary

***DSS1* encodes a small acidic protein shown in recent structural studies to interact with the DNA binding domain of BRCA2. Here we report that an ortholog of *DSS1* is present in *Ustilago maydis* and associates with Brh2, the BRCA2-related protein, thus recapitulating the protein partnership in this genetically amenable fungus. Mutants of *U. maydis* deleted of *DSS1* are extremely radiation sensitive, deficient in recombination, defective in meiosis, and disturbed in genome stability; these phenotypes mirror previous observations of *U. maydis* mutants deficient in Brh2 or Rad51. These findings conclusively show that Dss1 constitutes a protein with a significant role in the recombinational repair pathway in *U. maydis*, and imply that it plays a similar key role in the recombination systems of organisms in which recombinational repair is BRCA2 dependent.**

Introduction

The breast cancer susceptibility gene *BRCA2* encodes a protein that provides an important function in recombinational repair of DNA (Jasin, 2002; Venkitaraman, 2002). An early clue linking *BRCA2* to recombinational repair was suggested by the direct interaction of the protein with Rad51 (Sharan et al., 1997; Wong et al., 1997) which serves to provide the catalytic activity necessary for homologous pairing and DNA strand exchange during recombination events. The primary sites for Rad51 binding appear to be the BRC repeats (Chen et al., 1998; Wong et al., 1997), which comprise eight repetitive motifs of ~30 amino acids each distributed through the central third of the BRCA2 sequence.

Attempts to discover other proteins capable of interacting with BRCA2 led to the identification of DSS1, a highly acidic, 70 residue polypeptide that associates with a C-terminal portion of BRCA2 distal to the BRC region (Marston et al., 1999). The biological significance of this interaction was by no means clear, however. DSS1 was originally identified in studies of the developmental disorder split hand/split foot malformation (SHFM), which is a clinically heterogeneous syndrome characterized by missing and fused digits and by additional abnormalities affecting skin, genitourinary, and craniofacial structures (Zlotogora, 1994). An autosomal dominant form of this disorder (SHFM1) involves deletions of a minimal ~1.5 Mb locus that contains *DSS1* (Crackower et al., 1996). However, as this locus contains at least two additional genes of the homeobox family, the role of *DSS1* in this syndrome is uncertain. Based on RNA in situ hybridization analysis with the mouse ortholog, the *DSS1* gene appeared to be expressed predominantly in the limb and facial primordia during midgestation embryos and in the dermis of newborns (Crackower et al., 1996). Such a pattern of expression was markedly different from that of BRCA2, whose expression at the same stage was predominant in the fetal liver, midgut, and ventricular layer of the brain (Connor et al., 1997; Rajan et al., 1997). In adult tissue, no clear relationship between the specificity of expression of the *DSS1* and *BRCA2* genes was evident (Rajan et al., 1997; Sharan and Bradley, 1997; Wei et al., 2003), although both genes were noted to respond similarly in starved cells stimulated with serum (Bertwistle et al., 1997; Marston et al., 1999).

In an effort to learn more about the biological role of DSS1, attention was turned to the identification of orthologs in genetically tractable organisms. Database analysis revealed the gene was highly conserved in eukaryotes. Therefore, the genes encoding the DSS1 ortholog in budding and fission yeasts were disrupted and the mutants were characterized (Marston et al., 1999). The yeast mutants exhibited modestly reduced growth at high and low temperatures and an elongated morphology suggestive of cell cycle delay. However, no compelling phenotype suggestive of a role for DSS1 in recombinational repair was seen in these studies (Marston et al., 1999). Furthermore, with the isolation of the *DSS1* structural gene as a high-copy suppressor of certain yeast mutants defective in the secretory pathway (Jantti et al., 1999), the view that the interaction with BRCA2 could somehow be biologically significant seemed untenable. Nevertheless, during structural studies on BRCA2 it was found that expression of the highly conserved ~700 amino acid C-terminal domain in insect cells resulted in the production of largely insoluble protein unless DSS1 was simultaneous coexpressed (Yang et al., 2002). Subsequently, the crystal structure of the BRCA2 C-terminal domain bound to DSS1 revealed extensive interactions between BRCA2 and DSS1 through residues highly conserved in their respective orthologs (Yang et al., 2002). Furthermore, a subset of BRCA2 residues that interact with DSS1 were mutated in particular instances of cancer. Although these structural findings are consistent with an evolutionarily conserved role for the DSS1-BRCA2 interaction, they cannot rule out other possibilities.

BRCA2 was until recently thought to be confined to vertebrates, but by computational and genetic means homologs have been discovered in plants and invertebrates and a number of lower eukaryotes. *Ustilago maydis*, a basidiomycete fungus which is evolutionarily very dis-

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tant from the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, has a homolog of BRCA2, called *BRH2*, in contrast to those two yeasts. Mutants defective in *BRH2* are extremely sensitive to radiation, deficient in recombination and meiosis, and suffer from genome instability (Kojic et al., 2002). Close parallels between the *brh2* and *rad51* mutant phenotypes and evidence obtained supporting a direct interaction between Brh2 and Rad51 proteins raised the idea that important aspects of BRCA2 function are recapitulated in *U. maydis*, indicating that this genetically amenable fungus is a promising system for furthering knowledge of the cellular functions of BRCA2. Accordingly, since *U. maydis* offers the potential for explicit genetic analysis by molecular dissection, we have investigated the role of *DSS1* in relation to *BRCA2* function. In this paper we present evidence showing that DSS1 is indeed conserved in *U. maydis* and that it binds Brh2, and we provide definitive experimental evidence documenting its essential role in DNA repair, recombination, and genome maintenance.

Results

DSS1 Is Conserved in *U. maydis*

Brh2, with a predicted length of 1075 amino acids, is about one-third the size of BRCA2, yet contains both hallmarks of the BRCA2 sequence, an N-terminal BRC repeat and a C-terminal region with extended similarity to the ~700 amino acid BRCA2 DBD (DSS1/DNA binding domain) that binds DSS1 and single-stranded DNA (Yang et al., 2002). The BRCA2 DBD consists of a helical domain (HD), three oligonucleotide/oligosaccharide binding folds (OB1, 2, and 3), and a coiled-coil domain (Tower) inserted into OB2 (Figure 1A) (Yang et al., 2002). In the structure of the BRCA2 DBD-DSS1-ssDNA complex, DSS1 binds to the HD and OB1 domains, and ssDNA binds to the OB2 and OB3 domains. With the exception of the first half of the HD, which is variable across species, the HD and OB1 domains correspond to the most conserved region of BRCA2 (Yang et al., 2002). This is also true for the Brh2 DBD domain, which exhibits the highest sequence similarity to BRCA2 in the HD (39% identity) and OB1 (38% identity) domains (Figure 1A). The Brh2 OB2-Tower segment is less conserved (~17% identity), while the OB3 domain appears to be absent in Brh2 (Figure 1A). This conservation pattern extends among known BRCA2 homologs. For example, the *A. thaliana* Brca2 shows the highest conservation in its HD (44%) and OB1 domains (36%), while its OB2-Tower and OB3 domains are less conserved (25% and 18%, respectively; Figure 1A). In the BRCA2 DBD-DSS1 crystal structure there are 50 BRCA2 residues that contact DSS1 (Yang et al., 2002). With the exception of 10 residues that map to the variable part of the HD domain, the rest are highly conserved in Brh2: 24 residues are identical, 12 are conservatively substituted, and only 8 are different (Figure 1B). In view of the conservation of these residues, it seemed reasonable that if a gene encoding a DSS1 homolog were present in *U. maydis*, it would likely serve in a capacity similar to that in humans.

In the crystal structure, the 70 residue DSS1 binds to BRCA2 DBD in two extended segments separated by a disordered region. The BRCA2-interacting segments, 21 and 26 residues long, are highly conserved in DSS1 orthologs, while the intervening 11 residue disordered region has variable sequence and length in DSS1 orthologs (Yang et al., 2002). The binding of DSS1 is mediated by aromatic, hydrophobic, and charged amino acids, arranged in clusters of acidic residues with interspersed hydrophobic and aromatic residues (Figure 1C). A BLAST search of the *U. maydis* genome database using the human and yeast sequences as queries revealed a convincing DSS1 ortholog, although at 120 residues in length, it is somewhat larger than expected from the human (70 residues) and yeast (89 residues) proteins. It is significant to note that there are two regions of high similarity to the two BRCA2-interacting segments of DSS1 containing the characteristic clusters of acidic residues and interspersed aromatic and hydrophobic residues. Of the 25 human DSS1 residues that contact BRCA2, 17 are identical in the *U. maydis* ortholog, 7 are conservatively substituted, and only 1 is different. The insertions in the *U. maydis* sequence are in unstructured regions of DSS1, including the spacer between the segments (26 additional residues), and the N terminus (21 additional residues; Figure 1C). Analysis of the genomic sequence predicted the presence of a single intron in the middle of the gene, which was confirmed after PCR amplification of the coding sequence from a cDNA library. From this analysis it appears that *U. maydis* does contain a gene encoding a DSS1-related protein which most likely should bind and interact with Brh2. This notion was confirmed directly by coprecipitation of GST-tagged Brh2 and hexahistidine-tagged Dss1 expressed from baculoviruses in insect cells and by cochromatography of the untagged proteins by salt gradient elution from cation and anion exchange columns followed by molecular sieving on a gel filtration column (see Supplemental Figure S1 at http://www.molecule.org/cgi/content/full/12/4/1043/DC1).

dss1 Null Mutants Are Radiation Sensitive and Can Be Complemented by the Cloned *DSS1* Gene

U. maydis dss1 null mutants deleted of the coding region were constructed by gene disruption (Figure 2A). The mutants were extremely sensitive to UV and ionizing radiation (Figure 2C) and were comparable or even more severely compromised in DNA repair capacity than *brh2* and *rad51* null alleles. The radiation sensitivity could be rescued by introduction of self-replicating plasmids expressing the *DSS1* gene (Figure 2B). In one case, a genomic DNA fragment encompassing the *DSS1* locus and likely to include the natural promoter was used. In a second case, the cDNA of the complete open reading frame was driven by a heterologous promoter (*pgap*). Complementation analysis shows that the DNA repair phenotype associated with the disruption is indeed attributable to deletion of the *DSS1* gene.

Recombination Deficiency in *dss1* Cells

Since previous studies had shown that *BRH2* is required for mitotic and meiotic recombination, it was of immediate interest to learn whether the same was true for *DSS1*. Therefore, *dss1* homozygous diploids were constructed to enable analysis of recombination and meiosis. Recom-

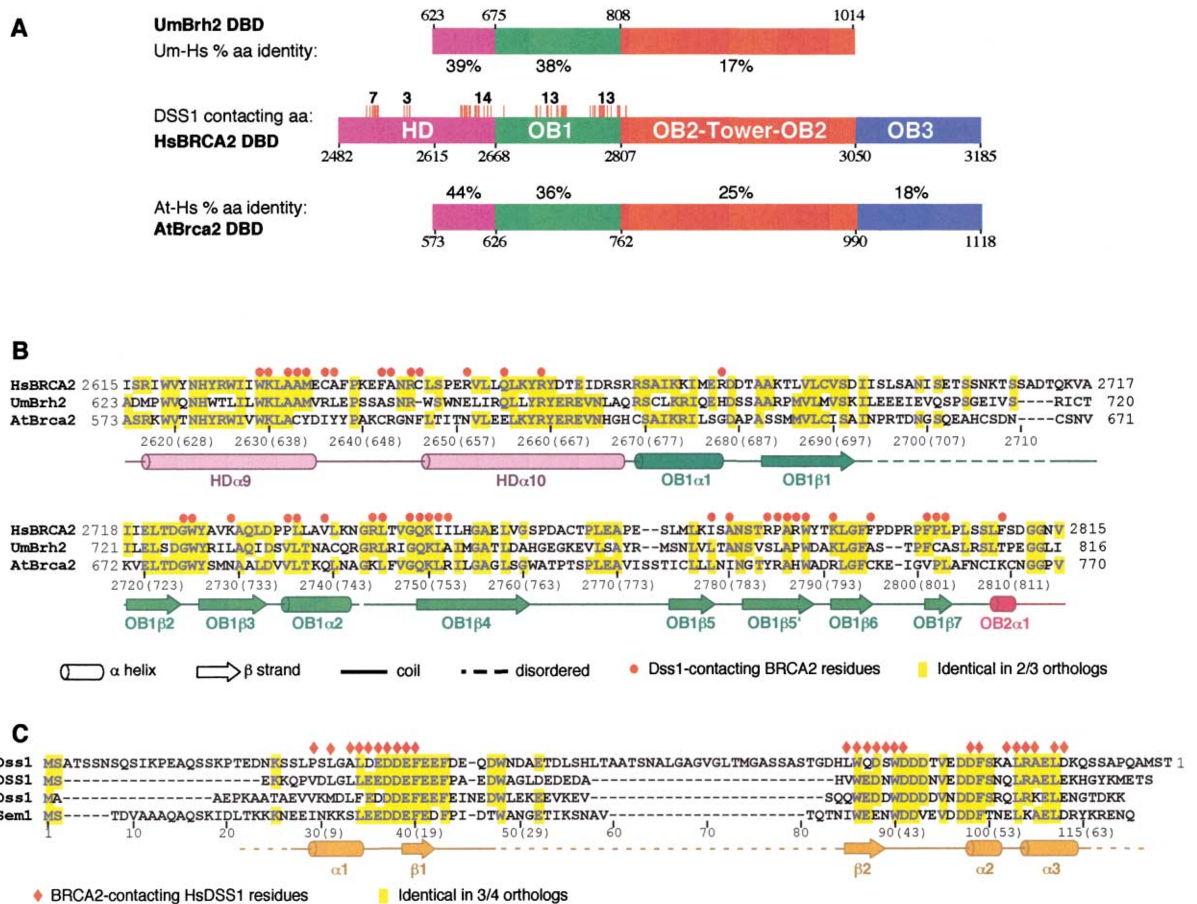

Figure 1. Identification of a DSS1 Ortholog in *U. maydis*

(A) Schematic representation of the Brh2 DNA/Dss1 binding domain (DBD) and its comparison to the DBDs of BRCA2 orthologs from human (Hs BRCA2) and *A. thaliana* (AtBrca2). The individual domains of the human BRCA2 DBD are according to the crystal structure of the BRCA2DBD-DSS1 complex (Yang et al., 2002). The approximate positions of BRCA2 residues that contact DSS1 are indicated by red lines, and their count is indicated above each cluster. The N-terminal portion of the helical domain (HD) is variable across species and cannot be reliably aligned for the UmBrh2 and AtBrca2 sequences (striped portion of the HD). Brh2 appears to lack the OB3 domain.

(B) Sequence alignment of the DSS1-interacting portion of human BRCA2 with the corresponding regions of UmBrh2 and AtBrca2. Residues identical in two out of three orthologs are shaded yellow, and HsBRCA2 residues that interact with DSS1are indicated by red dots. Residue numbers for HsBRCA2 and Brh2 (in parentheses) are shown below the sequence. Secondary structure elements of BRCA2 are according to the BRCA2DBD-DSS1 crystal structure (Yang et al., 2002).

(C) Yeast and human DSS1 sequences were used as queries to search the *U. maydis* genome. Multiple alignment shows Dss1 sequences from *U. maydis* (UmDss1 AY366406) human (HsDSS1, NP_006295), *A. thaliana* (AtDss1, NM_120241), and yeast (ScSem1, AAD 08804). Residues identical in three out of four orthologs are shaded yellow, and HsDSS1 residues that interact with BRCA2 are indicated by red diamonds. Residue numbers for UmDss1 and HsDSS1 (in parentheses) are shown below the sequence. Secondary structure elements of HsDSS1 are according to the BRCA2 DBD-DSS1 crystal structure (Yang et al., 2002), with dotted lines indicating disordered regions.

bination proficiency was tested using a standard assay for allelic recombination at the nitrate reductase (*nar*) locus (Holliday, 1967). Spontaneous allelic recombination was not detectable in *dss1* diploids (Table 1), and similarly, no recombinants were noted even after administering a small dose of UV light sufficient to induce allelic recombination about 40-fold in wild-type, but insufficient to kill more than 80% of the *dss1* diploid cells. Evidently, allelic recombination at *nar1* is abrogated to the same degree in *dss1* as it is in *brh2* and *rad51* null mutants.

Double-strand DNA break repair was measured using a plasmid-based transformation system (Ferguson and Holloman, 1996). The assay measures Leu^{+} prototroph formation in *leu1-1* derivative strains after introduction of a self-replicating plasmid containing the cloned *LEU1* gene inactivated by deletion of 0.7 kb from the coding sequence. Previously we had found that the frequency of gap repair in *brh2* and *rad51* mutants was reduced by a factor of 100 or more (Ferguson et al., 1997; Kojic et al., 2002); in the case of *dss1*, we found that gap repair was similarly reduced at least 100-fold.

Meiosis is abortive in *brh2* or *rad51* homozygous crosses due most likely to failed recombinational repair of induced double-strand DNA breaks. This is manifested in *U. maydis* by the inability of the meiotic teliospore to germinate (Kojic et al., 2002). By contrast with *brh2* or *rad51*, homozygous crosses of *dss1* were found to be sterile in that no meiotic teliospores were generated. This phenotype is the single obvious difference in

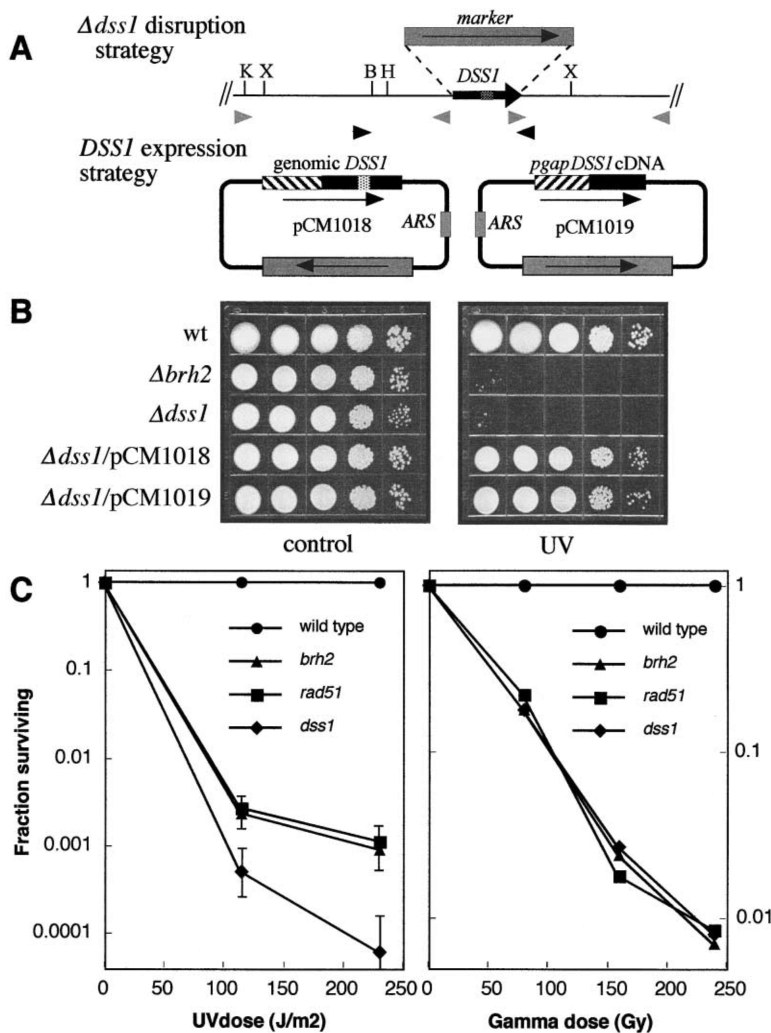

Figure 2. *DSS1* Disruption and Expression

(A) Shown schematically is a 4 kb sequence of *U. maydis* genomic DNA spanning the *DSS1* gene. The *DSS1* open reading frame is shown as the black bar with arrow indicating orientation and the stippled section indicates the small intron. The black carets mark the sites of primers used for PCR amplification of the 1.1 kb genomic sequence containing the *DSS1* gene used in pCM1018. The gray carets mark the sites of primers used for PCR amplification of 1.6 and 1.2 kb sequences proximal and distal to the *DSS1* gene that were used in constructing disruption vectors. The *DSS1* gene was replaced by one-step gene disruption (see Experimental Procedures). The following restriction enzyme sites used for orientation are shown: B (BamHI), H (HindIII), and X (XhoI). pCM1018 has the genomic fragment containing *DSS1* expressed under its own promoter (hatched box) and also contains an autonomously replicating sequence (*ARS*) and *HPH*. Similarly, pCM1019 has the *DSS1* cDNA expressed from the *gap* promoter (hatched box).

(B) Survival after irradiating 10-fold serially diluted samples with 120 J/m^2 of UV light was determined for the indicated strains: wt (UCM350), Δ*brh2* (UCM565), and Δ*dss1* (UCM591).

(C) Haploid strains with the indicated genotype were grown to late log phase, adjusted to a density of 2×10^7 cells per ml and irradiated with UV light or γ rays. Error bars are shown for three determinations. Survival was determined by counting colonies visible after incubation for 2 to 3 days. Strains: wild-type (UCM350), *rad51-1* (UCM342), *brh2-2* (UCM565), and *dss1* (UCM591).

otherwise identical characteristics noted between *dss1* and *rad51* or *brh2* cells and would appear to show that *DSS1* has a role in the developmental program leading to teliospore formation. In summary, *DSS1* is indispensable for proficiency in radiation resistance and recombination in *U. maydis* and as such can be added to *RAD51*, the paralog *REC2*, and *BRH2* as a constituent of the pathway of genes directing homology-dependent repair (see Table 1 for comparison).

Genomic Instability in *dss1* Cells

A large body of evidence has documented chromosome rearrangements and alterations in BRCA2-deficient mammalian cells (Kraakman-van der Zwet et al., 2002; Patel et al., 1998; Yu et al., 2000). Previously we had found that chromosome aberrations were associated with the *U. maydis brh2* mutant, and so we were interested to learn whether loss of *DSS1* disturbed chromosome integrity. Genomic stability was gauged by measuring

Table 1. Phenotype Comparison of *dss1*

Genotype	Mutation Rate[a]	Nar$^+$ Allelic Recombinants[b]		Gap Repair[c] Leu$^+$, (10^{-2})	Gall Formation	Teliospore Production	Teliospore Germination	Viable Progeny
		Spontaneous	UV					
Wild-type	1.2×10^{-9}	2×10^{-6}	82×10^{-6}	2.3	++	++	++	++
rec2	4×10^{-8}	1.7×10^{-6}	1.4×10^{-6}	0.033	++	++	++	−
rad51	$2.9 \pm 0.9 \times 10^{-8}$	$<10^{-8}$	$<10^{-8}$	≤0.020	++	++	−	−
brh2	$2.0 \pm 0.3 \times 10^{-8}$	$<10^{-8}$	$<10^{-8}$	≤0.020	++	++	−	−
dss1	$5.1 \pm 1.2 \times 10^{-8}$	$<10^{-8}$	$<10^{-8}$	<0.020	+/−	−	−	−

[a] Forward mutation to 5-fluoroorotic acid resistance.

[b] Frequency per viable cell. UV dose was 24 J/m^2.

[c] Determinations were standardized by comparison in transformation efficiency with pCM216 as described previously (Ferguson and Holloman, 1996). Gap repair frequency is defined as the ratio of Leu$^+$ recombinants per microgram of pCM291 DNA to Leu$^+$ transformants per microgram of pCM216 DNA.

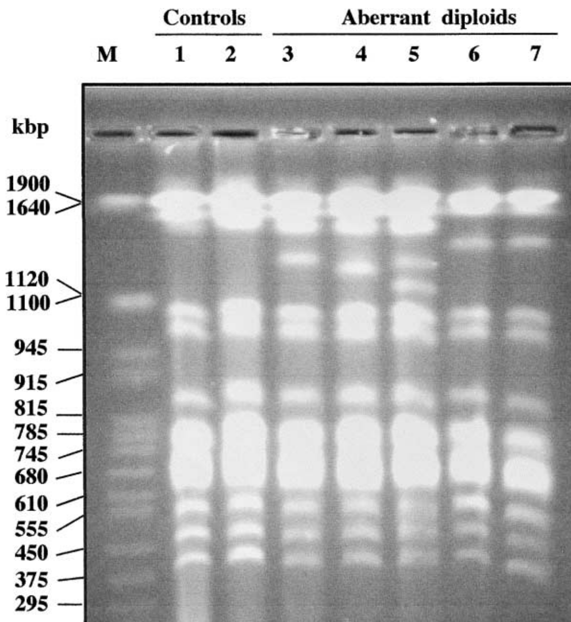

Figure 3. Molecular Karyotype Analysis

Chromosome-size DNA molecules were prepared from protoplasted cells and analyzed by CHEF gel electrophoresis. M, *S. cerevisiae* chromosome markers (BioRad Laboratories). Controls are as follows: lane 1, wild-type haploid (UCM350); lane 2, wild-type diploid (UCM33); lanes 3–7 show individual *dss1*/*dss1* diploids obtained from mating UCM589 × UCM591. The five shown were chosen from a larger set prescreened for loss of mating type heterozygosity. Additional bands representing aberrant chromosomes can be seen in the region of the gel corresponding to a size range of 1120–1900 kb.

mutator activity and by performing molecular karyotype analysis. In the first procedure, forward mutation to 5-fluoroorotic acid resistance was determined. By fluctuation analysis the rate of spontaneous mutation in *dss1* was found to be elevated about 40-fold, slightly higher than the other known *U. maydis* recombinational repair mutants (Table 1).

Molecular karyotype analysis was performed on *dss1*/*dss1* homozygous diploids by pulsed-field gel electrophoresis. Independent diploids picked for analysis were prescreened for likely candidates with genomic instability using a rapid genetic assay that measures loss of heterozygosity at one of the mating loci (Banuett and Herskowitz, 1989). Out of 52 diploids, 8 had lost mating-type heterozygosity and 5 of these exhibited chromosomal abnormalities (Figure 3). The aberrant molecules were clustered in a size range from 1000 to 2000 kb. The origin and structure of these aberrant chromosomes is unknown, but since the *b* mating type locus is known to reside on the largest chromosome (Dr. Peter Schreier, Bayer Crop Science AG, Monheim, Germany, personal communication, and http://www-genome.wi.mit.edu/annotation/fungi/ustilago_maydis/index.html), it seems likely that breakage or rearrangements associated with that chromosome gives rise to the patterns observed. In summary, both the elevated spontaneous mutator activity and the frequent appearance of aberrant chromosomes strongly support the conclusion that *DSS1* is required for maintenance of genome stability in *U. maydis*.

Discussion

The primary conclusion from these studies is that the *DSS1* gene is crucial for proficiency in DNA repair, recombination, and genome stability in *U. maydis*. As the recombinational repair system in *U. maydis* operates through a *BRCA2*-related gene and appears representative of that in higher eukaryotes, it seems reasonable to infer that the lessons learned from these studies can be generalized to more complex mammalian systems. In this regard it will be of great interest to learn whether *DSS1* functions as a genome caretaker in human cells as suggested by cytogenetic studies of a tumor suppressor gene mapping to the locus (for brief synopsis, see Supplemental Data at http://www.molecule.org/cgi/content/full/12/4/1043/DC1).

DSS1 first came to light as a candidate for the gene responsible for the developmental syndrome, split hand/split foot malformation. Evidence tying the gene to the disorder came from mapping studies where it was observed that individuals afflicted by an autosomal dominant form of this syndrome (SHFM1) carried translocations and deletions at chromosome 7q21.3-q22.1 spanning the *DSS1* locus. Uncertainty remained because two genes encoding homeodomain proteins important in limb development were also eliminated or else likely disturbed by those translocations and deletions. Nevertheless, the developmental expression patterns and tissue specificities of all three genes were so similar that it was not unreasonable to suppose that *DSS1* was indeed important for limb development. However, a shortcoming in the model of a purely developmental function for *DSS1* was the link to breast cancer, first suggested by the capture of DSS1 in yeast two-hybrid studies with BRCA2 as bait (Marston et al., 1999) and later implied by the cocrystallization of DSS1 with BRCA2 DBD (Yang et al., 2002). The findings reported here that inactivation of *DSS1* results in a phenotype as severe, if not more so, as in the *brh2* or *rad51* mutant, place the *DSS1* gene function squarely in the pathway for DNA repair and recombination. It remains a possibility that *DSS1* serves additional capacities, as suggested by the various studies on developmental regulation and tissue specificity that show patterns of expression overlapping but not coincident with *BRCA2*. However, a carefully controlled comparison of the two genes' expression pattern performed in parallel is necessary before any further conclusions can be drawn about the paradoxical role of *DSS1* in development.

The emerging paradigm of recombinational repair in BRCA2-dependent organisms holds that BRCA2 (or an equivalent) is a direct participant in Rad51-mediated homologous recombination. The findings reported here extend this picture further. From the above data, the strikingly similar phenotype of the *brh2* and *dss1* null mutants is most simply accounted for by the interaction of the two gene products. Removal of either one results in virtually identical phenotype leading to the pertinent and challenging question of the molecular mechanism by which Brh2 and Dss1 cooperate or combine their actions. Construction of double mutant combinations for assessment of epistasis relationships has not yet been successful (data not shown). Isolation by standard genetic methods has been problematical since *dss1*,

brh2, and *rad51* profoundly disturb meiosis, yielding aneuploids as the predominant class of meiotic products. So too, has been isolation by gene disruption, due presumably to reduced efficiency of targeted integration in a recombination deficient background. The possibility that Brh2 and Dss1 are permanent constituents of a holoenzyme dedicated to governing Rad51-mediated recombination is an intriguing one and is supported by the requirement for Dss1 in promoting or enabling production of a soluble form of Brh2 in insect cells, similar to the observation with DSS1 and the mammalian BRCA2 DBD (Yang et al., 2002). However, an alternative ad hoc model of Dss1 having a solely regulatory role could also be proposed. As a regulator, Dss1 may activate Brh2's Rad51 binding function, it may activate a preassembled but dormant Brh2-Rad51 complex, or it may modify the DNA binding activity of Brh2. The prospect should also be considered that Dss1 might serve as a transmitter or transducer of a DNA-damage signal, in which case transcriptional and/or posttranscriptional regulation of Dss1 itself could be an important issue for study. In any event, it is indeed remarkable that the biological activity of these molecules would be dependent on such a small protein as Dss1. Consequently, the most pressing question now concerns the molecular interplay of Dss1, Brh2, and Rad51.

In addressing these issues, we have taken the approach of isolating suppressors of *dss1*. Although any suppressor would be interesting, our immediate focus will be on hunting for mutant alleles of *BRH2*. It is hoped that a thorough investigation of the genetic properties of various alleles together with a detailed biochemical study will throw light on the mechanism of Brh2-Dss1 cooperation in DNA repair. We have already isolated some suppressors and have begun a detailed analysis of their properties. It would also be informative to examine the crystal structure of Brh2 with and without Dss1 bound to it so as to learn whether the binding of Dss1 induces either local or global conformational changes in Brh2. Elucidating the mechanism of Brh2-Dss1 interplay by genetic and structural means will likely have relevance for understanding the mammalian counterparts.

Experimental Procedures

U. maydis Strains and Methods

Strains used in this study are listed in Supplemental Table S1 at http://www.molecule.org/cgi/content/full/12/4/1043/DC1). Preparation of media and procedures for survival determinations, diploid construction, meiotic analysis, DNA transformations, gene disruptions, mutation rates, and other manipulations were carried out as described (Fotheringham and Holloman, 1989; Holliday, 1974; Kojic et al., 2001). Semiquantitative UV survival curves were performed by adjusting parallel cultures to 10^7 cells per ml, then spotting 10 μl aliquots of 10-fold serial dilutions onto plates containing solid medium. γ-radiation was performed using a Gammacell 220 ^{60}Co source (Nordion International, Kanata, ON, Canada). Gap repair of the *LEU1* gene was determined as described previously using the plasmids pCM216 and pCM291 (Ferguson and Holloman, 1996). Allelic recombination at the *nar1* locus was measured by determining Nar^+ prototroph formation (Holliday, 1967). Null mutants deleted of the *DSS1* gene were created by one-step gene disruption. Cassettes expressing hygromycin (*HPH*) resistance (Hyg^R) or nourseothricin (*NAT*) resistance (Nst^R) were used to replace the 532 bp fragment spanning the *DSS1* open reading frame within the genome. Disruption vectors contained the resistance marker flanked on either side by genomic sequences of 1.6 and 1.2 kb. Primer pairs used for PCR amplification of the 1.6 kb proximal and 1.2 kb distal sequences to the *DSS1* gene that were used in constructing disruption vectors were as follows: proximal forward, 5′-CTGAAGGATTCGTCGAGAGC-3′; proximal reverse, 5′-ACAAGGAGATCAAACGGTCGC-3′; distal forward, 5′-GAGCTTGACAAGCAGAGC-3′; and distal reverse, 5′-CAAGTTC GTCTTGCTCTGGC-3′. Plasmid pCM1018 is a self-replicating vector containing the *HPH* marker that expresses a 1.1 kb genomic fragment containing *DSS1* and its natural promoter. Plasmid pCM1019 is a self-replicating vector containing the *HPH* marker that expresses the *DSS1* cDNA under control of the glyceraldehyde 3-phosphate dehydrogenase (*gap*) promoter. Molecular karyotype analysis was performed by contour-clamped homogeneous electric field (CHEF) gel electrophoresis essentially as described previously (Kojic et al., 2002). Agarose gels (1%) were run using a CHEF-DR II drive module (BioRad Laboratories, Hercules CA) at 14°C at 200V for 17 hr with a pulse interval of 60 s and then switched to a pulse interval of 90 s for 9 hr. *dss1/dss1* homozygous diploids chosen for analysis after mating UCM589 and UCM591 were first selected on minimal medium, then were tested for loss of mating type heterozygosity by plating on charcoal medium (Banuett and Herskowitz, 1989).

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