

Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the *M26* meiotic recombination hotspot in *Schizosaccharomyces pombe*

NING KON*, MICHELLE D. KRAWCHUK*, B. GREG WARREN*, GERALD R. SMITH†, AND WAYNE P. WAHLS*‡

*Department of Biochemistry, Vanderbilt University School of Medicine, 621 Light Hall, Nashville, TN 37232-0146; and †Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N., Seattle, WA 98109

Communicated by Sydney Kustu, University of California, Berkeley, CA, October 13, 1997 (received for review July 11, 1997)

ABSTRACT Homologous recombination hotspots increase the frequency of recombination in nearby DNA. The *M26* hotspot in the *ade6* gene of *Schizosaccharomyces pombe* is a meiotic hotspot with a discrete, cis-acting nucleotide sequence (5'-ATGACGT-3') defined by extensive mutagenesis. A heterodimeric *M26* DNA binding protein, composed of subunits Mts1 and Mts2, has been identified and purified 40,000-fold. Cloning, disruption, and genetic analyses of the *mts* genes demonstrate that the Mts1/Mts2 heterodimer is essential for hotspot activity. This provides direct evidence that a specific trans-acting factor, binding to a cis-acting site with a unique nucleotide sequence, is required to activate this meiotic hotspot. Intriguingly, the Mts1/Mts2 protein subunits are identical to the recently described transcription factors Atf1 (Gad7) and Pcr1, which are required for a variety of stress responses. However, we report differential dependence on the Mts proteins for hotspot activation and stress response, suggesting that these proteins are multifunctional and have distinct activities. Furthermore, *ade6* mRNA levels are equivalent in hotspot and nonhotspot meioses and do not change in *mts* mutants, indicating that hotspot activation is not a consequence of elevated transcription levels. These findings suggest an intimate but separable link between the regulation of transcription and meiotic recombination. Other studies have recently shown that the Mts1/Mts2 protein and *M26* sites are involved in meiotic recombination elsewhere in the *S. pombe* genome, suggesting that these factors help regulate the timing and distribution of homologous recombination.

Meiosis and homologous recombination have vital roles in the evolution of eukaryotes. Meiosis and subsequent joining of haploid gametes generates new genotypes by shuffling linkage groups (chromosomes), and recombination generates new genotypes by shuffling alleles of genes within individual chromosomes. Both processes supply genetic diversity to provide eukaryotes a mechanism for sampling different combinations of newly arising genetic variants. Meiotic recombination rates are enhanced relative to mitotic rates, presumably to maximize the amount of genetic variability in the meiotic products or to ensure homolog disjunction or both. Recombination rates are higher than average in the vicinity of DNA sites called "recombination hotspots."

Homologous recombination hotspots are found in organisms ranging from bacteriophages to humans (1–3). Some eukaryotic hotspots function in mitosis (4), some function in meiosis (5–7), and some function in both (3, 8). Hotspots therefore influence the distribution of recombination along chromosomes and the timing of recombination during the life cycle. Recombination hotspots may contribute a significant fraction of total recombination in both prokaryotes and eukaryotes (9–11). Presumably, as for cis-acting transcriptional

regulatory elements, there are proteins that interact with hotspots to mediate their biological activity. In *Escherichia coli* the RecBCD enzyme interacts with Chi sites to enhance recombination (12). The *M26* recombination hotspot of the fission yeast *Schizosaccharomyces pombe* (5) is a well-characterized eukaryotic hotspot. The *M26* mutation is a single base pair substitution in *ade6* that increases meiotic recombination up to 20-fold relative to other *ade6* alleles, such as *M375* (Fig. 1) (5, 7, 13). Mutational analysis revealed that a specific 7-bp nucleotide sequence at *M26* is required for hotspot activity (14) (Fig. 1), suggesting that a protein might bind to the site to increase meiotic recombination above the basal levels.

To investigate the mechanism by which eukaryotic recombination hotspots function, we identified and purified 40,000-fold a heterodimeric protein that binds to the *M26* DNA site (15). The polypeptides are called Mts1 and Mts2 (*M*-twenty-six binding proteins). Optimal binding requires the 7-bp *M26* site (Fig. 1) and requires heterodimeric Mts1/Mts2 protein. Binding of Mts1/Mts2 *in vitro* (15) correlates almost precisely with hotspot activity *in vivo* (14) for single base pair substitutions in the region, suggesting that Mts1/Mts2 protein activates the *M26* hotspot.

We report here that binding of Mts1/Mts2 heterodimer to the *M26* site is essential for hotspot activation. While these genetic studies were underway, two laboratories described *S. pombe* genes identical to *mts1* (called *atf1* and *gad7*) and *mts2* (called *pcr1*), both isolated through different lines of research (16–18). Mts1 and Mts2 proteins have roles in developmental decisions and stress responses (Fig. 2), and the proteins regulate the transcription of a variety of genes (16–20) (N.K. and W.P.W., unpublished observations). However, we show that stress response and hotspot activation are genetically distinct, and hotspot activation is not a consequence of increased transcription at *ade6*. Thus, hotspot activation by the Mts1/Mts2 heterodimer is independent of the known transcriptional regulatory activities of the individual Mts1 and Mts2 proteins. We speculate that the Mts1/Mts2 protein activates the *M26* hotspot by altering the local chromatin structure or by directly recruiting recombination enzymes.

MATERIALS AND METHODS

***S. pombe* Strains, Media, and Genetic Analyses.** *S. pombe* strains used for this study are listed in Table 1. A complete genealogy is available upon request. Genetic nomenclature follows the recommendations of Kohli (21). Strains were cultured in nitrogen base liquid or on nitrogen base agar minimal medium (0.67% Difco yeast nitrogen base without

Abbreviation: bZIP, basic leucine zipper.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. U87869 (*mts1*) and U87870 (*mts2*)].

‡To whom reprint requests should be addressed. e-mail: wahlswp@ctrvx.vanderbilt.edu.

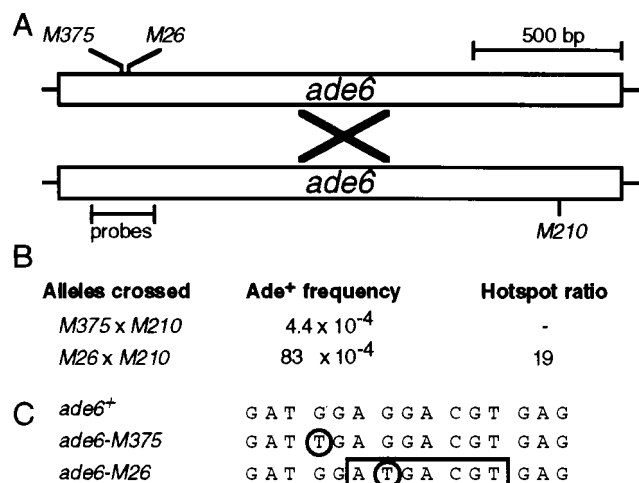


FIG. 1. Hotspot activity of *M26*. (A) Schematic diagram of the *ade6* gene showing positions of alleles and probes used for gel mobility shift experiments. Meiotic recombination (X) between a chromosome harboring the *ade6-M26* or the *ade6-M375* allele and a chromosome with the *ade6-M210* allele generates a wild-type, selectable *ade6⁺* gene. (B) Example of *M26* recombination hotspot activity. Data are from Table 2. Recombination in test crosses containing *M26* is increased up to 20-fold relative to recombination when using another allele, such as *M375* (5). (C) DNA sequence surrounding the *ade6-M26* recombination hotspot (7, 13). Both *M375* and *M26* are identical single base pair substitutions (circled) that generate stop codons. The *M26* mutation creates a 7-bp site (box) that is bound by the Mts1/Mts2 heterodimer (15) and is required for hotspot activity *in vivo* (14).

amino acids, 1% glucose, 2% agar for solid media) supplemented with the required amino acids, purines, and pyrimidines (100 μ g/ml). Synthetic sporulation agar was prepared as described (22). Strain constructions, meiotic crosses, and analyses of recombinant frequencies were as described (23) with the two following changes: matings were for 5 days, and the spore suspensions were treated with ethanol for 15 min.

Cloning and Sequencing *mts* Genes. From 400 liters of culture we obtained about 4,000 g of cells and we conducted multiple, large-scale purifications of protein as described (15). The amino acid sequences of tryptic fragments of Mts1 (amino acids 86–112, 130–158, 213–247) and Mts2 (amino acids 37–50, 52–65) were determined by Bill Lane at the Harvard Microchemistry Facility and were used to design degenerate oligo-

nucleotide primers for PCR amplification (24). Products of PCR amplification of genomic DNA were cloned into pBlue-scriptKSII+, sequenced (25) to confirm that they harbored the correct fragments, amplified, excised, and used as probes for colony hybridization (26) to identify multiple independent clones from a partial-digest, size-fractionated genomic DNA library (27). PCR analysis was used to identify one clone (pMts1-7) harboring a 4,376-bp insert with a centrally located *mts1* gene and one clone (pMts2-3) harboring a 3,642-bp insert with a centrally located *mts2* gene. A “primer-walk” approach was used to sequence (25) both strands of the entire genomic DNA inserts in pMts1-7 and pMts2-3 (GenBank accession numbers U87869 and U87870).

Protein Purification and Gel Mobility Shift Assay. Proteins were generated in *E. coli* by expression in the pET15b vector (Novagen), purified by nickel affinity chromatography in the presence of 6 M guanidine-HCl, renatured on the column, eluted with 500 mM imidazole, and dialyzed against storage buffer. Overexpression and purification were according to the manufacturer’s instructions (Novagen), and all other conditions were as described (15). The gel mobility shift experiments were as described (15) with probes indicated in Fig. 1.

RESULTS

The *mts1* and *mts2* Genes Encode the Heterodimeric *M26* Hotspot Binding Protein. To conduct further biochemical and genetic studies of the *M26* hotspot, we cloned and sequenced the genes encoding the Mts1/Mts2 protein (Fig. 3). The predicted polypeptides contain basic leucine zipper (bZIP) motifs characteristic of dimeric, sequence-specific DNA binding proteins. Both proteins have extensive sequence identity ($\approx 50\%$), restricted to the bZIP domain, to transcription factors of the activating transcription factor/cAMP response element binding protein (ATF/CREB) family, suggesting that the Mts1/Mts2 heterodimer is a transcription factor. To confirm that the cloned genes encoded *M26* recombination hotspot DNA binding proteins, we produced Mts1 and Mts2 in *E. coli*, purified the polypeptides, and characterized their binding affinity and specificity (Fig. 4). Heterodimeric protein bound specifically to DNA bearing the *M26* site; it bound poorly to control DNA molecules that differed by a single base pair substitution within the *M26* site (Fig. 4). The individual protein subunits also displayed hotspot-specific DNA binding activity, but with much lower affinity (data not shown). Thus, proteins expressed from the cloned *mts* genes exhibited DNA binding specificity similar to that of protein purified from *S. pombe* (15), confirming that the *mts1* and *mts2* genes encode the *M26* hotspot-specific DNA binding proteins.

Mts1/Mts2 Heterodimer Activates the *M26* Meiotic Recombination Hotspot in *ade6*. To determine whether the Mts1/Mts2 heterodimer is required to activate the *M26* hotspot, we used gene targeting to disrupt the genomic copies of *mts1* and *mts2* (Fig. 3). Although the mutants displayed a partial sterility phenotype ($\approx 15\%$ of wild-type mating efficiency), sufficient spores were formed to permit genetic analyses. Standard genetic techniques (5, 14, 28) were used to determine both basal and hotspot-mediated recombination levels. We measured recombination between two sets of *ade6* alleles (Fig. 1) in wild-type cells and in *mts* mutants. Crosses between strains with *ade6-M375* and *ade6-M210* alleles revealed the basal recombinant frequency, whereas crosses between strains with *ade6-M26* and *ade6-M210* alleles revealed the sum of basal plus hotspot recombinant frequencies (5). In cells wild-type for *mts1* and *mts2*, we observed a basal recombinant frequency of 4.4×10^{-4} and a hotspot-enhanced frequency of 83×10^{-4} (Table 2, line 1). The ratio of these two values, called the “hotspot ratio,” is a measure of the stimulation of recombination by *M26*. In wild-type (*mts⁺*) meioses we observed a

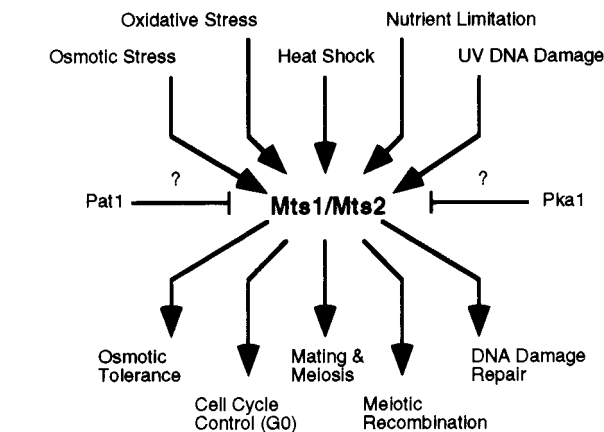


FIG. 2. Schematic representation of biochemical pathways by using Mts1 and Mts2 proteins. Upstream signals converge via the mitogen-activated protein (MAP) kinase cascade (19, 20) and likely via Pat1 and Pka kinases (16–20). Signals diverge at Mts1 and Mts2 to activate the appropriate developmental responses. Some effector functions require Mts1, some require Mts2, and some require both Mts1 and Mts2.

Table 1. *S. pombe* strains used in this study

Strain	Genotype	Source*
WSP599	<i>h⁻ ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP547	<i>h⁻ ade6-M210 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP550	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP571	<i>h⁺ ade6-M26 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP572	<i>h⁻ ade6-M26 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP578	<i>h⁺ ade6-M375 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP579	<i>h⁻ ade6-M375 ura4-D18 his3-D1 leu1-32</i>	This study [†]
WSP654	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	T of WSP550
WSP656	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	T of WSP550
WSP639	<i>h⁻ ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP654 × WSP599
WSP641	<i>h⁻ ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP656 × WSP599
WSP642	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP550 × WSP639
WSP643	<i>h⁻ ade6-M210 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP550 × WSP639
WSP644	<i>h⁺ ade6-M26 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP571 × WSP639
WSP645	<i>h⁻ ade6-M26 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP571 × WSP639
WSP646	<i>h⁺ ade6-M375 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP578 × WSP639
WSP647	<i>h⁻ ade6-M375 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺</i>	WSP578 × WSP639
WSP648	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP550 × WSP641
WSP649	<i>h⁻ ade6-M210 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP550 × WSP641
WSP650	<i>h⁺ ade6-M26 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP571 × WSP641
WSP651	<i>h⁻ ade6-M26 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP571 × WSP641
WSP652	<i>h⁺ ade6-M375 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP578 × WSP641
WSP653	<i>h⁻ ade6-M375 ura4-D18 his3-D1 leu1-32 mts2-D1::his3⁺</i>	WSP578 × WSP641
WSP671	<i>h⁺ ade6-M210 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP642 × WSP649
WSP672	<i>h⁻ ade6-M210 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP642 × WSP649
WSP674	<i>h⁺ ade6-M26 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP644 × WSP651
WSP675	<i>h⁻ ade6-M26 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP644 × WSP651
WSP677	<i>h⁺ ade6-M375 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP646 × WSP653
WSP678	<i>h⁻ ade6-M375 ura4-D18 his3-D1 leu1-32 mts1-D15::ura4⁺ mts2-D1::his3⁺</i>	WSP646 × WSP653

*Strains were constructed by transformation (T) as in Fig. 3 or derived from standard genetic crosses (×) of the indicated strains.

[†]Complete genealogies are available upon request.

hotspot ratio of about 19 (Table 2, line 1), similar to results reported previously (5).

Cells that were homozygous mutant for *mts1* (Table 2, line 3) or *mts2* (Table 2, line 5) exhibited basal recombinant frequencies that were statistically indistinguishable from those of wild-type (*mts⁺*) cells. We conclude that the *mts1* and *mts2* mutants are recombination-proficient and have an intact (wild-type) basal recombination machinery.

Although having normal basal recombination machinery, cells that were homozygous mutant for *mts1* (Table 2, line 3) or *mts2* (Table 2, line 5) entirely lacked recombination hotspot activity; the frequencies of recombinants in the hotspot crosses (*M26* × *M210*) were essentially the same as the frequencies of recombinants in the basal crosses (*M375* × *M210*). These observations, coupled with the hotspot-specific DNA binding activity of the heterodimer (Fig. 4), demonstrate that the Mts1/Mts2 heterodimer directly activates the *M26* meiotic homologous recombination hotspot, but this protein has no detectable role in *ade6* basal recombination. Furthermore, homodimeric Mts1 or Mts2 protein, observed to bind *M26* DNA with low affinity *in vitro* (15), does not activate the hotspot *in vivo*. Double mutants (*mts1⁻ mts2⁻*) had the same phenotype as either single mutant (Table 2, line 6), further indicating that a heterodimer of Mts1 and Mts2 is the functional unit in hotspot activation.

The *mts1* and *mts2* mutations were semidominant in crosses heterozygous for *mts1* or *mts2*: the hotspot ratio was intermediate between those of wild-type and homozygous mutant crosses (Table 2, lines 2 and 4). We estimate that there are ≥200 active Mts1/Mts2 protein molecules and about 300 *M26* sites per haploid genome (15), suggesting that the cis and trans components are present in approximately equal numbers. The semidominance supports those estimates and indicates that protein-DNA interaction within cells is limiting, at least when *mts1* or *mts2* is heterozygous.

Mts1, But Not Mts2, Is Required for Osmotic Stress Response in Mitotic Cells. The Mts1 and Mts2 proteins are

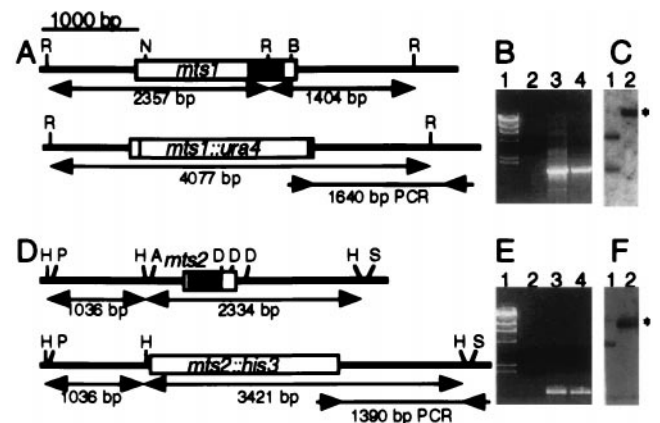


FIG. 3. Cloning, sequence analysis, and disruption of the *mts1* and *mts2* genes. (A and D) The genes encode proteins with bZIP dimerization and DNA binding motifs (shaded boxes) ≈50% identical to those in ATF/CREB proteins. R, *EcoRI*; N, *EcoNI*; B, *BallI*; H, *HincII*; P, *HpaI*; A, *AatII*; D, *NdeIII*; S, *BstEII*. Standard procedures were used to disrupt the genes. (B and E) Confirmation of gene disruption by PCR. Lanes: 1, λ DNA digested with *HindIII*; 2, PCR product of wild-type DNA; 3, PCR product of disrupted DNA (*mts1-D15::ura4⁺* or *mts2-D1::his3⁺*); 4, PCR product of positive control DNA (plasmids used to obtain gene targeting fragments). Primers were as shown by inward-pointing arrows in A and D. (C and F) Confirmation of gene disruption by Southern blot analysis. Lanes: 1, wild-type DNA; 2, disrupted DNA (*mts1-D15::ura4⁺* or *mts2-D1::his3⁺*). Restriction maps are shown in A and D, and the expected positions of altered restriction fragments are indicated (*). DNA was digested with *EcoRI* and probed with the 4,077-bp *EcoRI-EcoRI* fragment for *mts1* constructs or digested with *HincII* and probed with the 3,421-bp *HincII-HincII* fragment for *mts2* constructs.

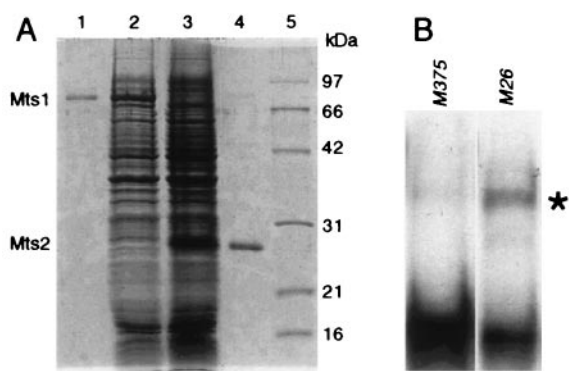


FIG. 4. Hotspot-specific DNA binding activity of Mts1/Mts2 heterodimer purified from *E. coli*. (A) Coomassie-stained, 12% SDS/PAGE analysis of proteins. Lanes: 1, purified Mts1; 2, whole-cell lysate containing Mts1; 3, whole-cell lysate containing Mts2; 4, purified Mts2; 5, molecular mass standards. (B) Gel mobility shift assay by using a mixture of renatured Mts1 and Mts2 polypeptides. Position of *M26*-specific complex is indicated (*). Gel shift conditions and probes were as described (15) and contained ≈ 5 nM probe, 25 nM Mts1, and 25 nM Mts2.

implicated in several biochemical pathways, including response to stress (Fig. 2), but the mutants exhibit no mitotic phenotype under normal laboratory culture conditions (data not shown) (16–20). We therefore tested the mutants for mitotic phenotypes under a variety of adverse conditions. Cells that were *mts1* mutant were highly sensitive to osmotic stress (Fig. 5). However, the *mts2* mutants were resistant to osmotic stress. We and others have observed similar differential responses to other cytotoxic and genotoxic agents (data not shown) (16, 18–20). The differential response to stress indicates that Mts1 and Mts2 have nonoverlapping functions in some pathways that sense environmental, cytotoxic, and genotoxic signals. Because hotspot activation requires a heterodimer of Mts1 and Mts2 (Table 2), we infer that the mechanism of hotspot activation is distinct from the mechanisms of developmental regulation (transcriptional transactivation of target genes) that require only the individual Mts proteins (Fig. 5).

Hotspot Activation Is Not a Consequence of Increased Transcription of *ade6-M26*. Because Mts1/Mts2 binding to *M26* activates the hotspot (Table 2) and the Mts1 and Mts2 proteins are transcription factors that induce some genes during meiotic differentiation (16–18), it is plausible that

hotspot activation might result from transcriptional transactivation of *ade6*. However, previous studies of transcript levels of *ade6-M26* (hotspot) vs. *ade6-M375* (basal recombination) during meiosis (29) revealed no differential levels of *ade6* expression. We have repeated those experiments to determine if *ade6* message levels were altered in an *M26*-dependent, Mts1/Mts2-dependent, meiosis-specific fashion. Steady-state *ade6* message levels were determined by Northern blot analysis of RNA from cells under a variety of conditions ($\pm M26$, $\pm Mts1$, $\pm Mts2$, \pm meiosis). The level of *ade6* mRNA was equivalent in all cases (data not shown), in agreement with the previous study (29). We conclude that increased transcription of the *ade6* gene, conferred by Mts1/Mts2 binding the *M26* site, is not the mechanism by which the hotspot is activated. These data, and the different Mts1 and Mts2 protein requirements for hotspot activation (Table 2) and stress response (Fig. 5), suggest that hotspot activation is a distinct function of the Mts1/Mts2 heterodimer.

DISCUSSION

We have cloned the genes, *mts1* and *mts2*, encoding the heterodimeric protein that binds the *M26* recombination hotspot (15). Disruption of either gene, or both, abolishes *M26* hotspot activity (Table 2), as predicted (15). While our genetic studies were underway, two laboratories described *S. pombe* genes identical to *mts1* (called *atf1* and *gad7*) and *mts2* (called *pcr1*), both isolated through different lines of research (16–18). Genetic analyses revealed that Mts1 (Atf1, Gad7) and Mts2 (Pcr1) proteins have roles in sexual development and stress response and the mutants exhibit altered induction patterns of several genes involved in those pathways (16–20). These data suggest a possible relationship between transcription and recombination. However, hotspot activity is not a consequence of increased transcription because there is no detectable difference in the level of *ade6* mRNA during meiosis in strains exhibiting normal (*M375*) vs. hotspot (*M26*) recombination (29) or in cells having or lacking the Mts proteins (data not shown). Furthermore, the factors required for stress response (Fig. 5) and hotspot activation (Table 2) are genetically distinct: Mts2 is dispensable for the stress response (Fig. 5) but not for hotspot activation (Table 2). [This separability has also been inferred for hotspots in *Saccharomyces cerevisiae*: some promoter mutations that alter gene expression do not alter recombination hotspot activity (30, 31)]. The Mts1 and Mts2 proteins acting together or separately therefore have multiple

Table 2. Requirement for Mts1 and Mts2 in *M26* hotspot but not basal meiotic recombination

Line	Relevant genotypes*		<i>ade6</i> alleles crossed and Ade ⁺ recombinant frequency ($\times 10^4$) [†]		Hotspot ratio [‡]	Hotspot ratio vs. wild-type basal [§]
	<i>mts1/mts1</i>	<i>mts2/mts2</i>	<i>M375</i> \times <i>M210</i>	<i>M26</i> \times <i>M210</i>		
1	+/+	+/+	4.4 \pm 0.6	83 \pm 7.6	19	19
2	+/-	+/+	5.6 \pm 1.6	45 \pm 9.5	8.0	10
3	-/-	+/+	5.3 \pm 1.4	5.4 \pm 0.0	1.0	1.2
4	+/+	+/-	7.6 \pm 0.1	27 \pm 0.4	3.6	6.1
5	+/+	-/-	5.3 \pm 0.6	4.9 \pm 0.8	0.9	1.1
6	-/-	-/-	5.7 \pm 1.1	7.0 \pm 1.1	1.2	1.6

*The (+) indicates *mts1*⁺ or *mts2*⁺, the (-) indicates deleted *mts1* (*mts1-D15::ura4*⁺) or deleted *mts2* (*mts2-D1::his3*⁺). In addition to the listed *mts1*, *mts2*, and *ade6* alleles, all strains had the genotype *leu1-32 ura4-D18 his3-D1* (Table 1).

[†]See Fig. 1 for positions of *ade6* alleles. Standard genetic crosses (5, 14, 28) were conducted, and spores were plated on supplemented NBA minimal medium containing adenine (100 μ g/ml) to determine the total viable spore titer (T) and on NBA medium lacking adenine to determine the *ade6*⁺ recombinant titer (R). The recombinant frequency in each experiment is R/T. Each cross was done with two pairs of strains bearing alleles in opposite configurations relative to the mating type alleles. No significant differences were observed, and the data were combined. Data are the mean \pm SD of recombinant frequencies from 2 to 4 experiments.

[‡]Ratio of recombinant frequency from the *M26* \times *M210* (hotspot) cross relative to that from the *M375* \times *M210* (basal recombination) cross.

[§]Ratio of recombinant frequency from the *M26* \times *M210* (hotspot) cross relative to that from the *M375* \times *M210* (basal recombination) cross in wild type (*mts1*⁺ *mts2*⁺).

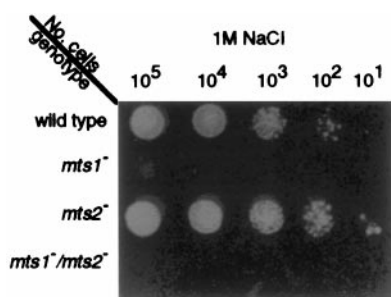


FIG. 5. Sensitivity of *mts*⁻ mutants to osmotic stress. The indicated number of cells from healthy, log-phase cultures were spotted onto supplemented NBA minimal medium containing 1 M NaCl and were incubated for 3 days at 32°C before being photographed.

biological roles: one in hotspot activation, one in regulating genes involved in sexual development, and one in regulating genes during stress conditions. In each case, the proteins are constitutively present (15) but apparently inactive during normal mitotic growth, permitting rapid induction of activity in times of crisis. Activation of the Mts1 protein for transcription involves signal transduction via mitogen-activated protein (MAP) kinase and cAMP-dependent kinase pathways, and perhaps others (Fig. 2) (16–20). This allows the cells to rapidly respond to environmental changes without requiring further protein synthesis. The posttranslational modifications of Mts1 and Mts2 that trigger the decision toward meiotic development (16–20) may overlap with those changes that result in meiotic hotspot activation.

Mts1/Mts2 heterodimer binding the *M26* site is required for hotspot activation. Although both Mts1 and Mts2 are involved in transcriptional regulation (16, 18, 20), the relevant cis-acting DNA sites have not been defined. Nor is it known whether Mts1 and Mts2 act together, or as homodimers, or as heterodimers with other bZIP proteins when regulating transcription. We expect that different homodimers and heterodimers have different related binding sites and different biological functions (Fig. 2). The use of the same factors for two different processes during meiosis, developmental gene regulation and *M26* recombination hotspot activation, provides a good example of the economy of nature.

That some transcription factors may activate recombination hotspots has also been inferred from studies with *S. cerevisiae* (32–34). For example, full activity of the *HIS4* hotspot requires elements of the promoter region that include binding sites for Bas1, Bas2, and Rap1 transcription factors (32, 35). A deletion that includes all the binding sites for these transcription factors abolishes hotspot activity, and replacement with Rap1 binding sites restores high hotspot activity, suggesting that Rap1 protein binding can activate the hotspot (32, 35, 36). A direct demonstration that Rap1 is required for hotspot activation has not been possible however, because the Rap1 protein is essential for viability. Similarly, a direct demonstration of a discrete nucleotide sequence defining the DNA site(s) has not been reported for the natural *HIS4* hotspot, but has been for the *M26* hotspot (14). The Rap1 protein, like Mts1/Mts2, is a multifunctional transcription factor that is constitutively expressed in mitotic and meiotic cells. Thus, for two hotspots in two different organisms, specific transcription factors have additional roles in meiotic hotspot activation. For other hotspots no single, discrete activating factors have yet been identified, but this could be caused by redundancy of sites clustered at hotspots or limitations of the experimental approaches used (11).

A current model for hotspot activation (10, 11, 34) proposes that the DNA within “open chromatin” becomes accessible to meiotic recombination enzymes that introduce a double-strand DNA break (37, 38). Alternatively, one or a few

components that are usually associated with open chromatin, rather than open chromatin itself, enhance recombination (39). Because Mts1/Mts2 protein binding to *M26* is required to activate the hotspot, the protein likely remodels chromatin, or recruits recombination enzymes, or both. Meiotically induced, *M26* site-dependent open chromatin is found at the *M26* hotspot (40) and is entirely dependent on the presence of the Mts1/Mts2 heterodimer (K. Ohta and W.P.W., unpublished observations). [The similarity to “architectural transcription factors” (41) and chromatin remodeling enzymes involved in gene regulation (42, 43) is striking. Perhaps some factors of those processes are also components of *M26* hotspot activation.] The current evidence is consistent with open chromatin being either the cause or the effect of hotspot activation: Mts1/Mts2 might create meiotically altered chromatin that serves as an assembly point for meiotic recombination enzymes, or the chromatin alteration might simply be a consequence of Mts1/Mts2 activating recombination while bound to *M26*. Because the Mts proteins are also transcription factors (16–20), and hotspot activation is not the result of increased transcription (29) (data not shown), we infer that the Mts1/Mts2 heterodimer has separate roles in transcriptional regulation and hotspot activation. It remains to be seen whether Mts1/Mts2 directly recruits recombination enzymes via protein–protein interactions, or whether recombination enzymes assemble at chromatin conformationally altered by Mts1/Mts2 binding to *M26* DNA, or both. In either case, binding of Mts1/Mts2 to *M26* sites increases recombination above basal levels, indicating that the hotspot with its binding protein regulates where and when recombination occurs within the genome.

Recombination serves a surveillance role in mitotic cells (to repair certain types of DNA damage) and recombination rates are elevated during meiosis, presumably to increase genetic diversity or to insure homolog disjunction or both. Part of the meiotic enhancement is caused by induction of meiotic recombination genes (27) and part may be because of Mts1/Mts2 protein binding to *M26* sites throughout the genome (15). Intergenic recombination in *mts* mutants is reduced as much as 50% in four intervals tested so far (N.K. and W.P.W., unpublished observations), and each of eight *M26* sites created by mutagenesis (involving one or a few base pair changes) at five sites in two widely separated loci has hotspot activity (44). These findings support the view that some natural *M26* sites are active in meiosis and may contribute to elevated meiotic recombination levels.

These findings permit a speculative calculation of the fraction of meiotic recombination attributable to Mts1/Mts2 heterodimer interacting with *M26* sites. There are an estimated 600 *M26* sites in the diploid *S. pombe* genome (15). If each *M26* site is, on average, as recombinogenic as *ade6-M26* and each promotes recombination in 5% of meioses [the level of gene conversion at *ade6-M26* (5)], then ≈30 conversion events per meiosis would be due to *M26* sites. About 65% of the conversion events of *ade6-M26* are accompanied by reciprocal exchange (28). Thus, ≈20 reciprocal exchange events per meiosis could be because of *M26* sites. Because the *S. pombe* meiotic map size is ≈2,200 cM (≈44 reciprocal exchanges) (45), the predicted 600 *M26* sites in the diploid genome could be responsible for ≈50% of meiotic homologous recombination. However, because no study has yet reported whether or not any naturally occurring *M26* sites are recombinogenic, this hypothesis remains unproven. The availability of *mts* mutants that abolish *ade6-M26* hotspot activity will be useful for determining what fraction of genomic *M26* sites are active.

We thank Charlie Albright, Joe Farah, Mary Fox, Ramsay McFarlane, Joachim Ostermann, Philippe Szankasi, Andrew Taylor, Tony Weil and Ron Wisdom for constructive comments and Steve Lindsey and Calley Hardin for laboratory assistance. Cloning of *mts1* was

supported by the Damon Runyon–Walter Winchell Foundation (W.P.W.) and the National Institutes of Health (G.R.S.), the remainder was supported by the National Institutes of Health and the Vanderbilt University Department of Biochemistry (W.P.W.). W.P.W. is a Leukemia Society of America Special Fellow.

1. Mosig, G. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 1177–1183.
2. Wahls, W. P., Wallace, L. J. & Moore, P. D. (1990) *Cell* **60**, 95–103.
3. Wahls, W. P., Wallace, L. J. & Moore, P. D. (1990) *Mol. Cell. Biol.* **10**, 785–793.
4. Voelkel-Meiman, K., Keil, R. L. & Roeder, G. S. (1987) *Cell* **48**, 1071–1079.
5. Gutz, H. (1971) *Genetics* **69**, 331–337.
6. Schuchert, P. & Kohli, J. (1988) *Genetics* **119**, 507–515.
7. Ponticelli, A. S., Sena, E. P. & Smith, G. R. (1988) *Genetics* **119**, 491–497.
8. Treco, D. & Arnheim, N. (1986) *Mol. Cell. Biol.* **6**, 3934–3947.
9. Smith, G. R. (1991) *Cell* **64**, 19–27.
10. Lichten, M. & Goldman, A. S. H. (1995) *Annu. Rev. Genet.* **29**, 423–444.
11. Wahls, W. P. (1998) in *Meiosis and Gametogenesis*, ed. Handel, M. A. (Academic, San Diego), pp. 37–76.
12. Kowalczykowski, S. C., Dixon, D. A., Eggleston, A. K., Lauder, S. D. & Rehrauer, W. M. (1994) *Microbiol. Rev.* **58**, 401–465.
13. Szankasi, P., Heyer, W. D., Schuchert, P. & Kohli, J. (1988) *J. Mol. Biol.* **204**, 917–925.
14. Schuchert, P., Langsford, M., Kaslin, E. & Kohli, J. (1991) *EMBO J.* **10**, 2157–2163.
15. Wahls, W. P. & Smith, G. R. (1994) *Genes Dev.* **8**, 1693–1702.
16. Takeda, T., Toda, T., Kominami, K., Kohnosu, A., Yanagida, M. & Jones, N. (1995) *EMBO J.* **14**, 6193–6208.
17. Kanoh, J., Watanabe, Y., Ohsugi, M., Iino, Y. & Yamamoto, M. (1996) *Genes Cells* **1**, 391–408.
18. Watanabe, Y. & Yamamoto, M. (1996) *Mol. Cell. Biol.* **16**, 704–711.
19. Shiozaki, K. & Russell, P. (1996) *Genes Dev.* **10**, 2276–2228.
20. Wilkinson, M. G., Samuels, M., Takeda, T., Toone, W. M., Shieh, J.-C., Toda, T., Millar, J. B. A. & Jones, N. (1996) *Genes Dev.* **10**, 2289–2301.
21. Kohli, J. (1987) *Curr. Genet.* **11**, 575–589.
22. Gutz, H., Heslot, H., Leupold, U. & Loprieno, N. (1974) in *Schizosaccharomyces pombe*, ed. King, R. C. (Plenum, New York), Vol. 1, pp. 395–446.
23. Ponticelli, A. S. & Smith, G. R. (1989) *Genetics* **123**, 45–54.
24. Compton, T. (1990) in *Degenerate Primers for DNA Amplification*, eds. Innis, M. A., Gelfand, D. H., Sninsky, J. J. & White, T. J. (Academic, San Diego), pp. 39–45.
25. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
26. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY).
27. Li, Y. F., Numata, M., Wahls, W. P. & Smith, G. R. (1997) *Mol. Microbiol.* **23**, 869–878.
28. Grimm, C., Bahler, J. & Kohli, J. (1994) *Genetics* **136**, 41–51.
29. Grimm, C., Schaer, P., Munz, P. & Kohli, J. (1991) *Mol. Cell. Biol.* **11**, 289–298.
30. Schultes, N. P. & Szostak, J. W. (1991) *Mol. Cell. Biol.* **11**, 322–328.
31. White, M. A., Detloff, P., Strand, M. & Petes, T. D. (1992) *Curr. Genet.* **21**, 109–116.
32. White, M. A., Dominska, M. & Petes, T. D. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6621–6625.
33. de Massy, B., Baudat, F. & Nicolas, A. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 11929–11933.
34. Wu, T. C. & Lichten, M. (1994) *Science* **263**, 515–518.
35. White, M. A., Wierdl, M., Detloff, P. & Petes, T. D. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9755–9759.
36. Fan, Q., Xu, F. & Petes, T. D. (1995) *Mol. Cell. Biol.* **15**, 1679–1688.
37. Keeney, S., Giroux, C. N. & Kleckner, N. (1997) *Cell* **88**, 375–384.
38. Bergerat, A., de Massy, B., Gabelle, D., Varoutas, P.-C., Nicolas, A. & Forterre, P. (1997) *Nature (London)* **386**, 414–417.
39. Fan, Q. Q. & Petes, T. D. (1996) *Mol. Cell. Biol.* **16**, 2037–2043.
40. Mizuno, K., Emura, Y., Baur, M., Kohli, J., Ohta, K. & Shibata, T. (1997) *Genes Dev.* **11**, 876–886.
41. Werner, M. H. & Burley, S. K. (1997) *Cell* **88**, 733–736.
42. Pazin, M. J. & Kadonaga, J. T. (1997) *Cell* **88**, 737–740.
43. Pazin, M. J. & Kadonaga, J. T. (1997) *Cell* **89**, 325–328.
44. Fox, M. E., Virgin, J. B., Metzger, J. & Smith, G. R. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 7446–7451.
45. Munz, P. (1994) *Genetics* **137**, 701–707.