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Cloning and Characterization of *DST2*, the Gene for DNA Strand Transfer Protein β from *Saccharomyces cerevisiae*

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The gene encoding the 180-kDa DNA strand transfer protein β from the yeast *Saccharomyces cerevisiae* was identified and sequenced. This gene, *DST2* (DNA strand transferase 2), was located on chromosome VII. *dst2* gene disruption mutants exhibited temperature-sensitive sporulation and a 50% longer generation time during vegetative growth than did the wild type. Spontaneous mitotic recombination in the mutants was reduced severalfold for both intrachromosomal recombination and intragenic gene conversion. The mutants also had reduced levels of the intragenic recombination that is induced during meiosis. Meiotic recombinants were, however, somewhat unstable in the mutants, with a decrease in recombinants and survival upon prolonged incubation in sporulation media. *spo13* or *spo13 rad50* mutations did not relieve the sporulation defect of *dst2* mutations. A *dst1 dst2* double mutant has the same phenotype as a *dst2* single mutant. All phenotypes associated with the *dst2* mutations could be complemented by a plasmid containing *DST2*.

The components of pathways of homologous recombination in fungi as well as other eukaryotes are now being studied both genetically and biochemically (5, 8, 10, 39). The association of DNA strands in close proximity (presynapsis) seems to be important to initiation of any recombination process (15, 31). Once presynapsis has occurred, a facilitated exchange of DNA strands is required for the formation of a synaptic complex, which in turn must be resolved in order that a successful recombinant molecule appear.

Recently, ATP-independent DNA strand transfer proteins have been found in *Saccharomyces cerevisiae* (6, 14, 22, 38) as well as in other eukaryotes (17, 25, 28). Their activities appear to be quite different from those of the well-studied RecA protein from *Escherichia coli* (5), the Rec1 protein from *Ustilago maydis* (21), and a human protein (11). The ability to augment biochemical studies of yeasts with classical genetic and molecular genetic approaches makes the yeast system an attractive one for analyzing the in vivo roles of enzymatic activities and as a model system for eukaryotic cells. To this end, the gene for the meiosis-activated DNA strand transfer protein (STPα) (38), called *DST1*, has been identified (3). In a *dst1* disruption mutant, intragenic recombination, which is normally induced up to 1,000-fold during meiosis, is induced only 10- to 50-fold, but sporulation is normal. STPβ, an activity similar to STPα, has been purified from mitotically growing cells (6). It has a molecular mass of approximately 180,000 Da and biochemical characteristics similar to those of STPα. To study its role in the cell, we have cloned and sequenced the gene for STPβ (*DST2*) and made deletion and disruption mutations. The characterization of the *DST2* gene and phenotypes of *dst2* mutants are reported in this paper.

MATERIALS AND METHODS

Strains. *E. coli* Y1090 (37) and DH5α (27) were used for propagating λ bacteriophages and subcloning the *DST2* gene, respectively. *S. cerevisiae* strains are listed in Table 1. New strain constructions are as follows. Strains resulting from crosses were not used until backcrossed at least four times to ensure efficient sporulation. CD202 was constructed by crossing MR966 with RSY6 and identifying *ura3-52 leu2-3,112 his3*::pRS6 *arg4-3 ade2-40* cells with rapid sporulation. CD206 was constructed by crossing MR93-28C with RSY433 and identifying *ura3-52, leu2-3,112, his3Δ200 ade2-101* cells with rapid sporulation. CD239 was constructed by crossing and backcrossing the *lys5* and *met13* markers from AB9 and into MR966. Spo-21c and Spo-20a were obtained by crossing MR966 with G757-2A and selecting strains which were rapidly sporulating for dyad spores. Spo50-1b and Spo50-13b were obtained by crossing Spo-21c with CG954, identifying cells that were methyl methanesulfonate (MMS)-sensitive and that produced dyad spores. CD202-1, CD206-5, KK1101 through KK2205, Spo-21cT, Spo-20aT, Spo50-1bT, and Spo50-13bT were constructed by transforming with plasmid DNA containing either *LEU2* or *URA3* insertions in the *DST2* gene digested with appropriate restriction enzymes, selecting for replacement by homologous recombination (34), and identifying the correct form by Southern hybridization.

Nucleic acids and gene libraries. Oligonucleotides were synthesized on a Dupont Coder 300 DNA synthesizer and purified with NENSORB columns (Amersham). The λgt11 library was constructed by using DNA from *S. cerevisiae* 20B-12 in this laboratory as published before (37). The λDASH library (Stratagene) consists of DNA from *S. cerevisiae* A364A, partially digested with *Sau*3A, and inserted into the phage *Bam*HI site. Bacteriophage λ DNA was purified with Lambdasorb kits (Promega). Plasmid DNA was isolated by the alkaline lysis procedure (27). Other general molecular techniques also followed the methods of Maniatis et al. (27). DNA sequencing was performed mainly with a Sequenase kit (U.S. Biochemicals) on double-stranded DNAs subcloned from the λDASH clones into the KS(+)

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TABLE 1. Yeast strains used in this work

Strain	Relevant genotype	Source
20B-12	*MATα pep4-3 prc1-1126 prb1-1122*	C. Peebles
AB9	*MATα lys5 gal2*	YGSC[a]
A364A	*MATa ade1 ade2 his7 tyr1 ura1 gal1*	YGSC
CD202	*MATα ura3-52 leu2-3,112 ade2-40 his3*::pRS6 *arg4-3*	This study
CD202-1	CD202 but *dst2-1*	This study
CD206	*MATa ura3-52 leu2-3,112 ade2-101 his3D200*	This study
CD206-5	CD206 but *dst2-1*	This study
CD239	*MATa ura3-52 leu2-1 trp1-289 lys5 met13 his1-1 ade3*	This study
CG378	*MATa ura3-52 leu2-3,112 trp1-289 ade5 can1*	C. Giroux
KK2101	CG378 but *dst2-1*	This study
KK2102	CG378 but *dst2-2*	This study
KK2103	CG378 but *dst2-3*	This study
KK2104	CG378 but *dst2-4*	This study
KK2105	CG378 but *dst2-5*	This study
CG379	*MATα ura3-52 leu2-3,112 trp1-289 ade5 his7-2*	C. Giroux
KK2201	CG379 but *dst2-1*	This study
KK2202	CG379 but *dst2-2*	This study
KK2203	CG379 but *dst2-3*	This study
KK2204	CG379 but *dst2-4*	This study
KK2205	CG379 but *dst2-5*	This study
MR966	*MATα ura3-52 leu2-1 trp1-289 his1-1 can1*	M. Resnick
KK1101	MR966 but *dst2-1*	This study
MR93-28c	*MATa ura3-52 leu2-1 trp1-289 his1-7 can1*	M. Resnick
KK1201	MR93-28c but *dst2-1*	This study
CG954	*MATa ura3-52 lys2 ho*::*LYS2 rad50 Hin*d-*Sal*I::*hisG*	C. Giroux
G538-16c	*MATα ura3 leu2 trp2 his1-7 hom3-10 rad54-3*	YGSC
RSY6	*MATα ura3-52 leu2-3,112 his*::pRS6 *ade2-40 trp5-27 arg4-3 ilv1-92*	R. Schiestl
RSY433	*MATa ura3-52 leu2-D98 his3-D300 ade2-101 lys2-801*	R. Schiestl
g757-2A	*MATa spo13-1 rad1-1 can1 HOM his1-1 trp2 len2*	J. Game
Spo-21c	*MATα ura3 leu2 trp1 spo13-1*	This study
Spo-21cT	Spo-21c by *dst2-2*	This study
Spo-20a	*MATa ura3 leu2 trp1 his1-1 spo13-1*	This study
Spo-20aT	Spo-20a but *dst2-2*	This study
Spo50-1b	*MATa ura3 trp1 can1 rad50 spo13-1*	This study
Spo50-1bT	Spo50-1b but *dst2-1*	This study
Spo50-13b	*MATα ura3 trp1 can1 rad50 spo13-1*	This study
Spo50-13bT	Spo50-13b but *dst2-1*	This study
XS144-S19	*MATa met13 aro2*	Ref. 36

[a] YGSC, Yeast Genetic Stock Center, University of California, Berkeley.

vectors (Stratagene). Oligonucleotides corresponding to sequences near gaps or designed to complete both directions of the sequence were synthesized and used as primers.

Protein blotting and immunodetection of STPβ. Antiserum against the purified STPβ (6) was incubated with proteins transferred to Immobilon filters (Millipore) at a 2,000-fold dilution with 2% bovine serum albumin in 50 mM Tris-HCl (pH7.5)–150 mM NaCl. Cross-reacting proteins were detected with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G secondary antibodies used as recommended by the supplier (Promega). For screening the λgt11 library, ^{125}I-protein A was used followed by autoradiography.

Protein sequencing and analysis. Purified STPβ (0.5 mg) (6) was digested at 37°C for 15 h with Lys-C endopeptidase. Proteolytic fragments were separated on an RPC5 column in a fast protein liquid chromatography (FPLC) system (Pharmacia) with water-acetonitrile as the solvent. Pools of peak fractions were obtained and were sequenced at the University of North Carolina-National Institute of Environmental Health Sciences protein-sequencing facility. One peak gave a sequence KAIENGDEIPKGEPFDSNSITP. We made a mixed oligonucleotide, 5′-GA(A/G)AA(T/C)GGIGA(T/C)G A(A/G)ATICC-3′ (I represents inosine), corresponding to the amino acids ENGDEIP. This oligonucleotide was then used for hybridization to genomic DNA as well as to potential STPβ λ clones.

Recombination analysis. Measurement of intragenic recombination at the *his1* locus and treatment with DNA-damaging agents were performed as reported previously (7, 38). The *ade2-40* and *ade2-101* alleles were crossed into the SK-1 background and treated as for the *his1* analysis. The DEL (deletion assay) intrachromosomal recombination sys-

TABLE 2. Temperature effect on sporulation

Strain background	*DST2* allele	Sporulation efficiency (%) at[a]:		
		24°C	30°C	35°C
CG378/CG379	+/+	23.0 ± 1.8	32.2 ± 2.3	ND[b]
CG378/CG379	*2-1*/+	27.4 ± 1.5	33.5 ± 3.5	ND
CG378/CG379	*2-1*/*2-1*	11.8 ± 9.4	0.4 ± 0.3	ND
MR966/MR93-28c	+/+	95.7 ± 1.0	93.2 ± 2.4	92.9 ± 2.0
MR966/MR93-28c	*2-1*/+	96.0 ± 1.0	96.1 ± 2.0	89.2 ± 2.8
MR966/MR93-28c	*2-1*/*2-1*	30.8 ± 4.7	16.5 ± 4.2	1.7 ± 1.4

[a] Cells at 24°C were examined after 5 days; cells at 30 and 35°C were examined after 4 days. Values are means ± standard deviations for five independent cultures of each strain.

[b] ND, Not determined.

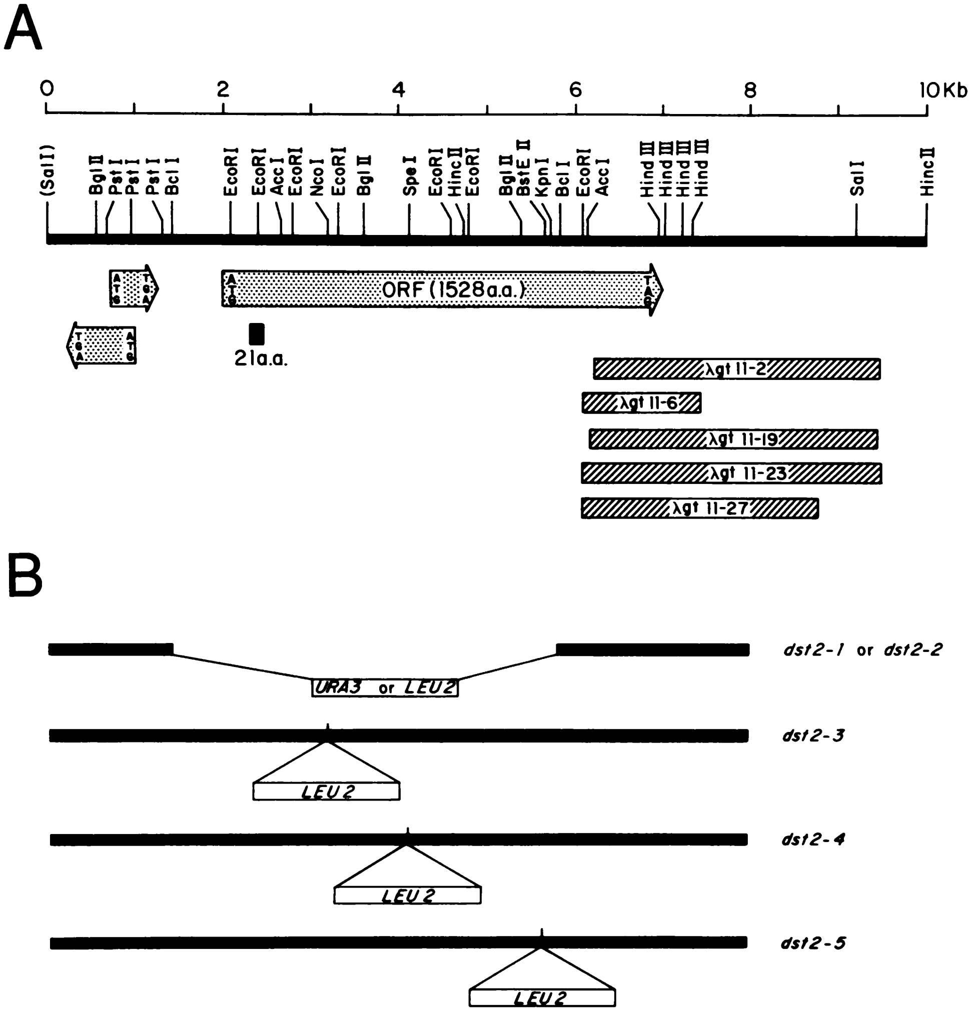

FIG. 1. Structure of *DST2*. (A) Restriction map of *DST2* showing the sequenced region, open reading frames translated from the sequence, location of λgt11 clones, and the site of the oligopeptide obtained from purified STPβ (labeled 21 a.a., where a.a. is amino acid). (B) Structures of deletion-insertion mutations of *dst2*.

tem (35) was also crossed into the SK-1 background. Analysis of DEL recombination between two partially duplicated *his3* genes with *LEU2* in the middle (insertion of pRS6 at *HIS3*) was performed by the method of Schiestl et al. (35). New strains constructed for this work are listed in Table 1 and described above.

Media, chemicals, and other methods. Standard media and techniques for bacterial (27) and yeast (36) culture were used except where indicated in the text. DNA strand transfer activity was measured as reported previously (38) on 0.5 M NaCl eluates of a MonoS column. The STPβ polypeptide is difficult to detect before chromatography through at least either MonoS or S-Sepharose (6). Protein concentrations were measured with the Bradford reagent from Bio-Rad Laboratories.

Nucleotide sequence accession number. The *DST2* sequence reported in this paper has been deposited in the GenBank data base as number M36725.

RESULTS

Cloning and sequencing the gene for STPβ. Antiserum against STPβ was prepared from a rabbit. This antiserum specifically recognized a 180-kDa STPβ polypeptide in MonoS fractions of yeast crude extracts (6) and was used to probe an *S. cerevisiae* genomic DNA λgt11 library as described previously (37). Eighteen plaques that produced products reacting with the STPβ antibody were detected and purified from approximately 10^7 plaques. Eleven had inserts which had homology to each other as determined by Southern hybridization. Figure 1A shows four representative insert DNAs from these. None of the inserts was large enough to encode the entire STPβ protein. The largest *Eco*RI fragment (3 kb) was therefore used to isolate a larger region of DNA from a λDASH genomic library with insertions of average size 10 kb. From this library, four clones were isolated from 2×10^6 phage plaques.

A sequence of 21 amino acids from a Lys-C endopeptidase cleavage fragment of STPβ protein was obtained. Mixed 20-mer oligonucleotides corresponding to all possible reverse translations of a portion of this amino acid sequence were synthesized and hybridized to the candidate λgt11 clones. The oligonucleotides failed to hybridize to any of the candidate λgt11 clones, but did hybridize to three of four λDASH clones as well as to chromosomal DNA. A 10-kb

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   1 AATGCCAATTTACCGCAGTTCTATATCAGAGAGAATACGGCAGTAATCCTCTAACTTCTTCTCGTACACATGAATTTTCTTTTGAAAGAATTTGTCTAAGTATTAGAAGAAAATTTTGCT
 121 TCCCGGAGTTAATAGTTGCTGTAAGAGCACCCGAAAAAGTTTGCGTGTCTAGAAGGTCCTCATCGATTAGCGATTTAAATGAGGATATTTTCTTCAAATCTTGCTTTAAATATTGACTTG
 241 TTGCAGATTCTGACTTCAGTAGGTATGCAACATCACGAGGAATGGAATCTATCAATTCATCATGATCGATTGTATCGGCCTTCAAATGGTGCGAGATCTGCACTTGTTTTTGAATATATT
 361 GATCTAGTTGTTCAATTTCTTGACGTATTTGAGGTGGTAATTCGGAAATCCGTGTCATTGGGGTAATAGGGAGCTGAGATAGATTTTGCAATGCGCACTGCATTTGTGGTTGTTGCTGCA
 481 GTTGCTGCTGCTGATTACCAAATAGCCCGCCTGACGCACTCATGATGTTGTTACTATTATTGTTATTGTTGTTGTTATTTGATGAACCAAACAAAGACGTTGCTCCTGTCGGTTTGGAGC
 601 CAAATAACCCTCCACCAGTGGTCGTATTACCTGCATTATTGCCGAATAGAGATGTACCAGCTGGTTTTGCACCAAACATGCCCTGGTTCTGCGCAGGGGCCGAAGAGGTGCTTGTATTAC
 721 CGAACAAAGAAGTGCCTCCTGCAGGTTTAGAGCCAAATAGTCCAGCGCTTGCATTATTCATGCCACTACTACTATTATTGATATTATTAGTGTTATTATTACCAAACAAACCACGACTCG
 841 TCTGTGCATTGTTGGAACCAAACAAACTGCCAGAATTACTGTTCGAATTAGCGCCAAATAACCCGCCCCCAGTGTTCGCCGTATTGTTAGGTTTGTTACCGAAAAGGCCCCCTCCGGTGG
 961 TGGCGCTTCCTCCAAATGCATTTGTCTGAGATTGTTGTTGCTGCTGACCAAATGATGCACCTAATCCTCCTGTAGATCCGGCTGGTTTAGCGCCAAATAGTCCACCTGTACTTGGGCCGG
1081 TGTTTTGTCCAGTTTGAGTCCCACCAAACGAAAACCCAGTATTCGCGTTTCCAGTGCTACGTCCGCTGGCCTGACCAAAGAGCCACCTGCAGGTGTCGAAGATGCTTTATTTAATCCAAA
1201 CATTGTGCCCACTACCCGTGAAAACCCTCTCAATTACTGTTTTTATCGTCTCATCACCTGATCAGTAACTATTCTCACGATTAATGGTCTTTTTCAACTACATTTATTTGAGAAAGCTGT
1321 AATACATTCCGTGTTGAGTGATATATACAACCTCCTTTGCTCGTCTTTTCGCCACCGCAGAGCAAGTAACAACAGAGACAAACAAGAGAGGTTAGAAAGCAATTTAAGGAGTAGTTTAAC
1441 GAGTACTGAAAACGTCAAACTAAGTACTTACGACTTCCAATTACCGGTTGTAAAGTTTATCTACGCAAATATAACAGTTTGCAGCTTGCTTTTTTTTTCAGCAGCTCTTCCGTTATTATA
                                                                                                                      ↓
1561 AAATCTGTTTGGCGTGCACAAGCGAACCGATTCTTTTATTATTTCTCTCTGCCTTTTATTTCCGTTCCTGTTAATAGTTTATTTTCTAAAGGATACTGTCTTCTTCCGTACTTATAATC
1681 GGGTTCACAATAAGCAATTGACTAATCCTAGGACGATTCGTGTACTATAAGGAGAAAAAAAATCAACACTTGTAACAACAGCAGCAACAAATATATATCAGTACGGTATGGGTATTCCAA
                                                                                                                   M  G  I  P  K   5
1801 AATTTTTCAGGTACATCTCAGAAAGATGGCCCATGATTTTACAGCTTATTGAGGGAACACAGATTCCTGAGTTTGATAACTTATACCTGGATATGAATTCGATTTTACATAATTGTACGC
        F  F  R  Y  I  S  E  R  W  P  M  I  L  Q  L  I  E  G  T  Q  I  P  E  F  D  N  L  Y  L  D  M  N  S  I  L  H  N  C  T  H  45
1921 ATGGTAACGACGATGATGTAACCAAGCGATTAACTGAAGAAGAGGTTTTTGCAAAAATCTGTACGTATATCGATCACCTTTTCAAACAATCAAGCCCAAGAAGATTTTCTACATGGCTA
        G  N  D  D  D  V  T  K  R  L  T  E  E  E  V  F  A  K  I  C  T  Y  I  D  H  L  F  Q  T  I  K  P  K  K  I  F  Y  M  A  I  85
2041 TTGATGGTGTGGCCCCTCGTGCCAAGATGAATCAACAAAGAGCTCGTAGATTCAGAACCGCTATGGATGCAGAAAAAGCCTTGAAGAAGGCTATTGAGAATGGTGACGAGATTCCTAAGG
        D  G  V  A  P  R  A  K  M  N  Q  Q  R  A  R  R  F  R  T  A  M  D  A  E  K  A  L  K  K  A  I  E  N  G  D  E  I  P  K  G  125
2161 GTGAGCCATTTGATTCGAATTCTATTACTCCAGGTACGGAGTTTATGGCCAAATTGACCAAAAACTTACAATATTTTATTCACGACAAGATTTCTAACGATTCCAAATGGAGGGAAGTGC
        E  P  F  D  S  N  S  I  T  P  G  T  E  F  M  A  K  L  T  K  N  L  Q  Y  F  I  H  D  K  I  S  N  D  S  K  W  R  E  V  Q  165
2281 AAATCATATTTTCTGGCCATGAAGTTCCAGGTGAAGGTGAACACAAGATCATGAACTTTATAAGGCATTTAAAATCCCAAAAGGATTTCAACCAGAATACGAGACATTGTATTTACGGTC
        I  I  F  S  G  H  E  V  P  G  E  G  E  H  K  I  M  N  F  I  R  H  L  K  S  Q  K  D  F  N  Q  N  T  R  H  C  I  Y  G  L  205
2401 TTGACGCAGATTTGATTATGCTGGGTTTGTCTACTCATGGGCCACATTTTGCGTTATTGAGAGAAGAAGTGACATTTGGTAGAAGAAATAGTGAAAAAAAATCGCTTGAACATCAAAATT
        D  A  D  L  I  M  L  G  L  S  T  H  G  P  H  F  A  L  L  R  E  E  V  T  F  G  R  R  N  S  E  K  K  S  L  E  H  Q  N  F  245
2521 TCTACTTATTACATCTTTCTTTATTAAGAGAATACATGGAGTTGGAATTCAAAGAAATTGCCGATGAAATGCAATTTGAATACAATTTTGAACGTATTTTGGATGATTTTATTCTTGTCA
        Y  L  L  H  L  S  L  L  R  E  Y  M  E  L  E  F  K  E  I  A  D  E  M  Q  F  E  Y  N  F  E  R  I  L  D  D  F  I  L  V  M  285
2641 TGTTCGTCATTGGTAATGATTTCTTGCCCAATTTGCCAGATTTGCACCTTAACAAAGGAGCATTTCCCGTTTTGTTACAAACGTTCAAAGAAGCTCTTTTACATACTGATGGCTACATTA
        F  V  I  G  N  D  F  L  P  N  L  P  D  L  H  L  N  K  G  A  F  P  V  L  L  Q  T  F  K  E  A  L  L  H  T  D  G  Y  I  N  325
2761 ATGAACATGGTAAAATAAATTTAAAGAGATTAGGTGTCTGGTTAAATTATCTGTCTCAATTTGAGTTATTAAATTTCGAAAAGGATGATATAGACGTTGAGTGGTTCAACAAGCAATTAG
        E  H  G  K  I  N  L  K  R  L  G  V  W  L  N  Y  L  S  Q  F  E  L  L  N  F  E  K  D  D  I  D  V  E  W  F  N  K  Q  L  E  365
2881 AGAATATTTCTTTGGAGGGTGAAAGGAAAAGACAGAGGGTTGGTAAAAAATTACTGGTAAAACAACAGAAGAAATTAATTGGAAGTATAAAACCATGGTTGATGGAGCAATTACAGGAAA
        N  I  S  L  E  G  E  R  K  R  Q  R  V  G  K  K  L  L  V  K  Q  Q  K  K  L  I  G  S  I  K  P  W  L  M  E  Q  L  Q  E  K  405
3001 AATTATCGCCTGATTTACCAGATGAAGAAATTCCAACTTTAGAGTTACCTAAGGACTTAGACATGAAAGATCATTTAGAATTTTTAAAAGAATTCGCTTTTGATTTGGGTCTTTTTATAA
        L  S  P  D  L  P  D  E  E  I  P  T  L  E  L  P  K  D  L  D  M  K  D  H  L  E  F  L  K  E  F  A  F  D  L  G  L  F  I  T  445
3121 CGCATTCCAAATCCAAAGGTAGTTATTCGCTAAAAATGGATCTTGATTCTATTAATCCTGATGAAACAGAAGAAGAATTTCAAAATCGTGTTAATTCTATCAGGAAAACAATAAAAAAAT
        H  S  K  S  K  G  S  Y  S  L  K  M  D  L  D  S  I  N  P  D  E  T  E  E  E  F  Q  N  R  V  N  S  I  R  K  T  I  K  K  Y  485
3241 ATCAAAATGCTATCATCGTGGAGGACAAAGAAGAATTGGAAACTGAAAAAACGATTTATAATGAAAGGTTTGAACGTTGGAAGCATGAGTATTATCACGACAAGTTAAAATTTACGACAG
        Q  N  A  I  I  V  E  D  K  E  E  L  E  T  E  K  T  I  Y  N  E  R  F  E  R  W  K  H  E  Y  Y  H  D  K  L  K  F  T  T  D  525
3361 ACAGTGAAGAAAAAGTGAGAGATCTTGCTAAAGACTACGTTGAAGGTTTACAATGGGTTCTATATTATTATTATAGAGGATGTCCATCTTGGTCGTGGTACTATCCGCACCATTATGCAC
        S  E  E  K  V  R  D  L  A  K  D  Y  V  E  G  L  Q  W  V  L  Y  Y  Y  Y  R  G  C  P  S  W  S  W  Y  Y  P  H  H  Y  A  P  565
3481 CAAGAATCTCCGACTTAGCCAAGGGTTTAGATCAAGACATTGAATTTGATTTGAGCAAACCATTTACTCCATTCCAGCAACTAATGGCAGTTTTACCGGAAAGGTCCAAAAATCTGATAC
        R  I  S  D  L  A  K  G  L  D  Q  D  I  E  F  D  L  S  K  P  F  T  P  F  Q  Q  L  M  A  V  L  P  E  R  S  K  N  L  I  P  605
3601 CTCCCGCCTTTAGGCCATTAATGTACGATGAACAGTCGCCAATCCACGATTTCTATCCCGCTGAGGTTCAACTTGATAAAAACGGCAAGACAGCTGATTGGGAAGCTGTGGTTTTGATAT
        P  A  F  R  P  L  M  Y  D  E  Q  S  P  I  H  D  F  Y  P  A  E  V  Q  L  D  K  N  G  K  T  A  D  W  E  A  V  V  L  I  S  645
3721 CGTTTGTAGATGAAAAAAGGTTGATTGAGGCTATGCAACCTTATTTGCGCAAGTTATCACCTGAAGAAAAAACGAGAAATCAATTTGGCAAGGACTTGATATATTCCTTTAATCCTCAAG
        F  V  D  E  K  R  L  I  E  A  M  Q  P  Y  L  R  K  L  S  P  E  E  K  T  R  N  Q  F  G  K  D  L  I  Y  S  F  N  P  Q  V  685
3841 TTGATAACCTTTATAAGAGTCCGTTGGGCGGCATTTTTTCTGATATTGAACACAATCATTGTGTCGAAAAAGAGTACATCACCATCCCATTGGACAGCTCCGAGATTCGGTATGGTTTAT
        D  N  L  Y  K  S  P  L  G  G  I  F  S  D  I  E  H  N  H  C  V  E  K  E  Y  I  T  I  P  L  D  S  S  E  I  R  Y  G  L  L  725
3961 TACCTAATGCTAAACTCGGTGCCGAAATGCTGGCGGGTTTCCCCACGTTATTGTCTTTACCATTTACTAGTTCACTGGAGTACAATGAGACAATGGTTTTCCAACAACCTTCTAAACAAC
        P  N  A  K  L  G  A  E  M  L  A  G  F  P  T  L  L  S  L  P  F  T  S  S  L  E  Y  N  E  T  M  V  F  Q  Q  P  S  K  Q  Q  765
4081 AATCAATGGTCTTACAAATAACTGACATATACAAAACGAATAATGTTACTTTGGAGGACTTTTCCAAGAGGCATTTAAACAAAGTGATTTATACAAGATGGCCATATTTAAGAGAATCCA
        S  M  V  L  Q  I  T  D  I  Y  K  T  N  N  V  T  L  E  D  F  S  K  R  H  L  N  K  V  I  Y  T  R  W  P  Y  L  R  E  S  K  805
4201 AATTGGTCTCTTTAACGGATGGTAAGACTATCTATGAATATCAGGAGTCCAATGATAAGAAAAAGTTCGGATTCATAACGAAGCCTGCGGAAACCCAGGACAAAAAACTTTTCAATAGTT
        L  V  S  L  T  D  G  K  T  I  Y  E  Y  Q  E  S  N  D  K  K  K  F  G  F  I  T  K  P  A  E  T  Q  D  K  K  L  F  N  S  L  845
4321 TGAAGAATTCAATGCTAAGGATGTATGCTAAACAGAAAGCTGTTAAAATAGGACCTATGGAAGCCATTGCTACCGTCTTTCCAGTGACTGGTTTGGTGAGAGACTCTGATGGTGGTTATA
        K  N  S  M  L  R  M  Y  A  K  Q  K  A  V  K  I  G  P  M  E  A  I  A  T  V  F  P  V  T  G  L  V  R  D  S  D  G  G  Y  I  885
4441 TTAAGACCTTTAGCCCTACCCCAGATTACTATCCATTGCAACTGGTTGTTGAATCTGTTGTCAACGAGGATGAAAGATATAAAGAAAGAGGACCCATTCCTATTGAAGAGGAATTTCCAT
        K  T  F  S  P  T  P  D  Y  Y  P  L  Q  L  V  V  E  S  V  V  N  E  D  E  R  Y  K  E  R  G  P  I  P  I  E  E  E  F  P  L  925
4561 TGAATTCAAAAGTTATTTTCTTAGGTGATTATGCCTATGGTGGTGAAACTACTATTGACGGTTACAGCAGTGACCGCAGACTAAAAATTACTGTAGAAAAGAAGTTTTTGGATAGTGAGC
        N  S  K  V  I  F  L  G  D  Y  A  Y  G  G  E  T  T  I  D  G  Y  S  S  D  R  R  L  K  I  T  V  E  K  K  F  L  D  S  E  P  965
4681 CCACCATCGGCAAAGAAAGGTTACAAATGGATCATCAAGCCGTTAAATATTATCCGTCTTATATTGTGTCCAAGAACATGCACTTACACCCCTTGTTTTTGTCTAAGATTACTTCCAAGT
        T  I  G  K  E  R  L  Q  M  D  H  Q  A  V  K  Y  Y  P  S  Y  I  V  S  K  N  M  H  L  H  P  L  F  L  S  K  I  T  S  K  F  1005
4801 TCATGATTACTGACGCTACTGGGAAGCATATCAATGTTGGTATCCCGGTTAAGTTCGAAGCTAGACACCAAAAGGTTTTAGGTTACGCGAGGAGGAACCCTAGGGGCTGGGAATACTCAA
        M  I  T  D  A  T  G  K  H  I  N  V  G  I  P  V  K  F  E  A  R  H  Q  K  V  L  G  Y  A  R  R  N  P  R  G  W  E  Y  S  N  1045
4921 ATTTAACTCTAAATTTACTGAAAGAGTATAGACAAACTTTCCCAGATTTTTTTTTCAGGTTGTCCAAGGTAGGTAATGATATCCCAGTTTTGGAAGATCTTTTCCCCGATACTTCCACTA
        L  T  L  N  L  L  K  E  Y  R  Q  T  F  P  D  F  F  F  R  L  S  K  V  G  N  D  I  P  V  L  E  D  L  F  P  D  T  S  T  K  1085
5041 AGGATGCCATGAATTTATTAGATGGTATCAAACAATGGCTAAAGTATGTCTCATCGAAGTTTATCGCGGTATCTTTGGAGTCTGACTCCTTAACTAAGACATCGATTGCTGCCGTGGAAG
        D  A  M  N  L  L  D  G  I  K  Q  W  L  K  Y  V  S  S  K  F  I  A  V  S  L  E  S  D  S  L  T  K  T  S  I  A  A  V  E  D  1125
5161 ATCATATCATGAAATACGCAGCTAACATCGAAGGTCATGAAAGAAAACAGTTAGCCAAAGTTCCTCGTGAGGCTGTTTTGAATCCAAGATCATCATTTGCACTCTTACGTAGTCAAAAGT
        H  I  M  K  Y  A  A  N  I  E  G  H  E  R  K  Q  L  A  K  V  P  R  E  A  V  L  N  P  R  S  S  F  A  L  L  R  S  Q  K  F  1165
5281 TCGATTTGGGTGACCGTGTTGTTTATATCCAAGATTCTGGTAAGGTACCAATTTTCTCAAAGGGTACAGTTGTTGGCTATACTACTCTCAGTTCATCATTATCAATTCAGGTCTTATTTG
        D  L  G  D  R  V  V  Y  I  Q  D  S  G  K  V  P  I  F  S  K  G  T  V  V  G  Y  T  T  L  S  S  S  L  S  I  Q  V  L  F  D  1205
5401 ATCATGAAATCGTGGCCGGTAATAATTTTGGCGGCAGGTTGCGCACGAATAGAGGCTTAGGGCTTGATGCCTCTTTCTTATTGAATATTACTAACAGGCAGTTCATTTATCACTCCAAGG
        H  E  I  V  A  G  N  N  F  G  G  R  L  R  T  N  R  G  L  G  L  D  A  S  F  L  L  N  I  T  N  R  Q  F  I  Y  H  S  K  A  1245
5521 CTTCCAAAAAGGCTTTGGAAAAGAAAAAGCAATCTAACAATAGGAACATTAATACCAAAACTGCTCACAAGACTCCTTCAAAGCAACAATCTGAAGAAAAACTGAGAAAAGAAAGGGCAC
        S  K  K  A  L  E  K  K  K  Q  S  N  N  R  N  I  N  T  K  T  A  H  K  T  P  S  K  Q  Q  S  E  E  K  L  R  K  E  R  A  H  1285
5641 ATGATTTATTGAATTTTATCAAAAAGGATACCAATGAAAAGAATTCTGAAAGTGTAGACAACAAGAGCATGGGATCGCAAAAAGATTCCAAACCCGCAAAGAAAGTTTTGTTAAAAAGAC
        D  L  L  N  F  I  K  K  D  T  N  E  K  N  S  E  S  V  D  N  K  S  M  G  S  Q  K  D  S  K  P  A  K  K  V  L  L  K  R  P  1325
5761 CAGCTCAGAAAAGCAGTGAAAACGTGCAAGTTGATTTGGCCAATTTTGAAAAAGCACCGCTTGATAATCCAACTGTTGCTGGATCTATTTTCAATGCCGTTGCAAATCAATATTCTGATG
        A  Q  K  S  S  E  N  V  Q  V  D  L  A  N  F  E  K  A  P  L  D  N  P  T  V  A  G  S  I  F  N  A  V  A  N  Q  Y  S  D  G  1365
5881 GTATAGGCAGTAATTTGAATATCCCAACTCCACCTCACCCAATGAATGTGGTTGGGGGTCCTATTCCTGGAGCGAATGATGTTGCAGATGTTGGTTTGCCGTACAATATTCCCCCAGGTT
        I  G  S  N  L  N  I  P  T  P  P  H  P  M  N  V  V  G  G  P  I  P  G  A  N  D  V  A  D  V  G  L  P  Y  N  I  P  P  G  F  1405
6001 TTATGACGCATCCTAATGGTCTTCACCCATTACACCCTCACCAGATGCCTTACCCTAATATGAATGGAATGTCTATTCCGCCACCAGCACCACATGGGTTTGGACAACCGATTTCCTTCC
        M  T  H  P  N  G  L  H  P  L  H  P  H  Q  M  P  Y  P  N  M  N  G  M  S  I  P  P  P  A  P  H  G  F  G  Q  P  I  S  F  P  1465
6121 CACCTCCACCTCCTATGACAAATGTTTCAGATCAAGGAAGTCGTATTGTTGTCAATGAAAAGGAAAGCCAAGATTTGAAAAAATTCATTAATGGTAAACAGCACAGCAATGGTTCAACTA
        P  P  P  P  M  T  N  V  S  D  Q  G  S  R  I  V  V  N  E  K  E  S  Q  D  L  K  K  F  I  N  G  K  Q  H  S  N  G  S  T  I  1485
6241 TTGGGGGAGAAACAAAGAACAGTAGGAAAGGCGAGATTAAACCTTCTTCTGGCACAAACTCTACTGAATGTCAATCGCCAAAGTCACAAAGCAATGCTGCTGACCGTGATAATAAAAAAG
        G  G  E  T  K  N  S  R  K  G  E  I  K  P  S  S  G  T  N  S  T  E  C  Q  S  P  K  S  Q  S  N  A  A  D  R  D  N  K  K  D  1525
6361 ACGAATCTACTTAGAACATACGACTAAAAACGAAGTATATTCGAGGTTACTTTAATAGTATATCTGAGACCTATATAAAGAGGGGTATTTGTATTTAATCGTTGATCTTGTTAAGAAAAA
        E  S  T                                                                                                                 1528
6481 GCTTATAACAAATGGGGATTGTCAAAGGGTATTTTTTACACAAAGCTTTCCGCATAGTTATATATTATCTCATTTTCAATAATCCTCCTGTAGGGTGTAGCTTTGCACTTTCTTCTCATT
6601 GATCTCATTTTGCTTAGCAAACCATTTTTCTTCAAAGCCATTTGATCTGTGACACCGTCCCATCTTGACCCAGGCATAATGGCAAACCTGTTCTCAGGTGCTGGCTTATCATAAAGCTT
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TABLE 3. Effect of *dst2* on sporulation; complementation by *DST2* on a plasmid in two strain backgrounds at 30°C

Strain background	*DST2* allele	Sporulation efficiency (%) with plasmid[a]:				
		None	YEp	YCp	pKb101	pKb102
CG378/CG379	+/+	39.9	36.7	37.3	37.5	42.0
CG378/CG379	+/*2-1*	42.4	39.5	44.2	43.9	43.2
CG378/CG379	*2-1*/*2-1*	2.35	1.1	0.7	28.1	37.7
MR966/MR93-28c	+/+	87.2	55.3 ± 8	61.8 ± 4.8	46.9 ± 6.5	53.6 ± 4.2
MR966/MR93-28c	+/*2-1*	86	63.9 ± 7.2	52.1 ± 10	50.8 ± 5.5	46.1 ± 5.8
MR966/MR93-28c	*2-1*/*2-1*	5.0	1.3 ± 1.2	2.3 ± 1.4	37.2 ± 5.3	37.8 ± 5.8

[a] YEp, YEplac181; YCp, YCplac22 (13); pKb101, YEp + *DST2*; pKb102, YCp + *DST2*.

*Sal*I fragment that hybridized to both λgt11 clones and the oligonucleotide probe was subcloned from one of the λDASH clones and sequenced. A large open reading frame was found, sufficient to encode an 180-kDa polypeptide (Fig. 1A). The DNA sequence and the deduced amino acid sequence of the open reading frame are shown in Fig. 2. The DNA sequence confirms the identity of the 21-amino-acid sequence determined by direct amino acid sequencing of the STPβ protein (doubly underlined in Fig. 2) and predicts a protein with 1,528 amino acids and a deduced molecular weight of 175,396 assuming that the first ATG is used to initiate translation. This predicted molecular weight matches that of the purified STPβ protein quite well, allowing us to conclude that this is the gene for STPβ. We named this gene *DST2* (DNA strand transferase 2). DNA-RNA hybridization studies identified a mRNA of approximately 5.4 kb (data not shown), consistent with the size of the *DST2* gene.

The predicted amino acid sequence shows no significant similarity to any sequence in the GenBank, Swiss nucleic acid, Swiss protein, or NBRF-PIR data bases or to any published recently, including *RAD50* (1), *RAD54* (4), and *U. maydis REC1* (16). Interestingly, the predicted amino acid sequence lacks similarity at the amino acid level to *E. coli* RecA, phage T4 UvsX, or yeast *DST1*, which has a similar DNA strand transfer activity. There are two possible nucleoside triphosphate-binding consensus sequences (12) in the middle of the sequence:

DFPAEVQLDKNGKTADWEAVVLISFV (amino acids 621 to 647)
ESKLVSLTDGKTIYEYQESNDKKK (amino acids 803 to 826).

However, the purified STPβ lacked detectable nucleoside triphosphatase activity (6).

Hydropathy analysis (not shown) suggests that the COOH-terminal region of the predicted protein is very hydrophilic and therefore more exposed to the environment. This would make it very susceptible to proteolysis, as well as more antigenic. This may explain why the antiserum against STPβ identified only λgt11 clones corresponding to the COOH-terminal region (Fig. 1A).

This same COOH-terminal region has a perfect set of yeast splice signals (Fig. 2, underlined nucleotides) (2, 24). If the mRNA were spliced, it would encode a protein of 132 kDa, a similar size reported for SEP1 (22). However, we saw no evidence of mRNA splicing. This conclusion came from polymerase chain reactions on mRNA isolated from cells in various growth conditions, including pretreated cells with MMS (data not shown). Furthermore, the size of *DST2* mRNA from cells in meiosis was the same as that from mitotic growing cells (data not shown).

Gene disruption of *DST2*. To determine the role of STPβ in the cell, several *dst2* mutations were constructed by disrupting or replacing the coding region with *URA3* or *LEU2* (Fig. 1B). *dst2-1* and *dst2-2* are replacements of the 4.4-kb *Bcl*I fragment with *LEU2* or *URA3*. *dst2-3* is an insertion in the unique *Nco*I site. *dst2-4* is an insertion in the unique *Spe*I site. *dst2-5* is an insertion in the unique *Bst*EII site. One of the wild-type *DST2* genes in a diploid strain (CG378 × CG379) was replaced with these mutant genes by homologous recombination (34). After confirming the correct replacement on one chromosome by Southern hybridization, the heterozygotes were sporulated and the spores were dissected. Four viable spores were obtained in each case, indicating that *DST2* is not essential for mitotic growth. All of the *dst2* mutants, however, grew more slowly: the doubling time of the haploid *dst2-1* strain was 1.5 times longer than that of its congenic *DST2* strain. *dst2-1* mutant strains no longer contained either STPβ activity or the 180-kDa polypeptide that reacts with STPβ antibodies (Fig. 3).

Homoallelic diploid strains containing the disrupted *dst2-1* gene were temperature sensitive for sporulation (Tables 2 to 5; Fig. 4). Similar results were seen in different strain backgrounds and with the several different *dst2* constructions (Fig. 1B). For example, strains related to SK-1 (19) normally exhibit rapid and efficient sporulation, but the sporulation frequency of the *dst2* mutants was reduced to 30% at 24°C compared with >95% in the congenic *DST2* strain and was reduced to 1 to 2% at 35°C (Table 2). The sporulation phenotypes associated with *dst2* were fully complemented by plasmids containing *DST2* (Table 3). Interestingly, an insertion mutation at the *Bgl*II site (Fig. 1A) resulted in 2:0 spore viability, suggesting that one of the two open reading frames upstream of *DST2* is an essential gene.

Genetic mapping of *DST2*. A ^{32}P-labeled *Sal*I fragment containing *DST2* was hybridized to yeast chromosomes separated by transverse alternating-field electrophoresis and transferred to nitrocellulose. Only chromosome VII hybridized to the probe (data not shown), indicating that *DST2* is located on chromosome VII. *DST2* was then mapped by tetrad analysis to 17 centimorgans (cM) from *lys5* and less than 2 cM from *rad54* (Table 6). *DST2* is clearly distinct from *RAD54* because their DNA sequences are completely different, at least at their upstream regions (4). Recently, the *rec1-1* mutation has been mapped very close to *RAD54* (9,

FIG. 2. DNA sequence of *DST2*. The region sequenced around the *DST2* gene is shown with the one-letter amino acid code below the deduced amino acid sequence. The potential transcriptional start at nucleotide 1672, as determined by primer extension (23) (data not shown), is indicated by an arrow. The region corresponding to the 21 amino acids identified by direct protein sequencing is doubly underlined. The two potential nucleotide-binding sites and the potential splice signals are underlined.

A. Genetic and biochemical analysis:

Spore	a	b	c	d
URA3	+	+	-	-
Strand transferase activity	-	-	+	+

B. Polypeptides that react with STPβ antibodies:

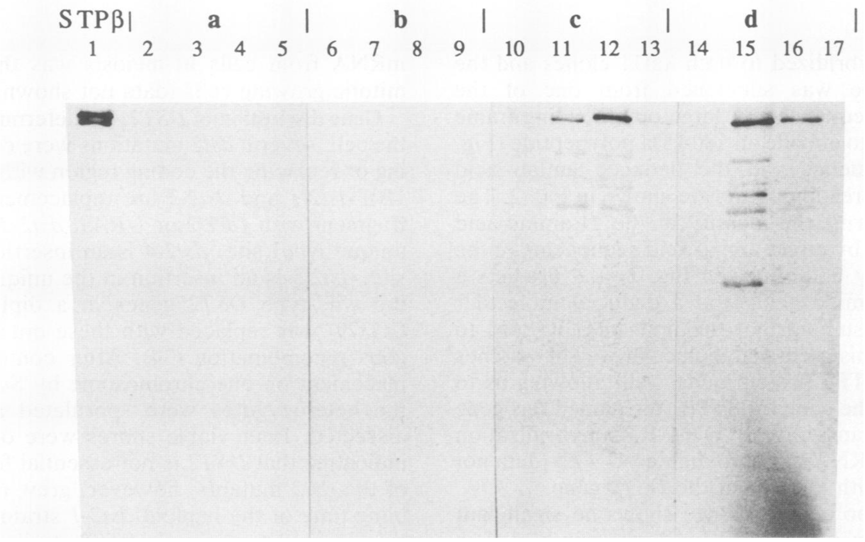

FIG. 3. Loss of antibody reactivity in *dst2* strains corresponds to loss of DNA strand transfer activity. The CG378/CG379 diploid strain was transformed with linearized DNA from a plasmid containing *dst2ΔBclI::URA3*. A Ura$^+$ transformant was tested for proper integration of the deletion-insertion mutation and sporulated. The analysis of one tetrad for DNA strand transfer activity and for antibody cross-reactivity is shown. (A) A Ura$^+$ spore should be deleted for the *DST2* gene and lose mitotic strand transferase activity. Such Ura$^+$ spores correspond to the *dst2-1* allele. (B) A small culture of each spore clone was grown, the cells were lysed, and MonoS columns (HR5/5; Pharmacia) were run before samples were analyzed for antibody reactivity. Four fractions from the 0.5 M NaCl elution are shown. Lanes: 1, 0.1 μg of purified STPβ; 2 to 5, fractions from spore a; 6 to 9, fractions from spore b; 10 to 13, fractions from spore c; 14 to 17, fractions from spore d.

29), but we have been unable to directly test for complementation of *rec1-1* by *DST2*.

Homologous recombination in *dst2* mutants. To observe the effect of *dst2* mutations on homologous recombination during both meiosis and mitotis, we measured recombination frequencies with several markers by using established techniques (33, 35). Figure 4 shows a time course of recombination induction between two heteroalleles at the *his1* locus during meiosis at 35°C in an SK-1 background strain. Induction of recombinants was delayed and was two- to threefold lower in the *dst2/dst2* strain than in the *DST2/DST2* strain. Furthermore, the recombinants in the mutant cells were unstable when measured after prolonged incubation in sporulation medium. In addition, cell survival fell considerably when incubation in sporulation medium was continued for more than 48 h at 35°C. Table 7 shows the results of intragenic recombination between two *his1* heteroalleles in the same strain as in Fig. 4 during meiosis at 30°C (a semipermissive temperature for sporulation), as well as for other markers. An SK-1 derivative strain with *ade2* heteroalleles was also used for comparison. Similar results were found for both markers: the initial recombination frequency was lower and the meiotic induction of recombination was delayed and somewhat unstable in the *dst2/dst2* strain when compared with the wild type.

A construction containing *LEU2* flanked by two partial *his3* genes (35) was used to measure DEL recombination, which is thought to occur by a pathway different from intragenic recombination (18, 35). DEL recombination between the overlapping region of the partial *his3* gene repeat should result in the His$^-$ Leu$^+$ cells becoming His$^+$ Leu$^-$ unless sister chromatid exchange occurs. Like intragenic recombination at *HIS1* and *ADE2*, excisive recombination induction during meiosis at *HIS3* was initially lower and somewhat delayed in the *dst2/dst2* mutant at 30°C (Table 7). These results indicate that recombinant induction in *dst2/dst2* mutant cells during meiosis is reproducibly severalfold lower than that of either *DST2/DST2* or *DST2/dst2* cells for both the *his1* and *ade2* heteroallelic markers, as well as for the excisive recombination, only because the initial recombination frequencies are lower.

Upon long-term incubation, mutant cell viability is reduced (Fig. 4), perhaps as a result of toxic, uncompletable initiated recombination products, as postulated for similar observations with *rad52* mutants (30, 32). Unlike *rad52* mutants, but like *dst1* mutants, *dst2* mutants were not sensitive to any DNA-damaging agents tested (UV, MMS, and γ-rays) (data not shown).

Mitotic recombination frequencies are reduced in *dst2* strains. Time zero (start) in Table 8 shows measurement of

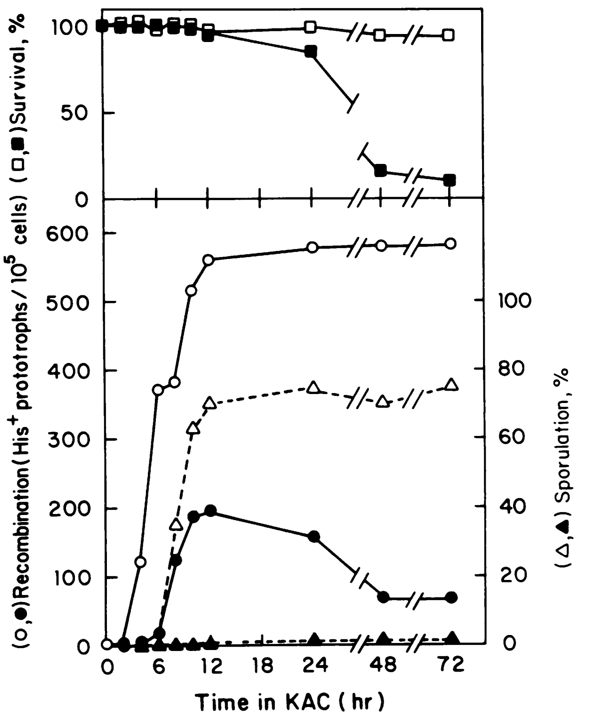

FIG. 4. Intragenic recombination during meiosis of *DST2* and *dst2* strains. Recombination between two *his1* heteroalleles was measured at various times after shifting to meiotic media (33) at 35°C in an SK-1 strain genetic background (19) relative to surviving cells. Sporulation was determined by phase microscopy. The *DST2* strain was a cross of MR966 with MR 93-28c (open symbols). The *dst2* strain was a cross of KK1101 with KK1201 (solid symbols). KAC, Potassium acetate buffer.

spontaneous, mitotic recombination in diploid *DST2/DST2*, *DST2/dst2*, and *dst2/dst2* mutant strains. In addition, a comparison of DEL recombination in *DST2* (CD202) and *dst2* (CD202-1) haploid strains was performed for 12 individual cultures of each strain. The DEL frequency was 9.79 × 10^{-4} for the *DST2* strain and 2.14 × 10^{-4}, or 21% of wild type, for the *dst2* strain. In each case, a significant reduction in recombination frequencies was seen for the *dst2* mutant. The cause of the variation among the tested alleles remains unknown. Reversion of the *ade2* and *his1* markers in the haploid state was also compared between *DST2* and *dst2* strains. No differences were seen, with the frequencies being $<10^{-7}$ for each.

TABLE 4. Effect of *dst2* on sporulation; effect of other insertion mutations on sporulation

Strain	Allele	Sporulation efficiency (%) at:	
		25°C	30°C
KK2103/KK2203	*dst2-3/dst2-3*	10.1 ± 3.2	<0.1
KK2104/KK2204	*dst2-4/dst2-4*	16.9 ± 8.4	1.0 ± 1.1
KK2105/KK2205	*dst2-5/dst2-5*	6.0 ± 2.2	0.7 ± 1.3
KK2103/CG379	*dst2-3/DST2*	37.3 ± 3.7	50 ± 3.6
KK2204/CG379	*dst2-4/DST2*	30 ± 3.1	37.2 ± 1.2

TABLE 5. Effect of *dst2* on sporulation; effect of multiple mutations on sporulation in *spo13* mutants

Strain	Mutation			Sporulation efficiency (%)[a] at:		
	dst2	*spo13*	*rad50*	23°C	30°C	35°C
Spo-21c/Spo-20a	+/+	−/−	+/+	90.0	71.3	8.3
Spo-21cT/Spo-20aT	−/−	−/−	+/+	57.2	28.4	<0.1
Spo50-1b/Spo50-13b	+/+	−/−	−/−	42.8	43.8	28.4
Spo50b-1bT/Spo50-13bT	−/−	−/−	−/−	15.3	10.0	<0.1

[a] Measured as dyad spores.

The *spo13* mutation does not allow bypass of the *dst2* sporulation defect. *spo13* mutants have previously been shown to bypass the first meiotic division and to complete a single, primarily equational division resulting in the production of two viable diploid spores (20). Many mutants defective in processes necessary for the first reductional division produce viable dyad spores in a *spo13* background (26). Therefore, a *spo13-dst2* double mutant was constructed to test whether the *spo13* mutation can rescue the temperature-sensitive sporulation phenotype of the *dst2* mutants. The double mutant was still temperature sensitive for sporulation (Table 5). A *rad52-1 spo13-1* double mutant does not sporulate, but a *rad50-1 rad52-1 spo13-1* triple mutant makes viable, nonrecombinant spores (26). This is possibly because a *rad50* mutation prevents the initiation of uncompletable recombination events. Unlike the results with *rad52*, a *spo13 dst2 rad50* triple mutant is still temperature sensitive for sporulation (Table 5). These results suggest that the *DST2* gene product (STPβ) is required not only for recombination processes, but also for later stages of meiotic processes, such as meiosis II chromosomal segregation or spore maturation.

DISCUSSION

We previously reported the purification and characterization of an ATP-independent DNA strand transfer activity (STPα) from meiotic cell extracts of the yeast *S. cerevisiae* (38). To determine whether this activity is required for meiotic homologous recombination, we have cloned and sequenced the gene (*DST1*) for STPα (3). Although disruption of *DST1* reduced meiotic homologous recombination by up to 90%, the cells proceed normally through meiosis and sporulation. One possible explanation for this result is that another strand transfer protein may substitute for STPα during meiosis. To test this possibility, we have purified and characterized an activity from mitotic cell extracts, which we call STPβ and find to be similar to STPα (6). In the work described in this report we have identified and sequenced the gene for STPβ, which we call *DST2*. This was accomplished by using antibodies against STPβ to probe a yeast genomic λgt11 library. The identification of *DST2* was confirmed by comparing an amino acid sequence from the purified polypeptide with the amino acid sequence deduced from the DNA sequence. The predicted molecular mass of the *DST2* gene product (175 kDa) is almost the same as that of the purified STPβ protein (180 kDa), suggesting that the purified protein is an undegraded monomer. Gene disruptions of *DST2* have been constructed in several different genetic backgrounds. All *dst2* strains so far constructed have lost the 180-kDa polypeptide that reacts with STPβ antibody and no longer contain any major mitotic DNA strand transfer activity. This result is also consistent with the conclusion that *DST2* encodes STPβ.

Genetic mapping of *DST2* hints that it may be allelic to

TABLE 6. Genetic mapping of *DST2*

Interval	Ascus type[a]			Map distance (cM)[b]
	PD	NPD	T	
dst2–lys5	20	0	11	16.9
dst2–rad54-3	30	0	0	<1.6
dst2–ade5	13	1	19	37.9
ade5–rad54-3	13	1	19	37.9

[a] The three classes of tetrads are represented by PD (parental ditype), NPD (nonparental ditype) and T (tetratype).

[b] Map distances were derived from tetrad analysis data by use of the equation: map distance (in centimorgans) = 50 [(T) + 6(NPD)]/(PD + NPD + T).

TABLE 8. Spontaneous mitotic recombination in various *DST2* strains[a]

Genotype	No. of cultures	Recombination frequency for:		
		10^{-6} *HIS1*	10^{-6} *ADE2*	10^{-4} *HIS3*
DST2/DST2	5	13	7.6	3.6
DST2/dst2-1	3	23	6.5	3.2
dst2-1/dst2-1	3	1.6 (0.123)	4.9 (0.64)	0.74 (0.20)
DST2	12			9.79
dst2-1	12			2.14 (0.21)

[a] Cultures were analyzed for spontaneous mitotic recombination as described in the text, using the same diploid strains described in Table 7. The haploid strains were CD202 and CD202-1. Reversion of the *ade2-40* allele was less than 10^{-7} for both strains. The values are the mean of the independent cultures at each genotype. Relative mitotic recombination in the *dst2* mutants relative to isogenic *DST2* strains is indicated in parentheses.

rec1-1 (9), which also maps to chromosome VII near *rad54*. Indeed, the *dst2* mutations exhibit genetic phenotypes somewhat similar to those reported for the *rec1-1* mutation. For example, *dst2/dst2* strains are temperature sensitive for sporulation, even when the complete polypeptide is deleted. Mitotic intragenic recombination frequencies are also reduced for both mutants. However, we note that there are some phenotypic differences between *dst2* and *rec1-1* mutants. The *dst2* mutants are fully recessive in their phenotypes, with both low- and high-copy-number plasmids of *DST2* being able to complement the defects seen in the various disruption mutations. The *dst2* mutants are insensitive to treatment with MMS, UV light, or γ-rays, but the *rec1-1* mutant is sensitive to X rays. The meiotic recombination phenotype has not been reported for the *rec1-1* mutant. A possible explanation for these differences may be found in the manner in which the mutations were constructed. None of the *dst2* mutants retain any mitotic DNA strand transfer activity, and those with a truncated gene product (Fig. 1B) do not seem to interfere with any cellular process(es) because the intact gene can complement all phenotypes tested (data not shown). It is possible that the *rec1-1* mutant produces a temperature-sensitive polypeptide that may become a poison at the restrictive temperature, thus explaining the semidominance and X-ray sensitivity of the *rec1-1* mutant (9).

The STPβ protein appears similar to SEP1 (22), even though their molecular masses are quite different. This is based on antibody comparisons and shared amino acid sequences. Monoclonal antibodies to SEP1 (21a) react with the 180-kDa STPβ protein (data not shown), but rabbit antiserum to STPβ does not strongly recognize SEP1. This may be partially explained by the observation that the antiserum made to STPβ identified only λgt11 clones that correspond to the COOH end of STPβ. If SEP1 lacks the COOH-terminal portion of STPβ, the antiserum to STPβ may not react significantly with the SEP1 polypeptide. The amino acid sequence that was determined for portions of SEP1, including the amino-terminal portion, corresponds to the amino acid sequence of STPβ as deduced from the DNA sequence (21a). We have investigated the possibility that the molecular mass differences could be due to differential splicing of the mRNA because there is a consensus splice signal that would give a 132-kDa polypeptide if the mRNA were spliced. Since no spliced mRNA was found by polymerase chain reaction analysis, we conclude that the molecular mass difference between STPβ and SEP1 is due to another factor, possibly proteolysis.

Insights into the timing of the action of STPβ during meiosis were obtained by comparing *spo13 dst2* double and *spo13 rad50 dst2* triple mutants. Because a *spo13* mutation has no effect on the *dst2* mutant phenotype, at least part of the requirement for *DST2* must occur later in meiosis, possibly during meiosis II chromosome segregation and/or spore maturation as well as during mitotic growth. This does not eliminate the possibility of an additional function during the first meiotic division. The slow mitotic growth may be a result of a reduced capability of cellular process(es) during mitosis.

TABLE 7. Recombination induction of *DST2* and *dst2* diploids at various loci[a]

Allele	10^{-6} *HIS1* frequency				10^{-6} *ADE2* frequency				10^{-4} *HIS3* frequency			
	No. of expt	Start	Max	Final	No. of expt	Start	Max	Final	No. of expt	Start	Max	Final
DST2/DST2	5	13	2,600 (48 h)	2,600	2	7.6	1,600 (24 h)	1,300	2	3.6	26 (12 h)	17
DST2/dst2-1	3	23	2,100 (48 h)	2,100	2	6.5	970 (12 h)	820	2	3.2	26 (12 h)	15
dst2-1/dst2-1	3	1.6	990 (24 h)	650	6	4.9	550 (48 h)	550	6	0.74	7.4 (24 h)	4.9
Ratio of *dst2-1/DST2*		0.12	0.38	0.25		0.63	0.34	0.42		0.20	0.28	0.28

[a] The diploids were exposed to sporulation medium (SPS) at 30°C for various lengths of time. Aliquots were removed and analyzed for frequency of prototroph formation, sporulation, and survival. "Start" refers to time zero, when the cultures were switched to SPS from presporulation medium and is equivalent to the mitotic frequencies. "Final" refers to 48 h in SPS, except for the *dst2/dst2* strains, for which it was 68 h. "Max" refers to the maximum values obtained at the time indicated in parentheses. Each value is the mean of all experiments. Recombination in *HIS1* was between *his1-1* and *his1-7*, using MR966 and MR93-28c and their derivatives for forming diploid cells. Recombination in *ADE2* was between *ade2-40* and *ade2-101*. Excisive recombination at *HIS3* was between the repeated region in *his3*::pRS6; in no case were His$^+$ Leu$^+$ recombinants (indicative of nondisjunction) found above a frequency of 0.1%. *ADE2* and *HIS3* recombination used CD202 and CD206 and their derivatives for forming diploid cells. The *ade2-101* allele reverted at a frequency of 1.45 X 10^{-7} and the *ade2-40* allele reverted at <1 × 10^{-8}.

Genetic studies with the isolated gene show that STPβ is required for the production of stable homologous recombinants during meiosis and sporulation. The temperature-sensitive sporulation phenotype of the *dst2* mutants is not yet understood. One possible explanation of this phenotype is that some other unidentified protein substitutes for STPβ at the permissive temperature, but not at high temperatures; however, it cannot be STPα because a *dst1 dst2* double mutant has the same phenotype as a *dst2* mutant (unpublished results). An equally plausible explanation is that the activity is required only to ensure that a reaction that can occur in the absence of any additional enzyme (DNA strand transfer) happens with the correct timing. At low temperature, the meiotic process may occur slowly enough for passive DNA interactions to suffice. A third possibility is that one or more steps in the recombination pathway become intrinsically temperature sensitive in the absence of *DST2*. Although an exhaustive search for an ATP-dependent DNA strand transfer protein (similar to *E. coli* RecA, phage T4 UvsX, or *U. maydis* Rec1) has not been conducted, it is clear from the results presented here that an ATP-independent DNA strand transfer reaction plays a major role in yeast homologous recombination.

The companion paper (3) describes the cloning and characterization of the gene (*DST1*) that corresponds to the meiosis-specific DNA strand transfer activity, STPα. The phenotype of the *dst1* gene disruption is quite subtle compared with that of the *dst2* mutants. It corresponds to an entirely meiosis-specific activity, and the residual activity can be attributed to another gene or genes. Since STPα is activated during meiosis and *dst2* mutants do not appear to complete normal meiotic processes at the restrictive temperature, STPα might not be activated in a normal way in a *dst2* mutant. Now that two major genes corresponding to RecA-like proteins from *S. cerevisiae* have been identified, other factors that are involved in various types of genetic recombination can be more easily identified and placed in perspective.

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ADDENDUM IN PROOF

While this paper was in press, we learned that the *DST2* gene is also the *KEM1* gene (J. Kim, P. O. Ljungdahl, and G. R. Fink, Genetics **126**:799–812, 1990) as well as the *XRN1* gene (F. W. Larimer and A. Stevens, Gene **95**:85–90, 1990).

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