A WD Repeat Protein, Rec14, Essential for Meiotic Recombination in *Schizosaccharomyces pombe*

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ABSTRACT

Mutations in the *Schizosaccharomyces pombe rec14* gene reduce meiotic recombination by as much as a factor of 1000 in the three intervals tested on chromosomes I and III. A DNA clone complementing the *rec14* mutation was shown by genetic and physical analysis to contain the *rec14* gene, which was functional in plasmid-borne inserts as small as 1.4 kb. The *rec14* gene contains two exons separated by a 53-bp intron, which was confirmed by analysis of *rec14* transcripts. The spliced transcript encodes a protein product of 302 amino acids, which contains six WD repeat motifs found in the G-beta transducin family of proteins and other proteins, including the *Saccharomyces cerevisiae* Ski8 (Rec103) protein. Although the *rec14* transcripts were present in mitotically dividing cells, *rec14* mutations had no detectable effect on mitotic recombination. The pattern of expression of *rec14* differs from that of previously analyzed *S. pombe rec* genes. Based upon mutant phenotypes and amino acid sequence similarities, we propose that *S. pombe* Rec14 is a functional homologue of *S. cerevisiae* Rec103.

DURING meiosis homologous recombination occurs at high frequency to aid pairing and proper disjunction of homologues into the haploid gametes and to increase genetic diversity among progeny formed by these gametes. Meiotic recombination is a complex process requiring multiple gene products. The elevated level of meiotic recombination, relative to that in mitotically dividing cells, likely results from the elevated level of expression of some of these genes. Elucidating these genes and determining their pattern of expression are key to understanding the mechanism and control of recombination.

Meiotic recombination-deficient mutants of *Schizosaccharomyces pombe* have been isolated and placed into 17 complementation groups (PONTICELLI and SMITH 1989; DEVEAUX *et al.* 1992). Based upon the reduction of intragenic *ade6* recombination, the *rec* mutations were grouped into class I (reduction by a factor of ~1000; *rec6, 7, 8, 12, 14,* and *15*), class II (reduction by a factor of ~100; *rec10, 11,* and *16*), and class III (reduction by a factor of three to 10; *rec9, 13, 17–21,* and *swi5*). Among the mutants tested (*rec6, 7, 8, 10, 11,* and *15*) some manifest a regional specificity: mutations in the *rec8, 10,* and *11* genes reduce recombination more dramatically on chromosome III than on either of the other two chromosomes (DEVEAUX and SMITH 1994; LIN and SMITH 1995a). The *rec* genes cloned and analyzed to date (*rec6, 7, 8, 10, 11 12, 15,* and *16*) are induced early in meiosis; transcripts are barely detectable or undetectable in mitotically dividing cells, and their abundance is maximal at 2 or 3 hr after induction of meiosis after which they are extensively degraded (LIN *et al.* 1992; LIN and SMITH 1994, 1995a,b). Meiotic DNA synthesis also occurs at 2–3 hr, and meiosis is completed in 6–8 hr under the conditions used (SZANKASI and SMITH 1992; LI and SMITH 1997; R. DING and G. R. SMITH, unpublished data). These observations indicate that the products of the *S. pombe rec* genes analyzed to date are induced transiently and specifically during meiosis. As expected from these observations, mutations in these *rec* genes have no detectable mitotic phenotype, such as sensitivity to DNA damaging agents or recombination-deficiency (DEVEAUX *et al.* 1992). We have cloned and analyzed the *S. pombe rec14* gene and found that its pattern of expression differs from that of the previously analyzed *rec* genes.

MATERIALS AND METHODS

Strains, culture media, and genetic methods: The *S. pombe* strains used are listed in Table 1. Culture media have been described previously (GUTZ *et al.* 1974; PONTICELLI and SMITH 1989). YEA and YEL are rich media; NBA, MMA, and EMM2* are minimal media; SPA is a solid sporulation medium. Minimal media and SPA were supplemented with required nutrients at 100 μg/ml. *S. pombe* was transformed using the LiOAc method (ITO *et al.* 1983), and *Escherichia coli* by electroporation (CALVIN and HANAWALT 1988). For the experiments in Table 4, 1 μg of each plasmid DNA was added to 10^8 cells, and the entire mixture was plated for transformants. Meiotic crosses were conducted and analyzed as described previously

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TABLE 1

***S. pombe* strains**

Strain	Genotype	Source[a] or reference
GP13	*h⁻ ade6-52*	PONTICELLI and SMITH (1989)
GP24	*h⁺ ade6-M26*	PONTICELLI *et al.* (1988)
GP32	*h⁺ ade6-M26 leu1-32*	LIN *et al.* (1992)
GP33	*h⁺ ade6-M26 ura4-294*	A. PONTICELLI[b]
GP331	*h⁻ ade6-M26 leu1-32 arg1-2 pat1-114 end1-458*	A. PONTICELLI[b]
GP363	*h⁺ ade6-M26 ura4-294 arg3-124*	PONTICELLI and SMITH (1989)
GP369	*h⁻ ade6-52 ura4-595 pro2-1*	PONTICELLI and SMITH (1989)
GP499	*h⁻ ade6-52 rec14-120*	DEVEAUX *et al.* (1992)
GP502	*h⁺ ade6-M26 ura4-294 arg3-124 rec14-120*	DEVEAUX *et al.* (1992)
GP505	*h⁻ ade6-52 ura4-595 pro2-1 rec14-120*	DEVEAUX *et al.* (1992)
GP599	*h⁺ ade6-M26 rec14-120*	DEVEAUX *et al.* (1992)
GP625	*h⁻ ade6-52 pat1-114 end1-458*	LIN and SMITH (1994)
GP720	*h⁻ ade6-52 ura4-294 leu1-32*	LIN *et al.* (1992)
GP879	*h⁻ ade6-52 ura1-171*	DEVEAUX and SMITH (1994)
GP930	*h⁺ ade-6M26 lys3-37 pro1-1*	DEVEAUX and SMITH (1994)
GP1163	*h⁻ ade6-52 leu1-32 his5-303*	This study[b]
GP1240	*h⁻ ade6-52 lys4-95 ura4-D18 leu1-32*	P. SZANKASI[b]
GP1327	*h⁹⁰ ade6-52 leu1-32 ura4-294*	LIN and SMITH (1995b)
GP1386	*h⁹⁰ ade6-M26 ura4-294 leu1-32*	Y. LIN[b]
GP1415	*h⁹⁰ ade6-M26 ura4-294 leu1-32* (pYL84)	LIN and SMITH (1995b)
GP1640	*h⁺ ade6-M26 ura1-171 rec14-120*	GP879 × GP599
GP1649	*h⁻ ade6-52 lys3-37 pro1-1 rec14-120*	GP930 × GP499
GP1664	*h⁻ ade6-52 lys3-37 pro1-1 ura4-294 rec14-120*	GP33 × GP1649
GP1706	*h⁹⁰ ade6-M26 ura4-294 leu1-32 rec14-120*	GP1327 × GP599
GP1708	*h⁹⁰ ade6-M26 ura4-294 leu1-32 rec14-120* (pYL84)	T of GP1706
GP1774	*h⁹⁰ ade6-M26 ura4-294 leu1-32 rec14-120* (pYL84) (pYFL101)	T of GP1708
GP1993	*h⁺ ade6-M26 leu1-32 rec14-161::LEU2*	T[c] of GP32
GP1994	*h⁺ ade6-M26 leu1-32 rec14-161::LEU2*	T[c] of GP32
GP2003	*h⁻ ade6-52 leu1-32 rec14-161::LEU2*	GP1993 × GP1163
GP2004	*h⁻ ade6-52 leu1-32 rec14-161::LEU2*	GP1994 × GP1163
GP2007	*h⁻ ade6-52 leu1-32 rec14-161::LEU2*	GP1993 × GP1240
GP2010	*h⁻ ade6-52 leu1-32 rec14-161::LEU2*	GP1994 × GP1240
GP2173	*h⁺ ade6-M26 ura4-294 leu1-32 rec14-161::LEU2*	GP720 × GP1994
GP2183	*h⁺ ade6-M26 ura4-294 leu1-32*	GP720 × GP1994

[a] ×, meiotic cross; T, transformation.
[b] Genealogies are available upon request.
[c] Transforming DNA was the 5.7-kb *Hin*dIII-*Bam*HI fragment of pDE13.

(PONTICELLI and SMITH 1989). Mitotic recombination was measured in five independent cultures grown in 5 ml of appropriately supplemented NBL to ~1 × 10^7 cells/ml. Ade⁺ recombinant frequencies were determined by differential plating of meiotic spores or mitotic cells on YEA and YEA plus guanine (200 μg/ml), which inhibits uptake of adenine (CUMMINS and MITCHISON 1967), or on NBA with and without adenine. These two methods gave indistinguishable results.

***S. pombe* genomic library:** The library, provided by LOUISE CLARK and JOHN CARBON (University of California at Santa Barbara) and constructed by ELLIOTT *et al.* (1986), contains ~10-kb partial *Sau*3AI digestion products inserted into the *Bam*HI site of plasmid pFL20 (LOSSON and LACROUTE 1983).

Screen for *rec14* clones: The method was similar to that described by LIN and SMITH (1995b) but used strain GP1708 [*h⁹⁰ ade6-M26 ura4-294 leu1-32 rec14-120* (pYL84)] as the host for plasmids to be screened. Plasmid pYL84 (LIN and SMITH 1995b) contains the *Saccharomyces cerevisiae LEU2* gene, which complements *S. pombe leu1* mutations (BEACH and NURSE 1981), and the *S. pombe ade6* gene bearing the *ade6-469* mutation, which recombines at high frequency with the chromosomal *ade6-M26* allele in *rec⁺*, but not *rec14* mutant, strains (GUTZ 1971; DEVEAUX *et al.* 1992). The *ura4-294* allele of strain GP1708 is complemented by the *S. cerevisiae URA3* gene (GRIMM *et al.* 1988) contained in plasmid pFL20 (LOSSON and LACROUTE 1983), the vector of the *S. pombe* library used. Ura⁺ transformants of strain GP1708 were selected on EMM2* plus adenine solid medium, on which homothallic (*h⁹⁰*) cells grow and switch their mating type. After ~6 days at 30°, cells in the colonies had depleted the nutrients, mated and sporulated. The colonies (~10,000 on 25 plates) were replicated to NBA lacking adenine and incubated for 2 days at 32° to identify clones that produced Ade⁺ recombinants at high frequency. The *rec⁺* control strain GP1415 produced ~20 Ade⁺ papillae per colony, and the *rec14-120* parental strain GP1708 produced one or none. Among the ~10,000 Ura⁺ transformants tested 10 produced Ade⁺ recombinants at high frequency. Ade⁻ cells from these clones were recovered from the initial EMM2* plus adenine plate, purified on NBA plus limiting adenine (10 μg/ml), on which Ade⁻ clones make red colonies, and retested for production of Ade⁺ recombinants. One, strain GP1774, produced a consistently high frequency

of Ade$^+$ recombinants. From it plasmid pYFL101 was recovered by transformation of *E. coli* strain DH5α [*supE44 ΔlacU169 (ϕ80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1*] to Amp^R. Transformation of *S. pombe* strain GP502 (*h$^+$ ade6-M26 ura4-294 rec14-120*) to Ura$^+$ and mating to strain GP499 (*h$^-$ ade6-52 rec14-120*) showed that pYFL101 complemented the *rec14-120* mutation for *ade6* intragenic recombination (see RESULTS and Table 3).

Gene subclones and exonuclease III (ExoIII) deletions: Standard methods for DNA manipulations were used (SAMBROOK *et al.* 1989). Noncomplementary DNA ends were ligated after filling in the ends using the Klenow fragment of DNA polymerase I. Plasmid pYFL101 was digested with *Hpa*I and *Eag*I, which cuts in the ColE1 *ori* region of the pFL20 vector, and was ligated to remove a 3.6-kb fragment, to yield pDE2. pYFL101 was digested with *Nhe*I, which cuts near one end of the *S. pombe rec14* insert and in the *tet* gene of the pFL20 vector, to produce a 4.1-kb fragment, 4.0 kb of which is *S. pombe* DNA; this fragment was ligated with pSP2 (COTTAREL *et al.* 1993) cut with *Spe*I, to yield pDE3. Using an Erase-a-Base Kit (Promega), ExoIII-generated deletions of pDE3 were obtained from one side after digestion with *Sal*I + *Eag*I and from the other side after digestion with *Kpn*I + *Bam*HI; plasmids with these deletions include pDE8, 9, 10, 12, and 15.

Insertion of *LEU2* into *rec14*: The 2.9-kb *Bgl*II fragment containing the *S. cerevisiae LEU2* gene of YEp13 (BROACH *et al.* 1979) was ligated into the unique *Bst*XI site of pDE12. Leu$^+$ transformants of *E. coli* strain C600 (*leuB6 thr-1 thi-1 lacY1 tonA21 supE44* λ^- F$^-$) were selected; the *LEU2* gene complements *leuB6*. From the recovered plasmid, pDE13, a 5.7-kb *Hin*dIII-*Bam*HI fragment was isolated and used to transform *S. pombe* strain GP32 (*leu1-32 rec$^+$*) to Leu$^+$. After purification, stable Leu$^+$ transformants were tested for complementation of the *rec14-120* mutation (see RESULTS and Table 3) and for homologous insertion of the *LEU2* fragment into the chromosomal locus carried by pDE12. DNA extracted from four transformants, including strains GP1993 and GP1994, was digested with *Dra*I or *Dra*I + *Kpn*I, and analyzed by electrophoresis and Southern blot hybridization. The probe was the 0.9-kb *Xho*I-*Nde*I fragment of pDE14 spanning the intronless *rec14* open reading frame (ORF) (see below). Upon digestion with *Dra*I the parental DNA produced a 1.3-kb fragment, and the four transformant DNAs produced only 4.2-kb fragments; upon digestion with *Dra*I + *Kpn*I, the parental DNA produced a 1.3-kb fragment, and the transformants produced only 1.5- and 2.7-kb fragments, as expected for a homologous replacement (data not shown).

Southern and Northern blot hybridizations: Southern hybridization procedures and the preparation of nucleic acids have been described (LIN *et al.* 1992; LIN and SMITH 1994). For the analysis of transcripts by Northern hybridization, RNA was extracted from strain GP625 (*pat1-114*) at 0.25, 2, 3, 4, and 5 hr after raising the temperature of an EMM2* liquid culture from ~20° to 34° and from strain GP331 (*pat1-114*) growing in EMM2* at 25°. RNA (10 μg per lane) was fractionated by electrophoresis through a 1% agarose gel in 20 mM MOPS (pH 7), 5 mM NaOAc, 1 mM EDTA, 18% formaldehyde and transferred to a Hybond-N membrane (Amersham). The RNA was crosslinked to the membrane by UV irradiation (125 mJ/cm^2) using a BioRad GS Gene Linker and hybridized to radioactive probe in a buffer of 0.5 M Na-PO$_4$ (pH 7.2), 2 mM EDTA, 7% SDS, and 1% bovine serum albumin. The probe for *rec14* was the 0.9-kb *Xho*I-*Nde*I fragment of pDE14 encompassing the intronless *rec14* ORF. The probes for *rec10* and *cyc1* were described by LIN and SMITH (1995b). Equal loading and transfer of rRNAs were confirmed by staining the gel with ethidium bromide and the membrane with methylene blue (data not shown). After hybridization at 62° for ~18 hr the membrane was washed at 62° for 30 min in 1× SSC + 0.1% SDS, dried, and analyzed on a PhosphorImager (Molecular Dynamics).

Nucleotide sequence determination and analysis: The nucleotide sequence of 3.0 kb of the ~4.4 kb *S. pombe rec14* insert in pDE3 was determined on both strands, using pDE3 and its ExoIII-generated deletion derivatives as templates and Applied Biosystems Dye-Terminator cycle sequencing reagents. Reactions used universal T3 and T7 primers and followed a protocol from Perkin-Elmer. Sequence analysis used the Sequencher 3.0 program (Gene Codes Corp.). Structural motifs were identified using the block search program (HENIKOFF and HENIKOFF 1994) based on Prosite 13 and Swiss-Prot 32 databases.

Determination of transcript 5′ ends and splicing junctions: A PCR-based method was used to place limits on the 5′ end of the *rec14* transcripts. One set of PCR reactions used plasmid pDE12 DNA as template, and the other used meiotically induced RNA (isolated from strain GP625 at 3 hr after induction), which was reverse transcribed before the PCR. RNA (100 μg) was digested with RQ1 RNase-free DNase (Promega) according to the supplier's protocol, extracted with phenol-CHCl$_3$, and precipitated with ethanol. The RNA plus 10 ng of primer V [5′CTCGAGTTCTGTAGCAGCAGCTC3′, complementary to nucleotides (nt) 2853–2837 in Figure 2 near the *rec14* translational stop] was dissolved in 0.25 ml of RT buffer (BRL), 10 mM DTT, and 0.5 mM each dNTP and incubated at 90° for 2 min and then at 37° for 10 min. M-MuLV reverse transcriptase (RT; 2000 units; BRL) was added, and the incubation continued for 1 hr. The mixture was extracted with phenol, and the nucleic acids were precipitated with ethanol and dissolved in 100 μl of H$_2$O.

The PCR was performed using primer I (5′TACGCCCGTC TACAGATGTG3′), which is complementary to nt 2326–2307 of the transcript to the right (3′ side) of the intron (Figure 2), in combination with primer II (5′ATTATAAAGTATTGG AAAGC3′; nt 1820–1839), III (5′CTCACCTTCTCATTTCTA CC3′; nt 1659–1678), or IV (5′AAACCTACTATTGCCAAAA G3′; nt 1597–1616), which are equivalent to the left (5′ side) of the intron. Each reaction contained 5 μl of RT reaction products or 5 ng of plasmid pDE12 DNA, 50 pmol of each primer, and 1.0 unit of *Taq* DNA polymerase (Promega) in 50 μl of *Taq* buffer containing 1.5 mM MgCl$_2$ (Boehringer). The reactions were subjected to 30 cycles of 95° for 30 sec, 55° for 30 sec, and 72° for 30 sec in a Perkin-Elmer Geneamp 2400 thermocycler. The products were analyzed by gel electrophoresis.

To determine the splicing junctions, RNA isolated from strain GP625 at 3 and 4 hr after meiotic induction was reverse transcribed and amplified by a PCR as described above, but using primers V and VI (5′TATTAGATACTATGAGGAAAGA GTATC3′; nt 1884–1910 in Figure 2) and 45° instead of 55° in the thermal cycles. The reaction products were fractionated by electrophoresis through an agarose gel. The shorter product (~950 bp) from the RNA-templated reaction was purified using glass milk (Bio101), and 100 ng was used for automated sequencing using primer VII (5′TTGCGGATTTGGACAGGA TG3′; nt 2122–2141 in Figure 2). A control reaction used 10 ng of plasmid pDE12 DNA as a template for the PCR.

Construction of an intronless *rec14* gene: RNA extracted from strain GP625 at 3 hr after induction was used as a template for an RT-PCR reaction with primers V and VI, as described above. The 0.9-kb product was ligated into pCRII (Invitrogen) to generate pDE14.

TABLE 2

Reduction of recombination on chromosomes I and III by a *rec14* mutation

		Recombinant frequency[a]				
rec	Experiment	*ade6-M26–ade6-52* (Ade$^+$/10^6)	*lys3–ura1* (%)	*ura1–pro1* (%)	*lys3–pro1* (%)	No. of spore colonies tested
+	1	2600	22 (50)	18 (40)	25 (57)	224
+	2	3300	25 (25)	34 (34)	43 (43)	100
14-120	1	<20	1 (1)	<1 (0)	1 (1)	112
14-120	2	5	2 (4)[b]	3 (6)[b]	1 (2)	185

[a] Intragenic *ade6* recombinant frequency was determined by plating spores on YEA for total viable spores and on supplemented NBA lacking adenine for Ade$^+$ spores; frequencies are Ade$^+$ spores per 10^6 viable spores. Intergenic *lys3-ura1-pro1* recombinant frequencies were determined by picking spore colonies from YEA plates to grids on YEA and replicating to appropriately supplemented NBA plates; frequencies are the fraction of recombinant types (observed numbers in parentheses) among the total tested. Strains for the *rec*$^+$ crosses were GP879 and GP930; strains for the *rec14* crosses were GP1640 and GP1649. LIN and SMITH (1995a) reported the *rec*$^+$ data for experiment 1, which was done concurrently with the *rec14* cross of experiment 1.

[b] Four of these were apparent double-exchange recombinants (Lys$^+$ Ura$^+$ Pro$^+$). One was apparently a diploid, as it sporulated (I_2-positive) on malt extract agar and segregated prototrophs during mitotic growth in *m*-fluorophenylalanine (KOHLI *et al.* 1977); the other three may be diploids homozygous at *mat* and hence nonsporulating.

RESULTS

Reduction of recombination by a *rec14* mutation: To determine whether the *rec14* gene product controls recombination in a region-specific manner, as do the *rec8, 10,* and *11* gene products (DEVEAUX and SMITH 1994), we tested intragenic recombination at the *ade6* locus on chromosome III and intergenic recombination in the linked *lys3-ura1-pro1* intervals on chromosome I. The *rec14-120* mutation strongly reduced recombination in the four intervals tested. At *ade6* the reduction was ~1000-fold (Tables 2 and 3), as reported previously (DEVEAUX *et al.* 1992). In the *lys3-ura1-pro1* intervals the reduction was >10-fold (Table 2). For the largest interval, *lys3-pro1,* the *rec*$^+$ mating gave 100/324 (31%) recombinants, but the *rec14* mating only 3/297 (1%). These data demonstrate that the *rec14* gene product does not manifest the regional specificity of the *rec8, 10,* and *11* gene products.

Viable spore yields in *rec14* mutants: The requirement for meiotic recombination for proper chromosome segregation predicts that a strongly recombination deficient mutant, such as *rec14,* should produce few spores with a haploid set of chromosomes. The three *S. pombe* chromosomes would be expected to segregate properly in 2^{-3} (=12.5%) of *rec14* meioses. Since *S. pombe* aneuploids are dead or poor-growing (NIWA and YANAGIDA 1985), only true haploids should be viable. In 18 independent *rec14* mutant crosses conducted on 5 days the viable spore yield in standard matings (PONTICELLI and SMITH 1989) was 15.6% (±3.7% SE) of that in side-by-side *rec*$^+$ matings. This level of viable spore yield is consistent with random chromosome segregation in *rec14* mutants.

Cloning of the *rec14* gene: We used a screening procedure (LIN and SMITH 1995b) to isolate a plasmid that complemented the *rec14-120* allele. As detailed in MATERIALS AND METHODS, strain GP1708 [*h*^{90} *ade6-M26 leu1-32 ura4-294 rec14-120* (pYL84)] was transformed to Ura$^+$ with plasmids from an *S. pombe* genomic library (ELLIOTT *et al.* 1986). Individual transformants were allowed to grow and self-mate, and were screened for recombination between the chromosomal *ade6-M26* mutation and the *ade6-469* mutation on plasmid pYL84. Among ~10,000 transformants tested, one produced Ade$^+$ recombinants at a frequency comparable to that of an isogenic *rec*$^+$ strain, GP1415. From this transformant we recovered plasmid pYFL101, which upon introduction into strain GP502 (*h*$^+$ *ade6-M26 ura4-294 rec14-120*) complemented the *rec14* mutation in heterothallic meiotic crosses (Table 3, line 6). We show below that this plasmid contains the *rec14* gene.

Location of the *rec14* gene in pYFL101: Plasmid pYFL101 contains an ~4.4-kb partial *Sau*3AI digestion product of *S. pombe* DNA (data not shown). To locate the *rec14* gene within this insert, we determined the minimal complementing region in subclones derived by deletion of restriction fragments from pYFL101 and by ExoIII-generated deletions of pDE3, a derivative of pSP2 (COTTAREL *et al.* 1993) containing ~4 kb of the *S. pombe* DNA from pYFL101. Deletions generated by ExoIII treatment of pDE3 complemented *rec14-120,* if they extended up to 2.6 kb from the left (plasmid pDE8; Figure 1; Table 3, line 8) but not if they extended farther from the left (plasmid pDE10; Table 3, line 9) or if they extended 250 bp from the right (plasmid pDE15; Table 3, line 10). These results confine the *rec14* gene to 1.4 kb near the right end of the *S. pombe* insert in pYFL101 (Figure 1).

Apparent mitotic lethality of certain *rec14* plasmids: We noted that two *rec14* plasmids with ~1.2 or 1.3 kb of DNA from the right end of the *S. pombe* DNA insert in pYFL101 failed, in six experiments, to trans-

TABLE 3

Genetic complementation by plasmids with the *rec14* gene and by *rec14* disruptions

Line	*ade6-M26* parent			*ade6-52* parent		Ade$^+$ recombinants per 10^6 viable spores[b]
	GP no.[a]	*rec*	Plasmid	GP no.[a]	*rec*	
1	24	+	—[c]	13	+	4000 (2700)[d]
2	363	+	—	499	*14-120*	2000, 2800 (4600)[d]
3	502	*14-120*	—	499	*14-120*	<100, <100, <160 (2)[e]
4	502	*14-120*	pFL20	499	*14-120*	59, 11
5	502	*14-120*	pSP2	499	*14-120*	<32, <32
6	502	*14-120*	pYFL101	499	*14-120*	1300, 1600, 6200, 6300 (4300, 3800)[f]
7	502	*14-120*	pDE3	499	*14-120*	5500, 7000, 7400, 9800 (4700, 5000)[f]
8	502	*14-120*	pDE8	499	*14-120*	7900, 6100
9	502	*14-120*	pDE10	499	*14-120*	<360, <80
10	502	*14-120*	pDE15	499	*14-120*	<160, <70
11	32	+	—	2004	*14-161::LEU2*	2800 (3700)[g]
12	599	*14-120*	—	2003	*14-161::LEU2*	<2 (1, 3, <1)[h]
13	1993	*14-161::LEU2*	—	499	*14-120*	<24 (<8)[i]
14	1994	*14-161::LEU2*	—	2004	*14-161::LEU2*	1, 2 (<4, <7, <2)[j]

[a] For plasmid-containing strains, the GP number is that of the parent strain.

[b] Strains were mated and recombinant frequencies determined as described in MATERIALS AND METHODS. Data from independent experiments are given. When 0 recombinants were observed, values of $<x$ are the 95% confidence limit from the Poisson distribution (assuming three recombinants in the sample plated).

[c] —, no plasmid.

[d] Values in parentheses were from crosses with GP32 as the *ade6-M26* parent.

[e] Value in parentheses was from a cross with GP599 in place of GP502.

[f] Values in parentheses were from crosses with GP1644 in place of GP499.

[g] Value in parentheses was from a cross with GP2007 in place of GP2004.

[h] Values in parentheses were from crosses with GP2004, GP2007, or GP2010 in place of GP2003.

[i] Value in parentheses was from a cross with GP1994 in place of GP1993.

[j] Values in parentheses were from crosses between GP1993 or GP1994 and GP2003, GP2007, or GP2010.

form *S. pombe* strain GP502 to Ura$^+$, although other *rec14* plasmids used concurrently did transform efficiently (data not shown). One nontransforming plasmid, pDE2, was generated by deleting from pYFL101 the 3.6-kb *Eag*I-*Hpa*I fragment, which removed the left end of the *rec14* insert (Figure 1). The other, pDE9, was generated by ExoIII deletion of 2.3 kb from the left end of the insert in pDE3. As a direct test of the inability of pDE2 and pDE9 to transform *S. pombe*, we mixed these plasmids, which contain the *URA3* gene, with plasmid pSP1, which contains the *LEU2* gene. The mixture was added to LiOAc-treated *S. pombe ura4 leu1* (*rec*$^+$ or *rec14* mutant) cells, and Ura$^+$ and Leu$^+$ transformants were selected independently. (*S. cerevisiae URA3* and *LEU2* complement *S. pombe ura4* and *leu1* mutations, respectively.) Control transformations used *rec14*$^+$ plasmids pYFL101 (the parent of pDE2), pDE3 (the parent of pDE9), and pDE8, as well as a *rec14*-noncomplementing plasmid pDE10. (See Figure 1 for the extent of *rec14* DNA in these plasmids). These control transformations gave both Ura$^+$ and Leu$^+$ transformants in each case, as expected (Table 4). With pDE2 and pDE9, however, only Leu$^+$ transformants were obtained. Thus, these two plasmids do not inhibit transformation, and the cells in these experiments were competent for transformation. Similar results were obtained with both *rec14*$^+$ and *rec14* mutant recipient cells. These observations suggest that plasmids carrying the *rec14* coding region, but with a disruption in its 5′ control region (see below), confer mitotic lethality.

Disruption of the chromosomal *rec14* gene: To determine whether the complementing DNA in pYFL101 and its derivatives contained the *rec14* gene or a high-copy suppressor of *rec14-120*, we inserted a 2.9-kb DNA fragment carrying the *S. cerevisiae LEU2* gene into the unique *Bst*XI site, near the right end of the *S. pombe* insert (Figure 1), in the region inferred to contain the *rec14* gene. (This insertion changes amino acids after residue 225; see below and Figures 1 and 2.) *S. pombe* strain GP32 (*rec*$^+$ *leu1-32*) was transformed to Leu$^+$ with a linear fragment bearing the insertion. Four stable Leu$^+$ transformants, including strains GP1993 and GP1994, were verified to have homologous replacements (see MATERIALS AND METHODS). The mutation in these transformants, designated *rec14-161::LEU2*, was recessive (Table 3, line 11) and failed to complement the *rec14-120* mutation (Table 3, lines 12 and 13). Crosses homozygous for the *rec14-161::LEU2* mutation

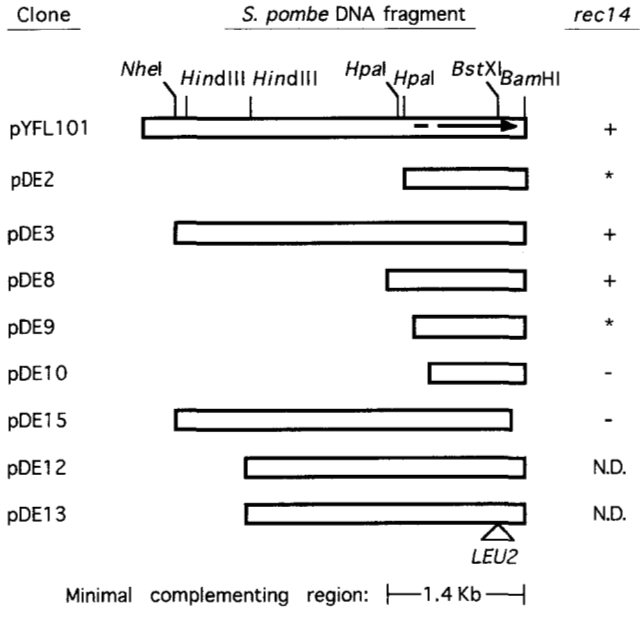

FIGURE 1.—Subclones of the *rec14* gene. Hollow bars represent the extents of the *S. pombe* DNA from the *rec14* region in the indicated plasmids. Plasmids pYFL101, pDE1, and pDE2 are derivatives of pFL20, and the others are derivatives of pSP2 (see MATERIALS AND METHODS). The interrupted arrow in pYFL101 represents the *rec14* exons separated by an intron (see Figure 2). + and -, the ability and inability, respectively, to complement the *rec14-120* mutation (see Table 3); *, no *S. pombe* transformants were obtained (see Table 4); N.D., not determined.

yielded Ade$^+$ recombinant frequencies (approximately two per 10^6 viable spores; Table 3, line 14) indistinguishable from those homozygous for *rec14-120* or heteroallelic for the two mutations. These results demonstrate that the cloned DNA contains *rec14* and that the *rec14-120* and *rec14-161::LEU2* mutations are likely to be null mutations. Both of these *rec14* mutations confer slightly slow growth on solid or liquid, rich or minimal media but no detectable sensitivity to DNA damaging agents (UV-light, methyl methanesulfonate, or γ-rays) or microtubule inhibitors (thiabendazole or methylbenzimidazol-2yl-carbamate) (DEVEAUX *et al.* 1992, data not shown).

Nucleotide sequence of the *rec14* gene and surrounding DNA: We determined the nucleotide sequence on both strands of 3.0 kb of DNA in the *rec14*-containing plasmid pDE3 and its ExoIII-generated derivatives (Figure 1). Two long ORFs separated by a putative intron of 53 nt (confirmed below) were identified as the *rec14* coding region, since one of these ORFs spanned the *Bst*XI site at which the null *rec14-161::LEU2* insertion was made (Figure 2). The exons and introns span 958 nt within the 1.4-kb region containing the functional *rec14* gene (Figure 1). Together, these exons encode 302 amino acids, with a predicted molecular mass for the *rec14* gene product of 32,915 Da. We discuss below additional features of the gene and its predicted product.

Within the sequenced region (nt 1–468) is an incomplete ORF that predicts amino acid sequence homology to a putative ATP-binding transmembrane protein of *S. cerevisiae* (GenBank accession number P40015; YEK9 YEAST). The function of this gene is unknown.

Induction of *rec14* transcripts during meiosis: To determine whether the pattern of expression of *rec14* is similar to that of the *rec* genes previously analyzed, we assayed the *rec14* transcript abundance at various times after meiotic induction. Cultures of *S. pombe* strains synchronously entering meiosis are conveniently prepared by raising the temperature of a *pat1-114* culture; the *pat1*$^+$ gene encodes a repressor of meiosis, which is temperature-sensitive in the *pat1-114* mutant (IINO and YAMAMOTO 1985; MCLEOD and BEACH 1986). RNA was

TABLE 4

Transforming ability of certain *rec14* plasmids

URA3 plasmid	*rec14*[b]	No. of transformants with recipient strain[a]			
		GP1386 (*rec*$^+$)		GP1706 (*rec14-120*)	
		Ura$^+$	Leu$^+$	Ura$^+$	Leu$^+$
pYFL101	+	54, 84, 17, 37	47, 71, 21, 60	296, 213, 41, 23	264, 146, 76, 55
pDE2	*	0, 0	40, 64	0, 0	125, 97
pDE3	+	197, 97	81, 56	197, 124	176, 132
pDE8	+	30, 33	58, 54	85, 28	157, 57
pDE9	*	0, 0	93, 35	0, 0	225, 73
pDE10	–	5, 26	52, 27	54, 20	225, 86

[a] The *URA3* plasmids, described in MATERIALS AND METHODS, contain all or part of the *rec14* gene (Figure 1). A mixture of the indicated plasmid and pSP1, which contains the *S. cerevisiae LEU2* gene (COTTAREL *et al.* 1993), was used to transform strain GP1386 or GP1706 as described in MATERIALS AND METHODS. Ura$^+$ or Leu$^+$ transformants were selected on appropriately supplemented NBA plates. Data from two to four independent experiments are listed.

[b] + and –, the ability and inability, respectively, to complement the meiotic recombination-deficiency of *rec14-120* (Table 3); *, apparent mitotic lethality (these data).

```
                          A ATTGTTCAAA TTTGAAAAAC CTACTATTGC CAAAAGTTTA DraI
1621 AACAATTTAA TTCAGTGTCA CGGTTAACAA CTAATAATCT CACCTTCTCA TTTCTACCTA HpaI
1681 CAGAAAAGAA TTATTTTCTT TCATTCATAC TTATAAATTC ATCTTTTCAC TAACATGTTT
1741 AGGGTATGTG CATAGACCGT GTATATCTAA GGAGAACGCA ACCACAGTAA ACTAAGTCCT
1801 GCTAGTCACA GGTTTAAGTA TTATAAAGTA TTGGAAAGCG CCAGCATTTT AATTACTAAT
1861 TTTGTTGTAT TATTTTCCTT ATTTATTAGA TACTATGAGG AAAGAGTATC TCGTTAGCCA
                                  M  R  K  E  Y  L  V  S  H
1921 TATAGAAGAA AATGCACATC AAGCAGACAT TTACTCTCTC AATGTAGTCG CAGGCAATCT
     I  E  E  N  A  H  Q  A  D  I  Y  S  L  N  V  V  A  G  N  L
1981 TTGGTCGGCT TCTGGGGATT CCAAAATAAA AAAATGGTCT ATAGGTGATG CTGAGCATAG
     W  S  A  S  G  D  S  K  I  K  K  W  S  I  G  D  A  E  H  S
2041 CTTAGTTGAA GAAATAGATA CCCCTCATAA ACTTGGCGTT CATCATCTAG CAACTAGCTT
     L  V  E  E  I  D  T  P  H  K  L  G  V  H  H  L  A  T  S  L
2101 GGATGAAAAC GTCGTCGTTT CTTGCGGATT TGGACAGGAT GTTTATGTTT GGAATCCTGA
     D  E  N  V  V  V  S  C  G  F  G  Q  D  V  Y  V  W  N  P  E
2161 AACTAATGAA TTTCGAGACT TGGgtacgaa ttactttacg aagaagctat gcatttctct
     T  N  E  F  R  D  L  G
2221 aacgaatatg atttagGTAA TAATGCTCAA CATCCCAGCG AATGCTGGTC TTCTTGCATT
                       N  N  A  Q  H  P  S  E  C  W  S  S  C  I
2281 AGCCCTGATG GACAAACGAT TGCTTTCACA TCTGTAGACG GGCGTATTGC TGTCTGGGAC
     S  P  D  G  Q  T  I  A  F  T  S  V  D  G  R  I  A  V  W  D
2341 AATCCAAGTG ATTGCAAAAT TTCCGAGTTA GATACCAAAG GTAAATTCGG TCTTTGTATA
     N  P  S  D  C  K  I  S  E  L  D  T  K  G  K  F  G  L  C  I
2401 GACTATTCTC CCAATGGAAG ATTTATCGTT TCTGGTCATC AAACTGGGCA GCTTTTCCTT
     D  Y  S  P  N  G  R  F  I  V  S  G  H  Q  T  G  Q  L  F  L
2461 ATAAGTACAG AAACAGGACG CTTGTTTCAT GTACTATCGG GCCACACTTC CCCTGTACGA
     I  S  T  E  T  G  R  L  F  H  V  L  S  G  H  T  S  P  V  R
2521 TCAGTGGCGT TCTCTCCTGG TTCAACACTT TTAGCTGCTG CCGGGGATTC TAAAATGATT
     S  V  A  F  S  P  G  S  T  L  L  A  A  A  G  D  S  K  M  I
2581 ACTATCTACG ACGTTTTATC AGGGGATCAA GTCGGCCAAC TTCGTGGTCA TGCTGCATGG BstXI
     T  I  Y  D  V  L  S  G  D  Q  V  G  Q  L  R  G  H  A  A  W
2641 ATTTTTGCAG TAGCTTTCAA TCCCGTAGGT GATTTGTTGC TTTCGGCTGA TGTTGAAGGA
     I  F  A  V  A  F  N  P  V  G  D  L  L  L  S  A  D  V  E  G
2701 AAAATCAAAA TCTGGGATAT TGACACCATG GAATGTATTA GTACACAAAG TGAAACGGAT
     K  I  K  I  W  D  I  D  T  M  E  C  I  S  T  Q  S  E  T  D
2761 GGAGCTATTT GGGCTGTGGC ATGGTATAAA AATGGGTTCA TCGTCGCAGG TGCTGATAAA
     G  A  I  W  A  V  A  W  Y  K  N  G  F  I  V  A  G  A  D  K
2821 AGTATTCGTT GGTATAGAGC TGCTGCTACA GAATAATTGC ACTCTTGTGC TTTCTTAATG
     S  I  R  W  Y  R  A  A  A  T  E
2881 ACCGATGGAA AAGTTTTTAA AAAATAGCAA AAAATTAAGG AGTTTCTAAT ATTTGGATTA DraI
2941 ATTATGAATA TCAACACTTT ACTCGTAAAA CGAAAGGATC c (BamHI)
```

Rec14
1-9
10-29
30-49
50-69
70-89
90-97
98-111
112-131
132-151
152-171
172-191
192-211
212-231
232-251
252-271
272-291
292-302

FIGURE 2.—Nucleotide sequence of the *rec14* gene and deduced amino acid sequence of its product. Numbers on the left refer to the nucleotide sequence, 1 being the first nucleotide of the sequenced region. Only the 1.4 kb corresponding to the minimal complementing region (Figure 1) is shown; the entire nucleotide sequence is GenBank accession number U39144. The last nucleotide (c) may derive from the *Bam*HI cloning site of the vector pFL20. Other lowercase letters are nucleotides in the *rec14* intron. Boxed nucleotides are the inferred translational start and stop signals. Underlined nucleotides indicate restriction sites used in the analysis here. Numbers on the right refer to the deduced amino acid sequence. The gray highlighting indicates nucleotides retained in the *rec14*$^+$ plasmid pDE8 and deleted from the toxic plasmid pDE2 (Figure 1, Tables 3 and 4).

extracted from strain GP625 (*rec*$^+$ *pat1-114*) at various times after induction of meiosis and analyzed by Northern blot hybridization, using as radioactive probes DNA fragments containing the *rec14* gene or, as controls, the *cyc1* and *rec10* genes. The *rec14* probe hybridized to two RNA species, whose mobilities during gel electrophoresis suggested sizes of ~1.2 and 1.3 kb (Figure 3). These species may be the spliced and unspliced forms of the *rec14* transcripts. Both transcripts were readily detectable before meiotic induction; this result was obtained in other experiments (strain GP331 in Figure 3; R. DING and G. R. SMITH, unpublished data). The abundance of the *rec14* transcripts increased during meiosis, to a maximal level at 4 hr of about two to three times that found in the uninduced culture, and decreased modestly thereafter (Figure 3; R. DING and G. R. SMITH, unpublished data). In contrast, the *rec10* transcript was barely detectable in the uninduced culture, reached its maximal abundance at 3 hr, and decreased thereafter; in previous reports (LIN and SMITH 1995b; LI and SMITH 1997) its abundance was maximal at 2 and 3 hr but decreased dramatically at 4 hr and later. The control *cyc1* transcript, encoding cytochrome C (RUSSELL and HALL 1982), was present in mitotic control cells and increased slightly in abundance during meiosis. These results show that the pattern of expression of *rec14* transcripts differs from that of the *rec* genes previously analyzed (see DISCUSSION).

Splicing of the *rec14* transcripts: The nucleotide sequence (Figure 2) predicted a 53-nt intron separating two exons. This putative intron has at its ends sequences similar to the 5′ and 3′ splice junction consensus sequences in *S. pombe* (ZHANG and MARR 1994). To test this prediction, we reverse transcribed RNA extracted from strain GP625 (*pat1-114*) at 3 and 4 hr after meiotic induction, amplified this cDNA in a PCR, and fractionated the products by electrophoresis through an agarose gel. We observed two products, whose mobilities relative to size markers indicated they were ~950 and 990 bp long (922 and 975 bp predicted; data not shown). The longer fragment, but not the shorter, was observed in a PCR using plasmid pDE3 containing a genomic *rec14* insert (Figure 1) as template. The nucleotide sequence of the shorter fragment from the RNA-

templated reaction confirmed that the predicted intron had been removed, as shown in Figure 2; no other introns in *rec14* were found.

Transcript 5′ ends: We placed limits on the transcriptional start site for *rec14* by an RT-PCR-based method. RNA extracted from meiotically induced cells was reverse transcribed using primer V, to the right of the intron and directing cDNA synthesis leftward (toward the 5′ end of the transcripts). The cDNA was then amplified in a PCR with primer I and one of three primers (II, III, and IV) ~60, 230, and 290 bp, respectively, to the left of the translational start site (ATG). A PCR product of the expected size (453 bp) was observed with primers I and II, but no product was observed with the other two primer pairs (Figure 4). All three primer pairs gave products of the expected sizes (506, 667, and 729 bp) with genomic DNA as a template. These results indicate that the *rec14* transcripts are complementary to primer II, but not to primer III or IV, and imply that *rec14* transcription begins in the interval from 60 to 230 bp to the left (5′) of the ATG translational start. Two ATG triplets are in this interval, but, since neither initiates an ORF as long as that identified in Figure 2 as the *rec14* translational start, we favor the assignment in Figure 2. The abolition of *rec14* function by a deletion (pDE15) and an insertion (*rec14-161::LEU2*) near the end of the long ORF (Figure 1) supports this assignment.

Mitotic recombination in *rec14* mutants: The presence of *rec14* transcripts during mitotic growth (Figure 3) raised the possibility that the *rec14* gene product (Rec14) is required for mitotic recombination. To test this possibility, we measured recombination between *ade6* alleles on the chromosome and on a plasmid in mitotically dividing cells. The Ade^+ recombinant frequency was measured in multiple independent cultures, and recombination rates were calculated according to the method of the median (LEA and COULSON 1949). No significant differences were found between rec^+ and *rec14-120* or *rec14-161::LEU2* strains, using two *ade6* allele pairs (Table 5). We conclude that Rec14 is required for *ade6* intragenic recombination during meiosis (Table 2; DEVEAUX *et al.* 1992) but not during mitosis.

DISCUSSION

We report here a molecular analysis of the *rec14* gene of *S. pombe*. The *rec14* gene was identified in a plasmid-borne library of *S. pombe* DNA fragments using a previously described screen for complementation of *rec* meiotic recombination-deficiency (LIN and SMITH 1995b). Insertion of the *S. cerevisiae LEU2* gene into an

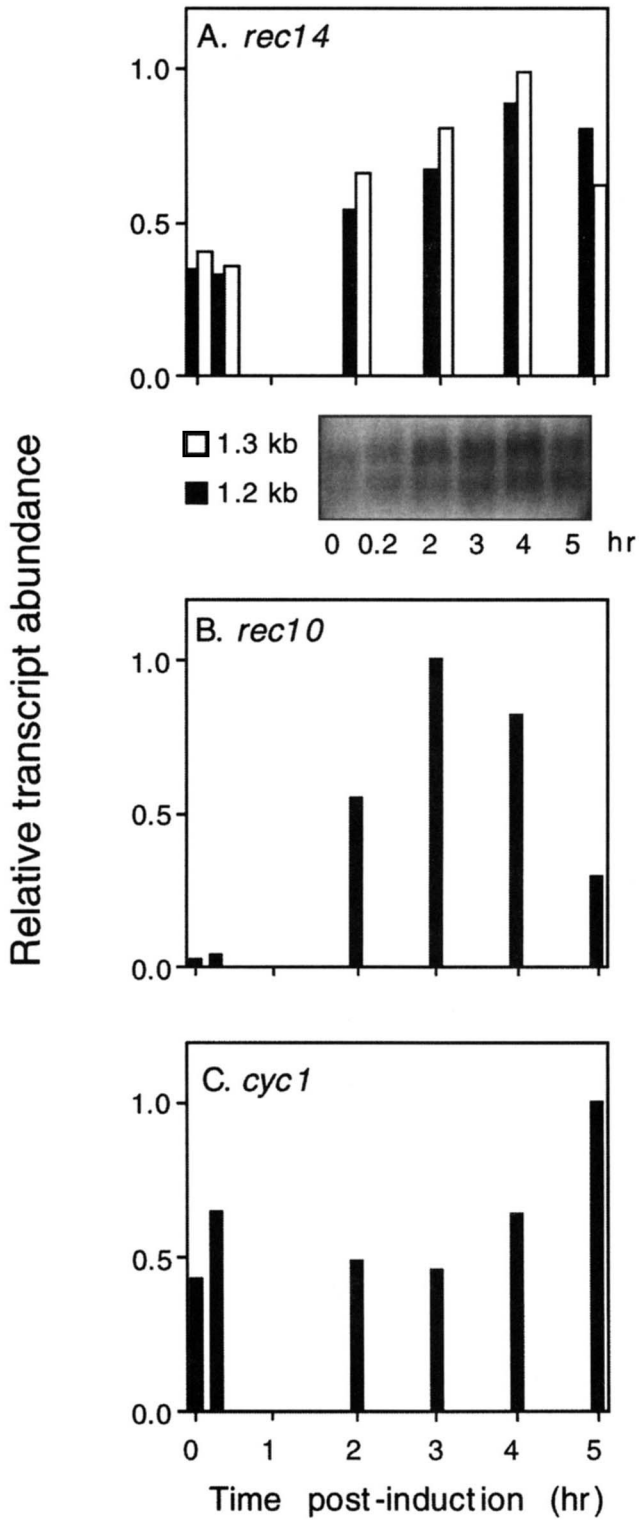

FIGURE 3.—*rec14* transcript abundance before and after meiotic induction. RNA was extracted from strain GP331 (*pat1-114*) growing at 25° (0 time point) and from strain GP625 (*pat1-114*) at the indicated times after raising the temperature to 34° to induce meiosis. The RNA was analyzed by Northern blot hybridization using probes for the *rec14, rec10,* and *cyc1* transcripts (see MATERIALS AND METHODS). The relative abundance of each transcript was quantitated by PhosphorImager analysis, setting the maximal abundance at 1. The sizes of the *rec14* transcripts were estimated from the positions of the 1.8 and 3.5 kb rRNA and RNA size markers (BRL).

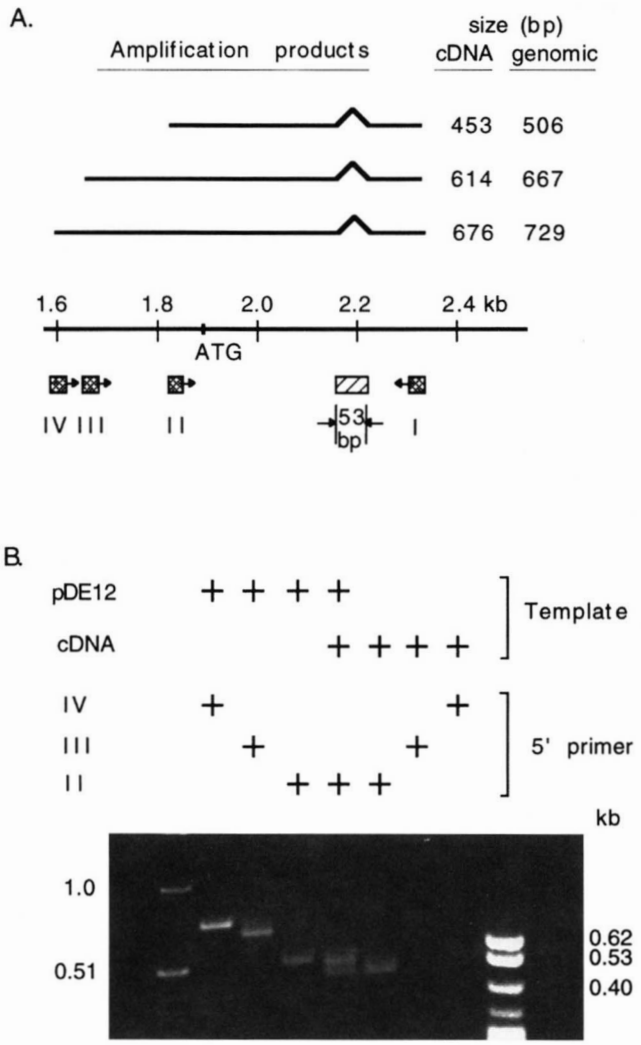

FIGURE 4.—Analysis of the 5′ ends of *rec14* transcripts. RNA extracted from strain GP625 at 3 hr after meiotic induction was reverse transcribed using primer V (near the *rec14* translational stop; Figure 2). The products, or plasmid pDE12 DNA, were subjected to a PCR using primer I in combination with primer II, III, or IV, as indicated (A); the PCR products were analyzed by gel electrophoresis (B). In the middle lane the PCR products were mixed before electrophoresis. Size markers are a 1-kb ladder (BRL; left) and *Msp*I-digested pBR322 DNA (right).

ORF on this fragment and homologous replacement into the chromosome generated a mutation that failed to complement the standard *rec14-120* allele; this result demonstrates that the fragment contains the *rec14* gene, rather than a high-copy suppressor of *rec14-120*. The nucleotide sequence of 3.0 kb of this fragment revealed two exons separated by a 53-nt intron (Figure 2). Together these exons encode a predicted protein of 302 amino acids (32,915 Da) with the WD repeat motif found in other proteins (see below).

The expression of the *rec14* gene appears unlike that of the other *rec* genes tested. Transcripts of *rec14* were readily detectable in mitotically dividing cells, increased about threefold to a maximum at 3–4 hr after meiotic induction, and declined slightly thereafter (Figure 3; R. DING and G. R. SMITH, unpublished data). Transcripts of *rec6, 7, 8, 10, 11, 12, 15* and *16*, however, are barely detectable, if at all, in mitotically dividing cells, increase many-fold to a maximum at 2–3 hr after meiotic induction, and decrease dramatically thereafter (LIN *et al.* 1992; LIN and SMITH 1994, 1995a,b; LI *et al.* 1997; LI and SMITH 1997; R. DING and G. R. SMITH, unpublished data). The expression of *rec14* is more similar to that of the *S. pombe exo1* gene, which encodes exonuclease I (ExoI), an enzyme that aids mutation avoidance during mitotic growth and mismatch correction during meiotic recombination (SZANKASI and SMITH 1992, 1995). Transcripts of *exo1* and ExoI activity are readily detectable in mitotically dividing cells, increase about fivefold at 4–5 hr after meiotic induction, and decline modestly thereafter.

The difference between the induction pattern of *rec14* and that of the other analyzed *rec* genes may reflect the absence of *Mlu*I or *Mlu*I-like sites near *rec14* (Figure 2) but their presence near *rec6, 7, 8, 10, 11, 12*, and *15* (LIN *et al.* 1992; LIN and SMITH 1994, 1995a,b; LI *et al.* 1997). The transcriptional activator Cdc10, complexed with other proteins, binds to DNA fragments with the *Mlu*I site (5′ ACGCGT 3′) or closely related sites (LOWNDES *et al.* 1991, 1992). This binding activates transcription of genes periodically in the mitotic cell cycle and, perhaps, transiently in meiosis (SUGIYAMA *et al.* 1994). The *rec14* gene and the *exo1* gene, which also lacks *Mlu*I sites (SZANKASI and SMITH 1995), may have a common mode of transcriptional regulation that differs from that of the other *rec* genes analyzed.

The presence of *rec14* transcripts in mitotic cells suggests that *rec14* mutations might have a mitotic phenotype. We searched for such a phenotype and found that *rec14* mutants differ from rec^+ cells only in their slightly slower growth (data not shown). Mitotic recombination and sensitivity to DNA damaging agents or to microtubule inhibitors were not detectably altered in *rec14* mutants (Table 5 and data not shown). The *rec14* gene product may, however, be lethal to cells when inappropriately expressed. We noted that two plasmids (pDE9 and pDE2) containing the entire *rec14* coding sequence but only ~50 and 250 bp, respectively, to the 5′ side of it failed to transform *S. pombe* (Figure 1; Table 4). A plasmid (pDE8) containing 320 bp to the 5′ side did transform. Thus, an element located between 250 and 320 bp to the 5′ side of *rec14* appears to be essential for mitotic viability, at least when coupled to the *rec14* coding region on a plasmid. This element might be a site of repression of *rec14;* deleting it may lead to overexpression of *rec14* and consequent cell death. If this interpretation is correct, then under- or overexpression of *rec14* is deleterious to mitotic growth.

Within the predicted 302 amino acid sequence of

TABLE 5

Mitotic recombination-proficiency of *rec14* mutants

ade6 alleles		Rate of Ade$^+$ recombination (events per 10^5 cell divisions)[a]			
		rec$^+$		*rec14*	
Chromosome	Plasmid	Strain	Rates	Strain	Rates
52	*M375*	GP369	2.3, 0.7, 1.3	GP505[b]	3.3, 0.7, 1.8
M26	*469*	GP363	2.4, 2.2	GP502[b]	3.7, 2.7
M26	*469*	GP2183	0.85, 1.0	GP2173[c]	0.81, 1.0

[a] Each datum is the rate calculated from the Ade$^+$ recombinant frequencies in five independent cultures of each strain, as described in MATERIALS AND METHODS. Data from two or three independent experiments are listed. The method of the median was used (LEA and COULSON 1949). Strains were transformants of the indicated strain bearing the pade6-M375 or pade6-469 plasmid (SZANKASI *et al.* 1988), as indicated.

[b] These strains contain the *rec14-120* mutation.

[c] This strain contains the *rec14-161::LEU2* mutation.

Rec14 we noted six repeated sequences with homology to a previously reported amino acid motif (Figure 5). This motif was first identified in the G-beta transducin family of proteins but later found in other functionally unrelated proteins (NEER *et al.* 1994). These "G-beta" or "WD" repeats probably facilitate protein-protein interactions (VAN DER VORN and PLOEGHT 1992; KOMACHI *et al.* 1994); these repeats are frequently found as tandem arrays of two to eight copies spaced ~40 amino acids apart. The crystal structure of rod transducin has identified amino acids that stabilize the repeats as "blades" in a β-propeller structure (SONDEK *et al.* 1996). These conserved amino acids and their spacing match quite well the six putative repeats we have identified in Rec14 (Figure 5). Because the G-beta motif is so widely distributed among proteins, many homologues are identified by a BLAST search (ALTSCHUL *et al.* 1990). The closest match we found in GenBank (re-

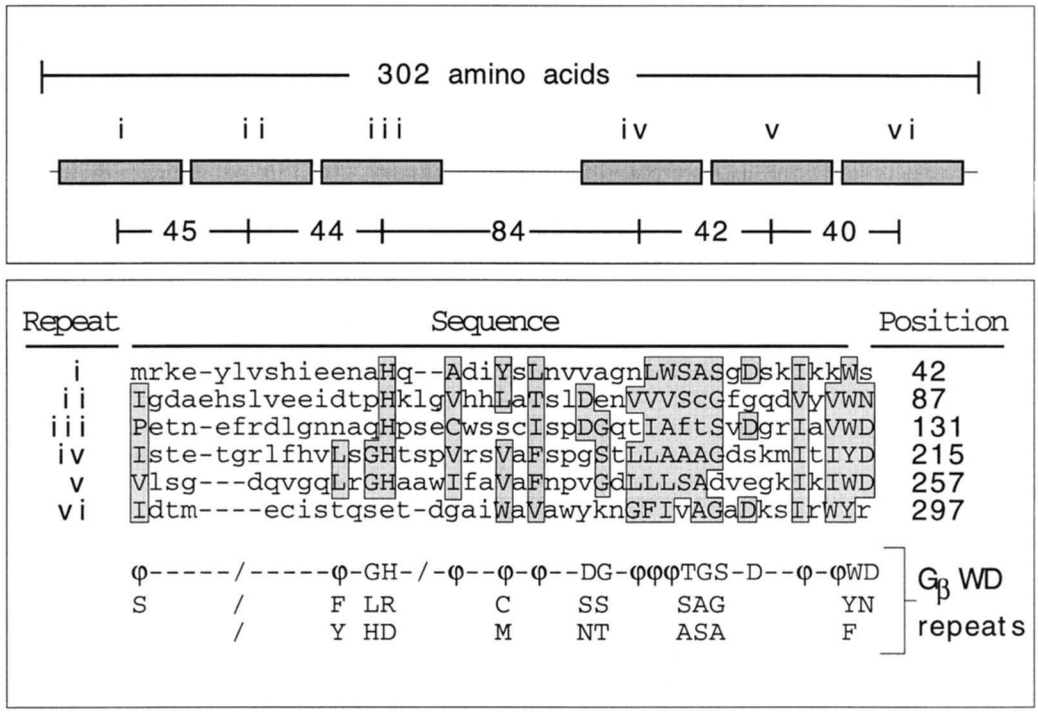

FIGURE 5.—G-beta transducin (WD) repeats in the deduced *rec14* gene product. Repeats i–v were detected using the block search program of HENIKOFF and HENIKOFF (1994) while repeat vi was identified by inspection. The bottom panel shows an alignment of these repeats along with their end points within the Rec14 polypeptide (at right). Boxes highlight amino acids that match a WD consensus sequence derived with the help of the crystallographic structure of rod transducin $G_{\beta\gamma}$ heterodimer (bottom; SONDEK *et al.* 1996). Where the $G_{\beta\gamma}$ structure shows a preference for one or more amino acids, these are shown with the most preferred residue on top. -, nonconserved positions; /, places where some length variation is tolerated within a WD repeat. Sites with a preference for hydrophobic amino acids are also indicated (φ). Although a seventh WD repeat would fit between repeats iii and iv, this region shares little sequence homology with the crystallographically derived consensus.

lease 92.0) was to a gene product controlling *Podospora anserina* vegetative incompatibility (SAUPE *et al.* 1995), but *S. cerevisiae* proteins, including the antiviral protein Ski8 (MATSUMOTO *et al.* 1993), also have high sequence homology. An alignment of Rec14 and Ski8, with eight gaps of 1–33 amino acids, showed 26% amino acid identity (data not shown). Recently, GARDINER *et al.* (1997) have shown that *SKI8* is identical to the *S. cerevisiae REC103* gene, one of several genes required apparently early in meiotic recombination (MALONE *et al.* 1991). Rec103 (Ski8) resembles *S. pombe* Rec14 in several ways. Like *rec14, REC103* is expressed in mitotic cells and is induced during meiosis. Moreover, *rec103* mutations, like *rec14* mutations, have no detectable effects on mitotic recombination or repair. The sequence homology and common phenotypes suggest that *REC103* and *rec14* encode functional homologues. The link between an antiviral protein and meiotic recombination remains unclear at present, but the availability of the *rec14* gene facilitates further genetic and biochemical analysis of Rec14 function.

We are grateful to MARCELLA CERVANTES for performing the experiment in Table 5 and to BOB MALONE for communicating results before publication. We thank LOUISE CLARK and JOHN CARBON for a sample of their *S. pombe* DNA library; RUBAI DING for unpublished data; YUKANG LIN, FRED PONTICELLI, PHILIPPE SZANKASI, and JEFF VIRGIN for strains; SUE AMUNDSEN, GLENN MANTHEY, and ANDREW TAYLOR for comments on the manuscript; and KAREN BRIGHTON for skillfully preparing it. This research was supported, in part, by a travel award from the Medical Research Council of Canada (D.E.), by funds provided to the Fred Hutchinson Cancer Research Center from the Sammamish Hills Guild (Y.F.L.), and by grant GM-32194 from the National Institutes of Health (G.R.S.).

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