MOLECULAR AND CELLULAR BIOLOGY, May 1991, p. 2576–2582
0270-7306/91/052576-07$02.00/0

Vol. 11, No. 5

Isolation, DNA Sequence, and Regulation of a *Saccharomyces cerevisiae* Gene That Encodes DNA Strand Transfer Protein α

ALAN B. CLARK, CHRISTINE C. DYKSTRA,† AND AKIO SUGINO*

Laboratory of Molecular Genetics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709

Received 21 September 1990/Accepted 14 January 1991

DNA strand transfer protein α (STPα) from meiotic *Saccharomyces cerevisiae* cells promotes homologous pairing of DNA without any nucleotide cofactor in the presence of yeast single-stranded DNA binding protein. This gene (DNA strand transferase 1, *DST1*) encodes a 309-amino-acid protein with a predicted molecular mass of 34,800 Da. The STPα protein level is constant in both mitotic and meiotic cells, but during meiosis the polypeptide is activated by an unknown mechanism, resulting in a large increase in its specific activity. A *dst1*::*URA3*/*dst1*::*URA3* mutant grows normally in mitotic media; however, meiotic cells exhibit a greatly reduced induction of both DNA strand transfer activity and intragenic recombination between *his1* heteroalleles. Spore viability is normal. These results suggest that *DST1* is required for much of the observed induction of homologous recombination in *S. cerevisiae* during meiosis but not for normal sporulation.

DNA strand transfer activities have been recently identified and studied in various organisms (2–4, 9, 13, 15, 20, 21, 26, 37). Although the DNA strand transfer reaction is thought to be a central event of genetic recombination, only the *Escherichia coli* RecA, bacteriophage T4 UvsX, and *Ustilago maydis* Rec1 proteins have been proved to be required for general recombination. Studies with *Saccharomyces cerevisiae* have identified mutants affecting recombination (see references 28 and 29 for reviews), but none of these has been correlated directly with any recombination-specific enzymatic activities. One way to approach this problem is to characterize and purify an activity which is expected to be required for genetic recombination, identify the gene encoding the activity, and observe effects of deleting the activity. Along with analysis of multiple mutants with known recombination defects, progress can be made in understanding this complex process. Since recombination frequencies are elevated nearly 1,000-fold during meiosis in *S. cerevisiae*, we anticipate higher levels of recombination enzymes from meiotic cell extracts.

From such meiotic *S. cerevisiae* cell extracts, we have identified and purified an activity (named STPα) that promotes the transfer of a strand from a duplex linear DNA molecule to a complementary circular single-stranded DNA in the presence of yeast single-stranded DNA-binding proteins (ySSB). We have also shown that DNA strand transfer activity increases specifically in *MAT***a**/*MAT*α diploid cells during meiosis concurrently with the observed large increase in homologous recombination frequencies (6, 37). Similar activities can be found in mitotic crude extracts that have been purified (2, 4, 5, 15, 20, 37). All of these activities do not require a nucleotide cofactor for their activity and seem to be different from the systems previously studied in prokaryotes (3) and *U. maydis* (19). Although we have eliminated the possibility that other activities associated with STPα and/or ySSB generate structures similar to those from a true DNA strand transfer reaction, genetic confirmation of the requirement for STPα in meiotic homologous recombination is needed. In this report, we describe the isolation, nucleotide sequence, and regulation of the gene (*DST1*) encoding STPα as well as the characterization of effects resulting from the disruption of *DST1*. From these results, we conclude that STPα is required only for meiotic induction of homologous recombination in *S. cerevisiae* and that it is active only in meiotic cells.

MATERIALS AND METHODS

Bacterial and yeast strains. *E. coli* Y1090 (36) was used for screening the λgt11 yeast genomic library with antibodies. GW5100 (*dam*::Tn*9*) was used for preparing unmethylated plasmid DNA susceptible to digestion by *Bcl*I. DH5α (23) was used for growth of subclones. The yeast strains used are listed in Table 1.

Chemicals, enzymes, and DNA. A λgt11 yeast genomic library was constructed by R. K. Hamatake in this laboratory by using reported methods (36). ^{125}I-protein A and α-^{32}P-labeled deoxyribonucleoside triphosphates were from either ICN or Amersham. Alkaline phosphatase-conjugated goat anti-rabbit antibodies, Nitro Blue Tetrazolium, and 5-bromo-4-chloro-3-indolyl phosphate were from Promega and were used as recommended by the supplier. Immobilon membranes for protein blotting were from Millipore. Nitrocellulose was from Schleicher & Schuell. STPα and the 26-kDa ySSB were purified as described previously (16, 37). Restriction enzymes, DNA ligase, and other enzymes were from either Bethesda Research Laboratories, International Biotechnologies, Inc., or New England BioLabs. Plasmid DNAs were purified by the alkaline lysis method (23).

Cloning, sequencing, and genetic methods. λgt11 yeast genomic clones producing antigens reactive to STPα polyclonal antibodies were isolated by published procedures (36). Subcloning of DNA fragments, DNA and RNA blot analyses followed published methods (23). The DNA sequence was determined by using the nested deletion method from a Cyclone kit to obtain overlapping fragments in M13mp18, and the Sequenase kit from United States Biochemical Corp. for the dideoxy-sequencing method with [α-^{35}S]dATP. ^{32}P-labeled probes obtained by the random

* Corresponding author.
† Present address: Department of Pathology, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7525.

TABLE 1. Yeast strains used in this work

Strain[a]	Relevant genotype	Source
CG950	*MATa ura3-52 lys2 leu2::hisG trp1::hisG ho::hisG*[b]	C. Giroux
CG953	CG950 but *MATα can1*[b]	C. Giroux
CG396	*MATa lys5 ade3 his5-2 ura1 leu1-c*	C. Giroux
D649	*MATa/MATα +/mal trp1/+ pet6/+ ade2/+ +/ade1 lys2/+ +/his4 leu2/+ +/thr4*	YGSC[c]
D649-11B	*MATα thr4 his4* spore from D649	This work
L119-7D	*MATα ura3 dbf2 trp1 trp2 ade1*	L. Johnston
MR966	*MATα ura3-52 leu2-3,112 trp1-289 his1-7*[b]	M. A. Resnick
MR93-28C	*MATa ura3-52 leu2-3,112 trp1-289 his1-1*[b]	M. A. Resnick
MR658	*MATa ade4*[b]	M. A. Resnick
MR43-25D	*MATa leu2 his1-7 can1 hom3-10 ade2-1 rad52-1 rad1-1*[b]	M. A. Resnick
MR45-43A	*MATα leu2 his1-1 trp2 lys1-1 tup7*[b]	M. A. Resnick
MR48	*MATa/MATα leu2/leu2 his1-7/his1-1 can1/+ hom3-10/+ +/trp2 +/lys1-1 ade2-1/+ rad52-1/+ rad1-1/+ +/tub7*[b]	37
RSY6	*MATa ura3-52 leu2-3,112 trp5-27 arg4-3 ade2-40 ilv1-92*	R. Schiestl (33)
S7	*MATa leu2 cdc62-1*	YGSC
K38-10A	*MATα ade6 ura3-1 arg4 aro7 asp5 met14 lys2 pet17 trp1*	YGSC
CD66	*MATa ura3-52 his1-7 can1*[b]	This work
CD101	MR966 but *dst1-1*[b]	This work
CD106	MR93-28C but *dst1-1*[b]	This work
CD143	*MATa his1-1 lys2 trp1 ura3 leu2*[b]	This work
CD240	*MATa ura3-52 ade6 met14*[b]	This work
CD241	MR966× CD106	This work
CD242	CD101 × CD106	This work
CD247	*MATa ura3-52 his4 thr4 lys1 trp1::hisG*[b]	This work
CD257	*MATa ura3-52 dbf2 leu2 ade1*	This work
CD251	*MATα ura3 leu2 trp1*[b]	This work
CD260	*MATa lys5 leu1 ade3 ura3-52*[b]	This work
CD261	*MATα dbf2 ade6 ura3-52 dst1-1*[b]	This work
CD262	*MATa ura3-52 dst1-1 leu2 trp1*[b]	This work
CD263	*MATα ura3-52 dst1-1 his4 thr4 trp1 lys1*[b]	This work
CD264	*MATa ura3-52 cdc62 ade4 trp1::hisG leu2::hisG can1*[b]	This work

[a] Strains were constructed as follows: CD66 is a CG950 × MR43-25D *ura3-52 his1-7 leu2 trp1 can1* spore that was backcrossed to CG953 and then MR658; CD101, CD106, CD262, and CD263 are Ura$^+$ transformants of MR966, MR93-28c, CD251, and CD247, respectively with pY106::*URA3* cut with *Eco*RI; CD136 is CG953 × MR43-25D; CD143 is CG950 × MR45-43A; CD240 is K38-10A × CD66 selecting for *MATα ade6 met14* and then backcrossing to CD66; CD247 is D649-11B × CG950 and backcrossed twice to CG658; CD251 is L119-7D × CD143 and backcrossed to MR658; CD257 is L119-7D × CD143; CD260 is CG396 × CG953 and backcrossed to CG950; CD261 is L119-7D × CD106 and backcrossed to CD66; CD264 is S7 × CD101 selecting for *MATα cdc62-1 ura3-52 leu2 trp1 can1*, backcrossing twice to MR658 selecting for *cdc62-1 ade4 ura3-52 leu2 trp1 can1*, each time selecting for rapidly sporulating cells.

[b] SK-1 background strains.

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

oligonucleotide method with a Prime Time kit (International Biotechnologies Inc.) were used for Southern and Northern (RNA) hybridizations. Yeast chromosomal DNA isolation, genetic crosses, and transformation were described previously (14, 35). Crude extracts of *S. cerevisiae* were made as described previously (37).

Protein sequencing. STPα (20 μg) was digested with Lys-C endopeptidase, and the resultant oligopeptides were isolated as published previously (5). Each oligopeptide was applied on an ABI automated protein sequencer. One oligopeptide gave a sequence: SN(D)AAVLEILHVL. The parentheses indicate an ambiguous amino acid.

Nucleotide sequence accession number. The *DST1* sequence reported in this paper has been deposited with the GenBank data base as number M60770.

RESULTS

Cloning of the *DST1* gene from a λgt11 yeast genomic library. Rabbit antiserum against the purified STPα protein (37) was used to identify the STPα gene contained in a λgt11 library prepared from *S. cerevisiae* genomic DNA. By screening approximately 200,000 recombinant λgt11 plaques with the antiserum, we identified 106 positive plaques. After purification, only 16 clones remained positive. Of these, there were 12 weakly positive clones that produced a fusion protein recognized by both STPα and β-galactosidase antibodies (data not shown). The four strongly positive clones produced a 38-kDa polypeptide (Fig. 1A) which reacted with the antiserum to STPα, but not with β-galactosidase antibodies (data not shown). Therefore, it is likely that the strongly positive clones have the intact STPα gene. The DNA from clones showing strong reactivity against STPα antibodies also shared a common region (Fig. 1B), confirmed by restriction mapping and DNA-DNA hybridization (data not shown). The region shared by all four λgt11 clones is about 1.6 kb (from the right end of the clone λgt11-13 or λgt11-46 to the left end of the clone λgt11-78 [Fig. 1]). This is sufficient to code for the 38-kDa STPα polypeptide and was sequenced. The sequence reveals an open reading frame of 927 nucleotides (309 amino acids) and the predicted molecular mass of 34,800 Da (Fig. 2). The predicted amino acid sequence matches the amino acid sequence determined from the purified STPα polypeptide (doubly underlined in Fig. 2), confirming that the cloned fragment contains the authentic STPα gene.

To confirm the presence of a transcript consistent with the expression of the open reading frame, Northern blots of total RNA from mitotic and meiotic cells were hybridized to the

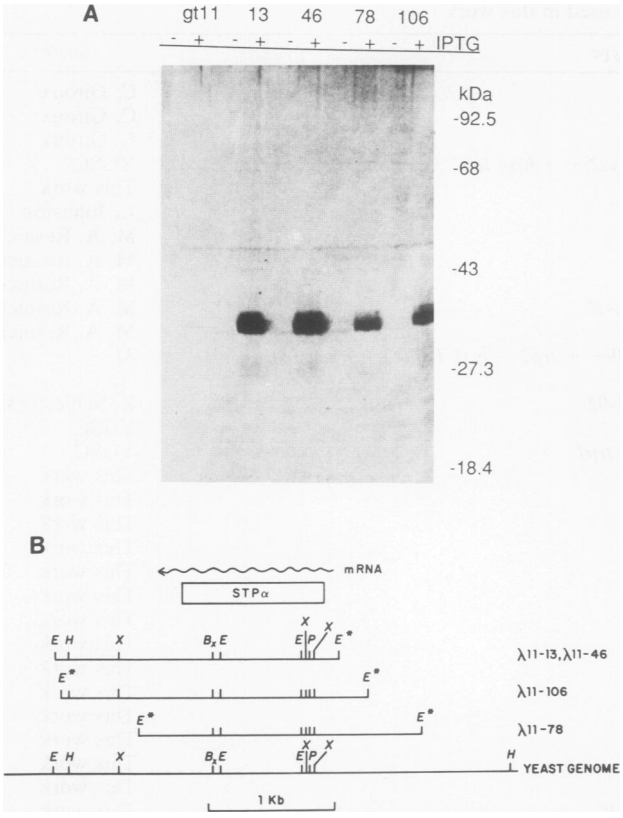

FIG. 1. λgt11 clones produce full-length STPα protein in *E. coli*. (A) Protein blot probed with a rabbit antiserum against the purified STPα protein. Each lane contains protein extracts (about 5 μg) from uninduced (lanes −) or isopropyl-β-D-thiogalactopyranoside (IPTG)-induced (lanes +) λgt11 lysogens. The two left-hand lanes contain extracts from λgt11 phage without any inserted DNA, and the remaining lanes contain extracts from clones isolated with STPα antiserum. (B) Limited restriction map of the four independent clones (λgt11-13, λgt11-46, λgt11-106, and λgt11-78), as well as a map of genomic DNA. Bx, E, P, and X represent restriction enzyme sites of *Bst*XI, *Eco*RI, *Pst*I and *Xba*I, respectively. E* shows an artificial *Eco*RI end generated by construction of the library and not found on the chromosome. The arrow indicates the direction of transcription.

^{32}P-labeled 0.64-kb *Eco*RI fragment of the clone λgt11-106 (Fig. 1). A transcript of about 1.4 kb, sufficient to code for a 35-kDa polypeptide, could be detected (Fig. 3A). This transcript hybridized only to the DNA strand complementary to that corresponding to the open reading frame as measured by using ^{32}P-labeled single-stranded DNA from a M13mp18 subclone, indicating that the open reading frame determined by the DNA sequence was expressed in the cells. We have named this gene *DST1* for DNA strand transferase 1. S1 nuclease analysis of RNA-DNA hybrids (data not shown) localized the 5′ end of the major *DST1* mRNA at −28 bases from an initiation codon (ATG) (Fig. 2, arrow). Analysis of the sequence predicts a codon bias index (34) of 0.125, characteristic of poorly translated genes, although protein levels appear fairly abundant, as determined by Western immunoblotting followed by incubation with antiserum (Fig. 3B). In addition, there is one consensus zinc finger DNA-binding structure (Fig. 2, circled amino acids) (1, 32), suggesting some DNA-binding ability but no consensus nucleotide-binding sequence (10) that would be expected if the protein needed a nucleoside triphosphate for activity. There is no significant homology at the protein level to either the *E. coli* RecA protein, the bacteriophage T4 UvsX protein from T4, *DST2* (5), or any other amino acid sequence in the NBRF-PIR protein data base or that predicted from nucleotide sequences in the GenBank data base.

Regulation of STPα activity during meiosis. Both the cloned DNA and the antiserum were used to analyze the expression of *DST1* during meiosis as well as during vegetative growth. Significant levels of *DST1* mRNA were seen in mitotically growing cells (Fig. 3A, $T = 0$). As cells underwent meiosis, mRNA levels stayed almost constant for 3 to 4 hours; they dropped significantly by the time that the maximum DNA strand transfer activity was seen in the SK-1 strain background (approximately 6 h after the shift to sporulation medium) (18, 37). No significant changes in the amount of the 38-kDa STPα protein were seen between mitotic cell extracts and extracts from meiotic cells despite the large increase in specific activity (Fig. 3B).

Gene disruption of *DST1*. To determine the role of *DST1* in vivo, we constructed a deletion-disruption mutant. A 0.57-kb *Bcl*I fragment from plasmid pY106 (covering the upstream regions, translation initiation site, and the middle of the coding regions of *DST1*) was replaced with a 1.2-kb *Bam*HI fragment that contains the yeast *URA3* gene (Fig. 4A and B). The resulting plasmid, pY106::*URA3*, was digested with *Eco*RI and used to transform both haploid and diploid yeast strains to Ura$^+$, replacing the wild-type *DST1* gene by the one-step gene replacement (31). The transformation frequency was similar for both diploid and haploid strains from two different sources of SK-1 background cells (CG950, CG953, MR966, and MR93-28C) (data not shown). The diploid Ura$^+$ transformants yielded meiotic tetrads, 83% of which showed four viable spores, segregating $2^+:2^-$ for the *URA3* marker.

DST1-disrupted transformants should lose a genomic *Eco*RI site that will correlate with *URA3* as well as the loss of cross-reacting STPα polypeptide. The insertion of *URA3* correlates with the disruption of the *DST1* gene and loss of the STPα antiserum cross-reacting STPα polypeptide in Ura$^+$ haploid cells (Fig. 4C and D). The minor background reactive bands did not change. This mutation was named *dst1-1*. After the transformants were backcrossed at least two times, homozygous mutant diploids (*dst1-1/dst1-1*) were formed. Sporulation appeared identical to that in the nondisrupted parental strains both in kinetics and in viability, confirming that the *DST1* gene is dispensable for meiosis and sporulation. The *dst1-1* strain did grow somewhat more slowly in both rich and defined media than the parental strain for unexplained reasons (data not shown), but it did not grow as slowly as the *dst2* mutants (5).

Intragenic meiotic recombination. *DST1* and *dst1-1* haploid strains in the SK-1 background and containing either *his1-1* or *his1-7* heteroalleles were mated to create diploids with or without the *DST1* gene. Freshly made diploids were essential to achieve a low background level of His$^+$ cells (below a frequency of 2×10^{-5}). Cells were examined at different times after the shift to meiotic media for the frequency of recombination between the *his1-1* and *his1-7* alleles in return-to-growth experiments (30). At the same time, extracts were made from cells harvested at the indicated times and assayed for DNA strand transfer activity as described previously (37). Strain CD242 (*dst1-1/DST1*) showed a greater than 500-fold increase in intragenic recombination and a more than 16-fold increase in DNA strand transfer-specific activity (Fig. 5). On the other hand, CD241 (*dst1-1/dst1-1*)

```
                                                                    ATTCCTC     (-361)
CCTCTAACAATTATTACAAGTGTTTCTGTTTAAAGCAAGTGCTAGAAGTGACTCGGTTCTCTTAGGAAATTCAAACGCAAGATTTCTCTT     (-271)
CTGGTGATTGTAACAATTATGAGATACTTCACTAGCCCACCTTAACTTTACGGACCTTCTTTTGAAACGATGCTTCGAATGAAACGCCTT     (-181)
TTTGATATATAATATCCAATTTCATTATAGGGAAATTTTTCAACTCTTACCCGCCCCACTATGCTGATATGACCAAGTGATCACTCGATG     (-91)
                                                                    ⇓
ATGGGACTACGTATTGAAAAATATTGAATGAAAAATTACTCAAGCAGCAGAACATTCACAGTGTAGTCAGTCCGCATAAGAGCATTCATC     (-1)

ATGGATAGTAAGGAAGTACTGGTACATGTTAAGAATCTAGAAAAGAACAAAAGTAATGATGCTGCAGTTCTAGAAATCTTACATGTCTTG     (+90)
M  D  S  K  E  V  L  V  H  V  K  N  L  E  K  N  K  S  N  D  A  A  V  L  E  I  L  H  V  L      30

GATAAAGAATTCTTCCCCACTGAAAAGTTACTGAGAGAAACAAAAGTTGGTGTGGAAGTCAACAAGTTTAAAAAATCCACTAATGTAGAG     (+180)
D  K  E  F  F  P  T  E  K  L  L  R  E  T  K  V  G  V  E  V  N  K  F  K  K  S  T  N  V  E      60

ATCAGCAAGCTCGTGAAGAAAATGATTAGCTCTTGGAAAGCGCAATTAATAAAAAATAAGCGTTGCAGGCAACCACAGCAGCATCATCAA     (+270)
I  S  K  L  V  K  K  M  I  S  S  W  K  A  Q  L  I  K  N  K  R  C  R  Q  P  Q  Q  H  H  Q      90

GATCATGCGCCAGGCAATGCAGAGGACAAGACAACTGTAGGTGAGTCCGTGAATGGTGTTCAACAGCCGGCCTCCTCCCAGTCAGATGCC     (+360)
D  H  A  P  G  N  A  E  D  K  T  T  V  G  E  S  V  N  G  V  Q  Q  P  A  S  S  Q  S  D  A      120

ATGAAACAAGACAAGTACGTCAGCACTAAACCAAGAAATAGTAAGAACGATGGTGTGGATACAGCTATATACCACCACAAATTACGTGAT     (+450)
M  K  Q  D  K  Y  V  S  T  K  P  R  N  S  K  N  D  G  V  D  T  A  I  Y  H  H  K  L  R  D      150

CAGGTACTAAAAGCACTCTACGACGTTTTGGCCAAGGAAAGTGAGCATCCACCTCAATCTATTTTGCATACTGCAAAGGCCATAGAAAGT     (+540)
Q  V  L  K  A  L  Y  D  V  L  A  K  E  S  E  H  P  P  Q  S  I  L  H  T  A  K  A  I  E  S      180

GAAATGAATAAAGTTAACAACTGTGACACCAATGAAGCCGCTTACAAAGCCAGGTATCGTATAATTTATTCAAACGTCATATCAAAGAAT     (+630)
E  M  N  K  V  N  N  C  D  T  N  E  A  A  Y  K  A  R  Y  R  I  I  Y  S  N  V  I  S  K  N      210

AACCCAGATCTCAAACATAAAATTGCCAACGGTGATATAACACCTGAATTCTTAGCTACATGCGATGCCAAGGATCTGGCACCAGCGCCC     (+720)
N  P  D  L  K  H  K  I  A  N  G  D  I  T  P  E  F  L  A  T  C  D  A  K  D  L  A  P  A  P      240

TTAAAGCAAAAGATAGAAGAAATTGCCAAGCAAAACTTATACAACGCACAAGGTGCCACCATAGAAAGGTCAGTCACCGATAGATTTACT     (+810)
L  K  Q  K  I  E  E  I  A  K  Q  N  L  Y  N  A  Q  G  A  T  I  E  R  S  V  T  D  R  F  T      270

TGTGGTAAATGTAAAGAGAAGAAGGTATCTTACTATCAATTGCAAACAAGATCTGCGGATGAACCATTGACTACTTTCTGTACATGTGAA     (+900)
Ⓒ  G  K  Ⓒ  K  E  K  K  V  S  Y  Y  Q  L  Q  T  R  S  A  D  E  P  L  T  T  F  Ⓒ  T  Ⓒ  E      300

GCATGTGGTAACAGATGGAAATTCTCTTAGAATAGAAAAAGATGAGAGTAGCCACGGACAGAGCAAAGGAAACAATAGGAGGAAGGGACA     (+990)
A  Ⓒ  G  N  R  W  K  F  S  *                                                                    309

GAAAAGCCTGTTCTGTTCGTAGTAGTATTTGACAGCCGGAATCCAGGAAAGAAAAGCC                                      (+1054)
```

FIG. 2. Nucleotide sequence and predicted amino acid sequence of the *DST1* gene. The region between the right end of the λgt11 clone 13 or 46 and the left end of clone 78 is sufficient for coding of *DST1* (Fig. 1). Thus, the nucleotide sequence of this region was determined. The predicted amino acid sequence from an open reading frame is shown by the one-letter code. The doubly underlined amino acids are the sequence matched to that determined from the purified STPα protein. The major transcriptional start site as determined by S1 nuclease analysis is indicated by the arrow. Initiation and termination codons are underlined (ATG and TAG). +1 nucleotide is the first base of the initiation codon ATG, and preceding nucleotides are minus numbers. The cysteines that indicate the consensus zinc finger DNA-binding structure are circled.

showed only a 50-fold increase in intragenic recombination and a 3- to 4-fold increase in DNA strand transfer specific activity. We conclude that more than 90% of the inducible recombination activity is absent in CD241. Virtually the same result was obtained from strains having two different SK-1 backgrounds as well as from a more slowly sporulating strain (data not shown). The *DST1* gene on either a multicopy or a single-copy plasmid restored the meiosis-inducible recombination as well as meiosis-inducible DNA strand transfer activity (data not shown).

Intergenic meiotic recombination. Some recombination-deficient mutants affect only recombination between short distances (28). To determine whether the *dst1* mutation affects only recombination between short distances, tetrad analysis was performed for a number of markers across the well-characterized chromosome III. Various mutant alleles were crossed into the *dst1-1* strains. Tetrads of diploid strains carrying either one or no copies of *dst1* and multiple markers were dissected. From the results of tetrad analysis, the genetic distance in centimorgans (cM) was calculated. A decrease in intergenic recombination frequencies would result in an apparent decrease in the genetic distance between genes. The recombination frequency between genes separated by a long distance was affected by the mutation (Table 2).

Intrachromosomal mitotic recombination. Since the experiments above with heteroalleles showed that baseline mitotic gene conversion rates do not change in the *dst1-1* mutant, we also measured intrachromosomal recombination frequencies between repeated sequences. The *DST1* gene was deleted from strain RSY6 (33) in which the *LEU2* gene is flanked by partially repeated *his3* genes. Intrachromosomal recombination across the *his3* region would result in a His^+ Leu^- colony. If sister chromatid exchange occurs, the colonies will become His^+ Leu^+. This process is inducible by DNA-damaging agents. We found no significant difference in mitotic intrachromosomal recombination frequencies between wild-type and *dst1-1* mutant cells. Furthermore, we found no differences between the mutant and wild-type cells before and after treatment with either methyl methanesulfonate, UV irradiation, or γ rays (data not shown). Therefore, no mitotic phenotype has been found for the *dst1-1* mutant.

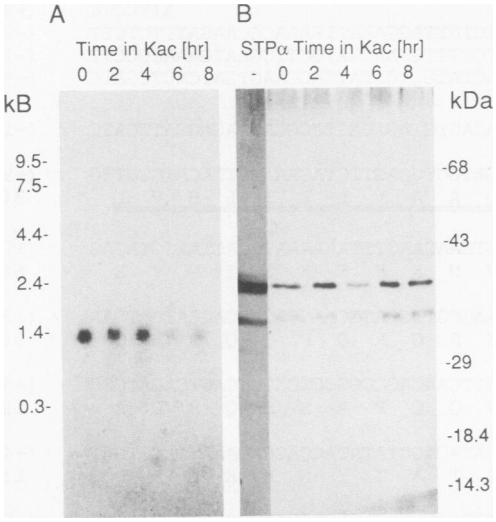

FIG. 3. Regulation of STPα activity during meiosis *S. cerevisiae* MR48 cells were grown in 25 ml of SPS medium as described previously (30). The cells were collected by centrifugation, resuspended in 25 ml of potassium acetate medium (1% potassium acetate [pH 7] plus required amino acids at 5 mM) and incubated at 30°C. At 0, 2, 4, 6, and 8 h after the media shift, 1 ml of culture was withdrawn and total protein was extracted (37) and another 1 ml was withdrawn and processed for total RNA determination. Cells were plated for survival along with His^+ recombinants to ensure that recombination induction occurred. (A) Hybridization analysis of the RNA levels found at different times. Each lane contains 11.5 μg of total RNA electrophoresed in a 1.5% agarose gel containing 3.7% formaldehyde. The RNA was transferred to a nitrocellulose filter, and the filter was probed with the ^{32}P-labeled 0.64-kb *Eco*RI DNA fragment from λgt11-106 (Fig. 1). The numbers to the left of the figure indicate molecular size marker RNAs. (B) Immunoblot of meiotic polypeptides found at different times. A 50-μg sample of protein was subjected to SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) followed by electrophoretic transfer to an Immobilon membrane. STPα antiserum-reacting polypeptides were analyzed. Lane 1 contains 10 ng of the purified STPα protein (fraction VI). Lanes 2 to 6 contain the proteins from the cells incubated for the indicated times in potassium acetate medium. The numbers to the right of the figure indicate the molecular mass marker proteins. The above experiment has been done at least three times, and the results were essentially the same as shown in this figure.

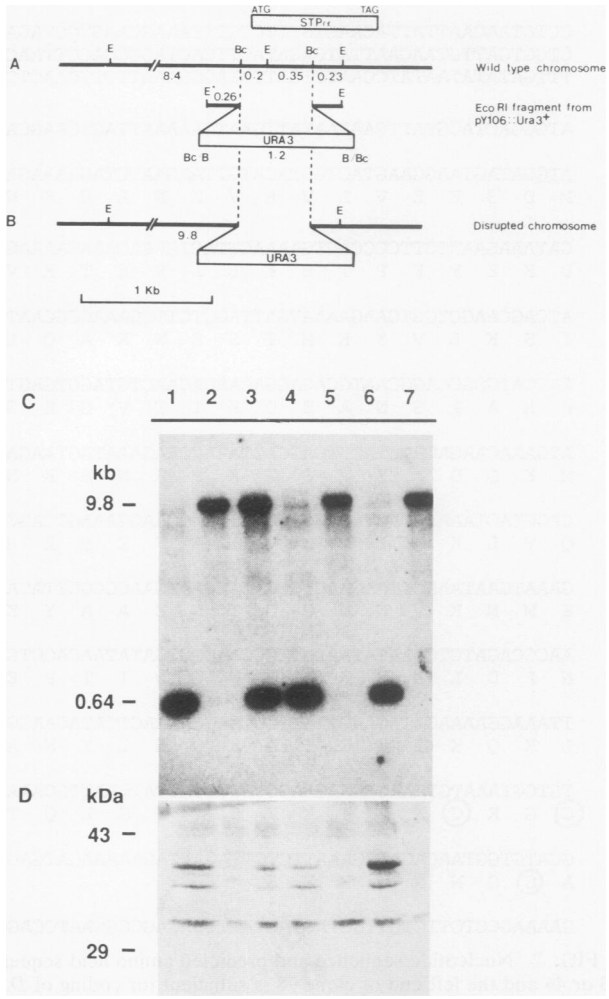

FIG. 4. Construction of a *DST1* gene disruption. (A) The *Sac*I-*Kpn*I fragment of λgt11-106 (Fig. 1) that contains the intact *DST1* gene along with flanking λgt11 DNA was subcloned into pUC18. The resulting plasmid, pY106, was grown in GW5100 to obtain unmethylated DNA, which could be digested by *Bcl*I. The *Bcl*I fragment (nucleotides −40 to +499 in Fig. 2) was excised from pY106 and replaced with the *Bam*HI fragment containing the yeast *URA3* gene from plasmid pGB310 (obtained from C. Giroux). (B) The resulting plasmid, pY106::*URA3*, was cut by *Eco*RI and used to transform haploid and diploid cells. (C) Ura^+ transformants and wild-type control cells were selected. Chromosomal DNA was extracted, digested with *Eco*RI, and analyzed by Southern transfer and DNA-DNA hybridization with the ^{32}P-labeled 0.64-kb *Eco*RI fragment from λgt11-106 as the probe (Fig. 1B). (D) Cells were grown in YPD medium (35) to 5×10^7 cells per ml, total protein was extracted, and the STPα polypeptide was analyzed by Western blotting followed by incubation with antiserum as described in the legend to Fig. 3B. In both panels C and D, molecular size markers are indicated on the left. Lanes: 1, MR966; 2, CD101; 3, CD242; 4 to 7, four spore isolates from CD242.

Genetic mapping. By use of pulsed-field gel electrophoresis, the *DST1* gene was located on chromosome VII (data not shown). *DST1* was then mapped by tetrad analysis (35). It was found on the right arm of chromosome VII between the centromere and *ADE6*, and closer to *ADE6* than to the centromere (Table 3). No gene has been reported in this region (27).

DISCUSSION

We have previously purified a DNA strand transfer activity (STPα) that increases during meiosis in *S. cerevisiae*. STPα does not require a nucleotide cofactor and, unlike other systems, is not associated with a nucleoside triphosphatase activity (37). In the present work we used antibodies to identify the gene (*DST1*) encoding STPα, constructed a gene disruption mutant, and studied the mitotic and meiotic phenotypes associated with the *dst1* mutant. Disruption of *DST1* resulted in a 90% reduction in meiosis-specific intragenic recombination between *his1* heteroalleles and a loss of most of the meiosis-inducible DNA strand transfer activity (Fig. 5). Similar results were seen for intergenic recombination (Table 2), but there was no effect on basal mitotic intragenic recombination frequencies. This suggests that the *DST1* gene is specifically required for the observed levels of meiosis-inducible homologous recombination in *S. cerevisiae*. Since both survival and spore viability are normal in the *dst1-1* strain, much of the observed inducible recombi-

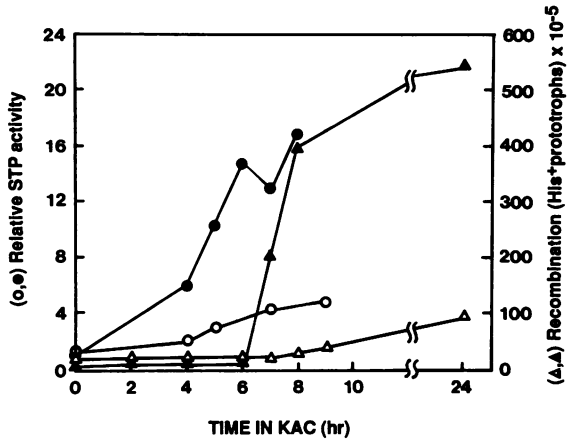

FIG. 5. Reduction in the induction of both STPα activity and homologous recombination during meiosis in a diploid homozygous for *dst1-1*. Cultures (1 liter) of heterozygous *DST1/dst1-1* (CD242) and homozygous *dst1-1/dst1-1* (CD241) congenic strains containing *his1* heteroalleles were grown and processed for sporulation as described previously (37). Samples were removed at various times after the transfer from presporulation to sporulation medium. Recombination levels were measured by testing for His^+ recombinants between the *his1-1* and *his1-7* heteroalleles. These are for CD242 (▲) and CD241 (△). Extracts representing each time point were prepared from approximately 100 ml of cells. After partial purification (fraction II), the specific activity was measured by the agarose gel assay, followed by scanning with a laser densitometer (37). The circles indicate DNA strand transfer specific activity for CD242 (●) and CD241 (○). For both strains, spores first appeared between 9 and 10 h after the medium shift. KAC, Potassium acetate.

nation that occurs during meiosis is probably dispensable. This is contrary to results seen for other recombination-specific genes identified to date, including *dst2* mutants (5, 28, 29).

Some meiosis-inducible recombination as well as DNA strand transfer activity remains in the *dst1* mutants as a result of an as yet unidentified activity. We have purified another DNA strand transfer protein, STPβ, from mitotically growing cells and identified its gene (*DST2*) (5). Mutants deficient in this activity are temperature sensitive for sporulation, with reduced sporulation frequencies even at the permissive temperature (24°C). We have found by antibody experiments that the level of STPβ protein increases two- to threefold during meiosis (5). It is possible, therefore, that this protein is responsible for the remaining inducible

TABLE 2. Intergenic recombination on chromosome III during meiosis

Interval[a]	Distance (cM) for:		Ratio (*dst1-1/DST1*)
	dst1-1	*DST1*	
HIS4–LEU2	35	15	2.33
HIS4–THR4	34	65	0.52
HIS4–MAT	30	45	0.66
LEU2–THR4	28	50	0.56
LEU2–MAT	11.9	30	0.56
MAT–THR4	17	20	0.85

[a] The diploids were sporulated at 30°C, dissected, and tested for the above markers. A total of 116 and 105 tetrads were dissected, and 84 (72%) and 98 (93%) tetrads gave four viable spores for the *DST1* and *dst1-1* strains, respectively. The crosses used for dissection were CD262 × CD263 for *dst1-1* and CD247 × MR966 for *DST1*.

TABLE 3. Genetic mapping of *DST1*

Interval	Ascus type[a]			Map distance[b] (*cM*)	Cross
	PD	NPD	T		
DST1[c]*–ADE6*	58	1	18	15.5	CD240 × CD101
DST1–LEU1	13	0	15	26	CD260 × CD263
DST1–CDC62	10	2	24	53	CD264 × CD101
DST1–DBF2	4	1	10	50	CD261 × MR93-28C
DBF2–ADE6	14	0	15	13	CD261 × MR93-28C
ADE6–CDC62	8	0	9	26	CD264 × CD240

[a] PD, Parental ditype; NPD, nonparental ditype; T, tetratype.
[b] Map distance was calculated as described previously (35).
[c] The *DST1* marker was followed by the *URA3* insertion.

recombination. When *DST2* is disrupted, about one-third of the level of intragenic recombinants between the *his1* heteroalles can be observed during meiosis, but the recombinants are unstable. Taken together, these observations indicate that *DST1* is specific for meiosis, but is dispensable for sporulation as long as the *DST2* gene is present. However, the *DST1* gene cannot replace the requirement for the *DST2* gene for sporulation.

In both antiserum and RNA hybridization experiments (Fig. 3), we did not observe an increase in protein or RNA levels even though the specific activity of STPα increased more than 15-fold during meiosis (37) (Fig. 5). A plausible explanation for this observation is that there is a constant level of inactive STPα protein in vegetatively growing cells that is activated by some sort of posttranslational modification during meiosis, such as phosphorylation or adenylation. A similar observation has been seen with the *CDC28* gene, which is constitutively expressed but is activated in a cell-cycle-dependent manner (24). Consistent with this hypothesis is the lack of DNA strand transfer activity in *E. coli* strains carrying the intact *DST1* gene, even though high levels of the protein were expressed (Fig. 1). The availability of the *DST1* gene should allow us to identify factors responsible for any activation.

The analysis of the *DST1* DNA sequence has shown several interesting correlations with the biochemical observations. (i) The predicted molecular weight confirms sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis data on the purified protein. (ii) The DNA strand transfer activity does not require a nucleotide cofactor and is not a nucleoside triphosphatase, and the DNA sequence indicates the absence of a consensus nucleotide-binding site (10). (iii) DNA-binding analysis has shown that STPα does bind DNA, and this correlates with a consensus zinc finger DNA-binding structure (1) found near the carboxyl end of the *DST1* protein. Among the known DNA strand transfer proteins (*E. coli* RecA, phage T4 UvsX, STPα, and STPβ), little amino acid sequence similarity can be found by computer analysis (data not shown). This has been confirmed by the use of antiserum against each protein (RecA, UvsX, STPα, and STPβ) (our unpublished results). Some amino acid sequence similarity between some domains of RecA and UvsX has been reported (11), but the significance of this is not known.

The identification of the *DST1* and *DST2* genes will enable us to make considerable progress in defining biochemical components of the recombination process. Of interest will be observation of phenotypes of strains with multiple mutations between genes already implicated in the early steps of recombination (such as *rad50* [30], *hop1* [17], *mer1* [7], *mei4*

[25], and *spo11* [8]), as well as later steps (*rad52* and *rad57* [12]), to determine the timing of its action. We would like to know whether the reduction in inducibility of recombinants is also a reduction in recombination initiation at some time after pairing and hence might partially relieve the spore inviability of *rad52* and *rad57* mutants (12) in much the same way that a *spo13* mutation alleviates defects in meiosis I processes (22) but not defects in later steps (29). It will be interesting to see whether any residual DNA strand transfer activity is found in either mitotic or meiotic cells and whether the double mutant can sporulate at all; this may be done by constructing a double mutant of *dst1* and *dst2*.

ACKNOWLEDGMENTS

We thank C. Giroux, M. A. Resnick, and R. Schiestl for providing various yeast strains. We also thank M. A. Resnick and J. W. Drake for their critical reading of this manuscript.

C.C.D. was the recipient of a National Research Council Associateship.

REFERENCES

1. **Berg, J. M.** 1988. Proposed structure for the zinc-binding domains from transcription factor IIIA and related proteins. Proc. Natl. Acad. Sci. USA **85**:99–102.
2. **Cassuto, E., L.-A. Lightfoot, and P. Howard-Flanders.** 1987. Partial purification of an activity from human cells that produces homologous pairing and the formation of heteroduplex DNA in the presence of ATP. Mol. Gen. Genet. **208**:10–14.
3. **Cox, M. M., and I. R. Lehman.** 1987. Enzymes of general recombination. Annu. Rev. Biochem. **56**:229–262.
4. **Dykstra, C. C., R. K. Hamatake, and A. Sugino.** 1990. DNA strand transfer protein β from yeast mitotic cells differs from strand transfer protein α from meiotic cells. J. Biol. Chem. **265**:10968–10973.
5. **Dykstra, C. C., K. Kitada, A. B. Clark, R. K. Hamatake, and A. Sugino.** 1991. Cloning and characterization of *DST2*, the gene for DNA strand transfer protein β from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**:2583–2592.
6. **Dykstra, C. C., J. Nitiss, M. A. Resnick, and A. Sugino.** 1988. ATP-independent DNA strand transfer catalyzed by proteins from the yeast *Saccharomyces cerevisiae*, p. 231–236. *In* E. C. Friedberg and P. C. Hanawalt (ed.), Mechanism and consequences of DNA damage processing. Alan R. Liss, Inc., New York.
7. **Engebrecht, J., and S. G. Roeder.** 1989. Yeast *mer1* mutants display reduced levels of meiotic recombination. Genetics **121**: 237–247.
8. **Esposito, R. E., and S. Klapholz.** 1981. Meiosis and ascospore development, p. 211–288. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
9. **Fishel, R. A., K. Detmer, and A. Rich.** 1988. Identification of homologous pairing and strand-exchange activity from a human tumor cell line based on Z-DNA affinity chromatography. Proc. Natl. Acad. Sci. USA **85**:36–40.
10. **Fry, D. C., S. A. Kuby, and A. S. Mildvan.** 1986. ATP-binding site of adenylate kinase: mechanistic implications of its homology with ras-encoded p21, F1-ATPase, and other nucleotide-binding proteins. Proc. Natl. Acad. Sci. USA **83**:907–911.
11. **Fujisawa, H., T. Yonezaki, and T. Minagawa.** 1985. Sequence of the T4 recombination gene, uvsX, and its comparison with that of the recA gene of *Escherichia coli*. Nucleic Acids Res. **13**:7473–7481.
12. **Game, J. C., T. J. Zamb, R. J. Braun, M. A. Resnick, and R. M. Roth.** 1980. The role of radiation (*rad*) genes in meiotic recombination in yeast. Genetics **94**:51–68.
13. **Ganea, D., P. Moore, L. Chekuri, and R. Kucherlapati.** 1987. Characterization of an ATP-dependent DNA strand transferase from human cells. Mol. Cell. Biol. **7**:3124–3130.
14. **Gietz, R. D., and A. Sugino.** 1988. New yeast-*Escherichia coli* shuttle vectors constructed with in vitro mutagenized yeast genes lacking six-base pair restriction sites. Gene **74**:527–534.
15. **Halbrook, J., and K. McEntee.** 1989. Purification and characterization of a DNA-pairing and strand transfer activity from mitotic *Saccharomyces cerevisiae*. J. Biol. Chem. **264**:21403–21412.
16. **Hamatake, R. K., C. C. Dykstra, and A. Sugino.** 1989. Presynapsis and synapsis of DNA promoted by the STPα and single-stranded DNA-binding proteins from *Saccharomyces cerevisiae*. J. Biol. Chem. **264**:13336–13342.
17. **Hollingsworth, N. M., and B. Byers.** 1989. *HOP1*, a yeast meiotic pairing gene. Genetics **121**:445–462.
18. **Kane, S. M., and R. Roth.** 1974. Carbohydrate metabolism during ascospore development in yeast. J. Bacteriol. **118**:8–14.
19. **Kmiec, E. B., and W. K. Holloman.** 1986. Homologous pairing of DNA molecules by Ustilago Rec1 protein is promoted by sequences of Z-DNA. Cell **44**:545–554.
20. **Kolodner, R., D. H. Evans, and P. T. Morrison.** 1987. Purification and characterization of an activity from *Saccharomyces cerevisiae* that catalyzes homologous pairing and strand exchange. Proc. Natl. Acad. Sci. USA **84**:5560–5564.
21. **Lowenhaupt, K., M. Sander, C. Hauser, and A. Rich.** 1989. *Drosophila melanogaster* strand transferase: a protein that forms heteroduplex DNA in the absence of both ATP and single-strand DNA binding protein. J. Biol. Chem. **264**:20568–20575.
22. **Malone, R. E.** 1983. Multiple mutant analysis of recombination in yeast. Mol. Gen. Genet. **189**:405–412.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a Laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Mendenhal, M. D., C. A. Jones, and S. I. Reed.** 1987. Dual regulation of the yeast CDC28-p40 protein kinase complex: cell cycle, pheromone, and nutrient limitation effects. Cell **50**:927–935.
25. **Menees, T. M., and G. S. Roeder.** 1989. *MEI4*, a yeast gene required for meiotic recombination. Genetics **12**:675–682.
26. **Moore, S. P., and R. Fishel.** 1990. Purification and characterization of a protein from human cells which promotes homologous pairing of DNA. J. Biol. Chem. **265**:11108–11117.
27. **Mortimer, R. K., D. Schild, C. R. Contopoulou, and J. A. Kans.** 1989. Genetic map of *Saccharomyces cerevisiae*, edition 10. Yeast **5**:321–403.
28. **Petes, T. D., R. E. Malone, and L. S. Symington.** *In* The molecular and cellular biology of the yeast *Saccharomyces*, in press. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Resnick, M. A.** 1987. Investigating the genetic control of biochemical events in meiotic recombination, p. 157–210. *In* P. B. Moens (ed.), Meiosis. Academic Press, Inc., New York.
30. **Resnick, M. A., A. Sugino, J. Nitiss, and T. Chow.** 1984. DNA polymerases, deoxyribonuclease, and recombination during meiosis in yeast. Mol. Cell. Biol. **4**:2811–2817.
31. **Rothstein, R.** 1983. One step gene disruption in yeast. Methods Enzymol. **101**:202–211.
32. **Schleif, R.** 1988. DNA binding by proteins. Science **241**:1182–1187.
33. **Schiestl, R. H., S. Igarachi, and P. J. Hastings.** 1988. Analysis of the mechanism for reversion of a disrupted gene. Genetics **119**:237–247.
34. **Sharp, P. M., T. M. F. Tuohy, and K. R. Mosurski.** 1986. Codon usage in yeast: cluster analysis differentiates highly and lowly expressed genes. Nucleic Acids Res. **14**:5125–5143.
35. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
36. **Snyder, M., S. Elledge, D. Sweetser, R. A. Young, and R. W. Davis.** 1987. λgt11: gene isolation with antibody probes and other applications. Methods Enzymol. **154**:107–128.
37. **Sugino, A., J. Nitiss, and M. A. Resnick.** 1988. ATP-independent DNA strand transfer catalyzed by protein(s) from meiotic cells of the yeast *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **85**:3683–3687.