MOLECULAR AND CELLULAR BIOLOGY, Apr. 1985, p. 610–618
0270-7306/85/040610-09$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 5, No. 4

Expression of the *Escherichia coli dam* Methylase in *Saccharomyces cerevisiae*: Effect of In Vivo Adenine Methylation on Genetic Recombination and Mutation

MERL F. HOEKSTRA AND ROBERT E. MALONE*

Department of Microbiology, Loyola University of Chicago, Stritch School of Medicine, Maywood, Illinois 60153

Received 15 October 1984/Accepted 8 January 1985

The *Escherichia coli* DNA adenine methylase (*dam*) gene has been introduced into *Saccharomyces cerevisiae* on a yeast-*E. coli* shuttle vector. *Sau3A*I, *Mbo*I, and *Dpn*I restriction enzyme digests and Southern hybridization analysis indicated that the *dam* gene is expressed in yeast cells and methylates GATC sequences. Analysis of digests of total genomic DNA indicated that some GATC sites are not sensitive to methylation. The failure to methylate may reflect an inaccessibility to the methylase due to chromosome structure. The effects of this in vivo methylation on the processes of recombination and mutation in mitotic cells were determined. A small but definite general increase was found in the frequency of mitotic recombination. A similar increase was observed for reversion of some auxotrophic markers; other markers demonstrated a small decrease in mutation frequency. The effects on mutation appear to be locus (or allele) specific. Recombination in meiotic cells was measured and was not detectibly altered by the presence of 6-methyladenine in GATC sequences.

Methylation of DNA bases has been demonstrated to play an important role in the processes of DNA replication, repair, and recombination in procaryotes and gene expression in eucaryotes (1, 4). In *Escherichia coli* a methyl group is added, after replication, to the N6 position of adenine in 5′-GATC-3′ sequences by DNA adenine methylase produced by the *dam* gene (15, 28). It has been proposed that the transient undermethylation of newly replicated strands allows a mismatch repair system to preferentially remove the new (incorrect) information when replication errors occur (13, 37, 42). Recent experiments using heteroduplexes of phage λ DNA, methylated in vitro and transformed into *E. coli*, have confirmed that the mismatched base on the undermethylated strand is preferentially repaired (36).

In *E. coli*, the consequences of losing the ability to methylate adenine are profound. The lack of methylation in *dam*$^-$ strains leads to increased frequencies of recombination and spontaneous mutation (2, 27, 29) and increased sensitivity to methyl methanesulfonate (29) and UV (27). Furthermore, the *dam* mutation is lethal in combination with *recB*/*C* or *lexA* mutations (2, 29). All of these phenotypes can be understood in terms of the mismatch correction system being unable to distinguish which strand to attack when a mismatch is created during replication. Specifically, increased mutation could occur when the correct base was removed, and increased recombination could result from single-strand gaps and breaks or from double-strand breaks generated when excision tracks on both DNA strands overlap (37). Overproduction of the *dam* enzyme also leads to increased mutation frequencies (19). Herman and Modrich argued that increased levels of the *dam* methylase would result in a DNA molecule (19) rapidly methylated after replication on both strands, producing fully methylated DNA resistant to mismatch repair (19). This is supported by the results of Pukkila et al. (36), which indicate that λ DNA fully methylated in vitro is not subjected to mismatch repair when transformed into *E. coli*.

The yeast *Saccharomyces cerevisiae* contains undetectable amounts of methylated adenine (<0.05%) (17). By using the same chromatographic technique, it was shown that approximately 1% of deoxycytosine in yeast DNA contained methyl groups at the 5 position (17). More recently, Proffitt et al. (35) have shown by high-pressure liquid chromatography and by Southern analysis that 5-methylcytosine is present at <0.03% of the cytosine residues in the endogenous yeast 2μm plasmid or in chromosomal DNA.

The *dam* gene in *E. coli* has been cloned and more recently inserted into a yeast-*E. coli* shuttle vector that can replicate in yeast cells (3; Kostriken, personal communication). Brooks et al. (3) state that the *dam* gene is expressed and methylates yeast DNA. We have modified the original vector and transformed yeast strains with the cloned *dam* gene to confirm that it is expressed and to ask what effect adenine methylation has on recombination and mutation in *S. cerevisiae*. To our knowledge these are the first experiments to measure the effect of 6-methyladenine on recombination and mutation in eucaryotes.

(This study has been submitted by M.F.H. in partial fulfillment of the Ph.D. requirements of Loyola University of Chicago.)

MATERIALS AND METHODS

Strains and culture conditions. The strains used in this study are listed in Table 1. To monitor the effect of *dam* on recombination, the yeast diploid strain MH16 was constructed containing six heteroallelic loci and two recessive drug resistance loci. The former allow us to examine mitotic gene conversion and the latter allow us to measure mitotic crossing-over (6). To examine the effect of *dam* on mutation, the transformed MH16 diploid was dissected by using standard techniques (5) generating the haploids MH16-4C, MH16-A-10B, and MH16-B-24B with and without plasmid pMFH1 containing the *dam* gene (Table 1). All yeast media have been previously described (14). *E. coli* media are described in reference 24.

Construction of pMFH1. To introduce an appropriate selectable marker into the *dam*-containing pRK99, plasmid

* Corresponding author.

TABLE 1. List of strains

Strain	Genotype	Source
Yeast strains[a]		
MH16	III *MATa*/*MATα* II *lys2-2 tyr1-2 his7-1*/*lys2-1 tyr1-1 his7-2* V *CAN1ˢ ura3-1*/*can1ʳ ura3-13*	This Work
	VII +/*ade5* *met13-c cyh2ᴿ trp5-c leu1-c*/*met13-d CYH2ˢ trp5-2 leu1-12* XV *ade2-1*/*ade2-1*	
MH16-4C	*MATa* *lys2-2 tyr1-2 his7-2* *CAN1ˢ ura3-1*	This work
	ade5 met13-d cyh2ᴿ trp5-c leu1-c *ade2-1*	
MH16-A-10B	*MATα* *lys2-2 tyr1-1 his7-2* *CAN1ˢ ura3-1*	This work
	+ *met13-d CYH2ˢ trp5-c leu1-c* *ade2-1*	
	Containing pMFH1	
MH16-B-24B	*MATa* *lys2-2 tyr1-2 his7-2* *CAN1ˢ ura3-1*	This work
	ade5 met13-d CYH2ˢ trp5-c leu1-c *ade2-1*	
	Containing pMFH1	
Bacterial strain (*E. coli*)		
MC1006	*hsdR hsdM⁺ leuB6 lacX74 galU galK*	M. Casadaban
	StrAʳ *trpC9830 pyrF*	

[a] The circle represents the centromere and the line represents a chromosome linkage group. The roman numerals refer to the chromosome number. Gene symbols are as defined by Plischke et al. (34).

pMFH1 was constructed. The construction scheme and plasmid maps are given in Fig. 1. Briefly, the 1.1-kilobase (kb) *Hin*dIII fragment containing the *URA3* gene from YEp24 was introduced into the unique *Hin*dIII site of pRK99. The resulting plasmid pMFH1 was obtained by selecting for Leu$^+$ Ura$^+$ Amp^r transformants of *E. coli* strain MC1006 (38) on minimal media containing tryptophan. Recombinant plasmids were isolated and subjected to *Hin*dIII-*Pvu*II double digests for verification of the construct.

Measurement of recombination and mutation frequency. The mitotic recombination frequency was measured as described in Malone and Hoekstra (24). Mutation frequencies were determined by using a similar approach except that the initial inoculum, as determined by hemacytometer count, was lower (100 cells per ml) and the cells were allowed to grow to midexponential phase (approximately 5×10^7 cells per ml) before harvesting and plating on the appropriate media. The meiotic recombination analysis (see Table 3) results from standard tetrad analysis (31).

Transformation, DNA purification, and hybridization analysis. The procedures for spheroplast transformation and DNA isolation from yeast cells have been previously described (20) and were used with minor modifications. Transformation of *E. coli* cells was mediated by $CaCl_2$, using standard protocols (26). In all experiments described, the yeast DNA has been CsCl purified and phenol extracted. Southern analysis (40) was carried out as previously described (25) except the gel was pretreated with 0.25 M HCl for 20 min before blotting to enhance transfer of larger DNA fragments. Transfer of DNA was judged to be complete by ethidium bromide stain of the gel. Prehybridizations and hybridizations were carried out in the presence of 5× Denhardt reagent and the hybridization mix contained 5% dextran sulfate (46) (1× Denhardt reagent is 0.02% each Ficoll 40,000, polyvinylpyrrolidone, and bovine serum albumin). Restriction enzymes were purchased from Bethesda Research Labs, Gaithersburg, Md., and New England Biolabs, Beverly, Mass., and were used as recommended by the vendors.

DNA fragments used as nick-translated probes were radiolabeled as described in Malone and Hyman (25). Before labeling, the fragments were purified from low-melting-temperature agarose (Bethesda Research Labs) by a procedure modified from Gafner et al. (11).

RESULTS

Expression of the *dam* gene in *S. cerevisiae*. The 1.1-kb *Hin*dIII fragment containing the *URA3* gene from YEp24 was inserted into the *Hin*dIII site of pRK99 (Fig. 1). The

resulting plasmid, pMFH1, was used to transform the yeast diploid MH16 by selecting for Ura$^+$ colonies. Plasmid pMFH1 contains the 2μm origin of DNA replication and is a relatively high-copy-number plasmid. To determine whether the *E. coli dam* gene product was capable of methylating adenine in GATC sequences in yeast cells, DNA from transformants was compared with DNA from the nontransformed parental strain. The restriction enzyme isoschizomers *Sau3A*I, *Mbo*I, and *Dpn*I were used to compare the susceptibility of the various DNAs to digestion. Samples were taken after various times of digestion and examined by agarose gel electrophoresis. Figure 2A illustrates that DNA from a transformant containing the *dam* gene was refractory to cleavage by *Mbo*I; this enzyme will not cleave at GATC sequences containing 6-methyladenine (12). *Sau3A*I digested DNA from the transformant with similar kinetics and to a similar end product as it did the nontransformed parental DNA (Fig. 3A). None of the DNAs was digested by *Sau3A*I to the same extent as the DNA from the nontransformed parent cleaved by *Mbo*I (see Discussion). The pertinent observation, however, is that *Sau3A*I digested both DNAs equally, whereas *Mbo*I was inhibited on the DNA from the transformant containing the *dam* gene (Fig. 2A and 3A). This confirms that the *dam* gene is present and indicates that it is expressed and active in yeast cells. These experiments have been repeated with a second independent transformant and demonstrate the same kinetics and endpoints as those shown in Fig. 2, 3, and 4 (data not shown).

To further substantiate this conclusion, DNA from the transformant and the parent was digested with *Dpn*I (Fig. 4A). The *Dpn*I restriction nuclease only cleaves DNA when

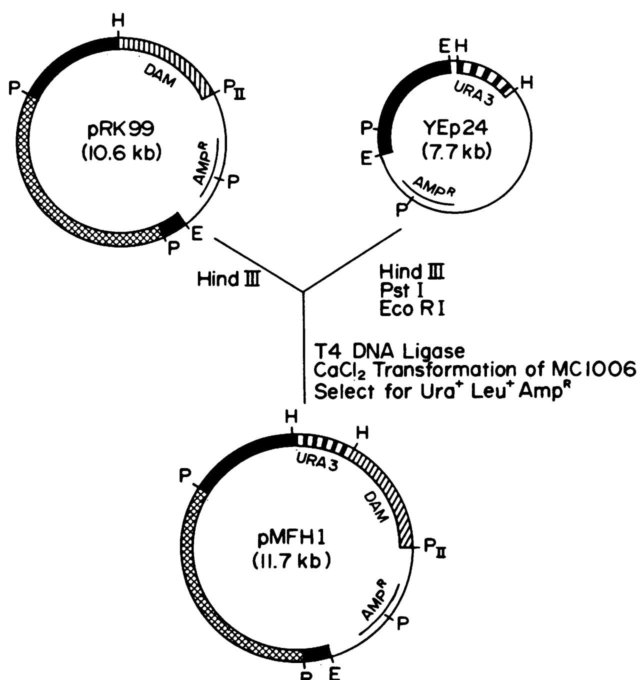

FIG. 1. Construction of pMFH1. The 1.1-kb *URA3* gene from YEp24 was introduced into pRK99 at the unique *Hin*dIII site to produce pMFH1. E refers to a restriction site for *Eco*RI; H, for *Hin*dIII; P, for *Pst*I; and PII, for *Pvu*II. The heavy solid line is the 2μm origin of replication; the thin line is pBR322 sequences. The yeast *URA3* gene and *E. coli dam* are indicated, and the checkered area is the yeast *LEU2* gene. Only relevant restriction sites are shown.

the GATC sequence is methylated at adenine (15, 21, 22). The results illustrate that the *dam* gene is expressed and methylates GATC sequences in yeast cells. The data in Fig. 4A also indicate that not all GATC sequences are methylated; there exist high-molecular-weight DNA bands, as well as a considerable amount of DNA which bands at a position expected for completely undigested DNA (>25 kb). The undigested DNA may exist because some cells have lost pMFH1, even though the cells were grown under selective conditions for the plasmid (42). Cells without plasmid will contain no *dam* methylase and their DNA will appear like the parental yeast strain. The large (5 to 25 kb) bands are not expected from a restriction enzyme which recognizes a 4-base pair (bp) sequence; this observation is also consistent with the hypothesis that not all GATC sequences are methylated. That unique bands appear suggests that specific GATC sequences might be resistant to methylation in yeast cells (see Discussion).

Examination of a specific DNA sequence in the presence of *dam*. The experiments above examine the response of the total yeast genome to methylation by the *dam* methylase at GATC sites. To extend these observations and examine methylation patterns in a specific segment of DNA, we have used Southern hybridization analysis (40) (Fig. 2B, 3B, and 4B). The probe was a 1.4-kb *Eco*RI fragment containing the *TRP1* gene. (A partial restriction map of the *TRP1* gene is shown in Fig. 5; see reference 44 for the sequence.) The results show that transformants containing the *dam* gene have GATC sites in and around the *TRP1* region which are methylated. If complete digestion occurred at all GATC sites, the expected products would consist of 885- and 515-bp fragments and a small fragment of >84 bp (Fig. 5). The latter fragment is not visible with the electrophoresis conditions used. The *Mbo*I digest (Fig. 2B) demonstrates that DNA from a *dam*-containing strain is refractory to cleavage, whereas the same DNA is digested by *Sau3A*I (Fig. 3B) with similar kinetics and endpoint as the parental DNA. *Dpn*I digests show that the *dam* gene methylates specific sequences in and around the *TRP1* region (Fig. 4B). Together, the data from the specific DNA fragment indicate that GATC sequences are methylated.

Some of the *TRP1* DNA appears to have been incompletely methylated (Fig. 2B and 4B); as in the total genomic digests, it is possible to account for this by the cells in the population which have lost pMFH1. Alternatively, hemimethylation (methylation of A residues on one strand only) does not create *Dpn*I-sensitive sites (22); this may also be a cause of the incomplete *Dpn*I digestion. We have digested DNA from *dam* transformants under the same conditions as given in Fig. 4 for as long as 18 h with no change in the restriction pattern or Southern hybridization profile. Therefore, the failure to obtain limit digests does not appear to be due to incomplete cleavage of sites which are, in fact, sensitive.

We have attempted to determine the fraction of *TRP1* DNA completely digested by *Dpn*I by calculating the amount of DNA in the 885- and 515-bp fragments (densitometer tracings not shown). From these, we determine that the fraction of the total *TRP1* DNA in the partially digested or undigested positions for limit digests in Fig. 4B (i.e., bands larger than 885 bp) is approximately 55%. The average fraction of cells which do not contain the plasmid after selective growth is 31% (averaged from 14 cultures; data not shown). Thus, plasmid loss can account for much of the undigested DNA; hemimethylation of GATC may also contribute to the lack of complete digestion by *Dpn*I (Fig. 4).

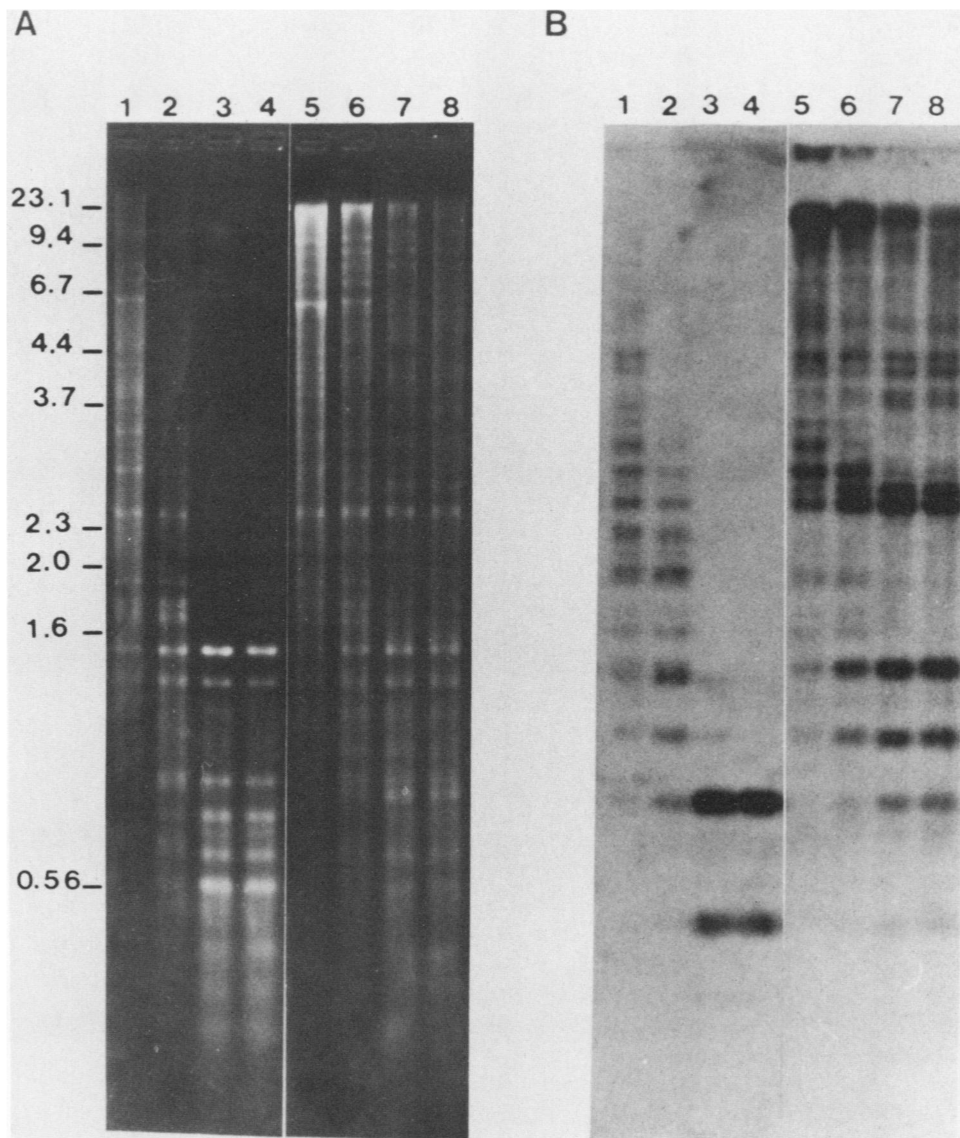

FIG. 2. Illustration that yeast DNA from a *dam* (pMFH1) transformant is refractory to cleavage by *Mbo*I. From a reaction mixture containing 24 μg of DNA and 20 U of *Mbo*I, 4-μg samples were removed at 15 (lanes 1 and 5), 30 (lanes 2 and 6), 60 (lanes 3 and 7), and 120 (lanes 4 and 8) min and run overnight at 30-mA constant current in a 1.5% agarose gel, using Tris-Borate-EDTA (23) as the running buffer. (A) Ethidium bromide-stained gel. (B) Southern blot probed by 1.4-kb *Eco*RI *TRP1* DNA. In both (A) and (B), DNA in lanes 1 to 4 is from the untransformed MH16, whereas lanes 5 to 8 contains samples from the corresponding pMFH1-containing *dam* transformant. The size standards were *Hin*dIII and *Ava*I digests of λ DNA (not shown).

Effect of *dam* methylation on spontaneous mitotic recombination. Given the major effects that adenine methylation has on recombination in *E. coli* and its phages (2, 27, 36), we have asked what effect the heterologous expression of the *dam* gene would have on mitotic recombination in *S. cerevisiae*. The data in Table 2 indicate that transformants containing the *dam* gene have a small, but significant, increase in the frequency of mitotic recombination compared with the control strain [χ^2 = 282(d.f. = 7); $P < 0.001$]. If we make a correction for the loss of the plasmid, and assume that only cells with the plasmid contribute to the observed increase, the frequency of mitotic gene conversion and crossing-over is elevated at seven of eight loci by adenine methylation in vivo [χ^2 = 2,104(d.f. = 7); $P << 0.001$].

Effect of *dam* methylation on meiotic recombination. Diploids containing the *dam* plasmid pMFH1 were sporulated and dissected, and a recombination map was calculated by the empirically derived formula of Ma and Mortimer (23) and by the Perkins' formula (33). During sporulation, plasmids containing the 2μm origin of replication are lost from the cell (42). This generates tetrads that segregate in a non-4:0 fashion for the *URA3* marker on pMFH1. Therefore, we have calculated two maps, one from all tetrads originating from the transformed MH16 and one from only those tetrads that segregated 4:0, 3:1, or 2:2 for the plasmids (Table 3). There appear to be no major differences among the three maps in Table 3, and we conclude that 6-methyladenine has little effect on meiotic recombination.

Effect of *dam* methylation on mutation. In *E. coli*, overproduction of the *dam* methylase leads to increased mutation rates (2, 24). Both forward and reverse mutation frequencies

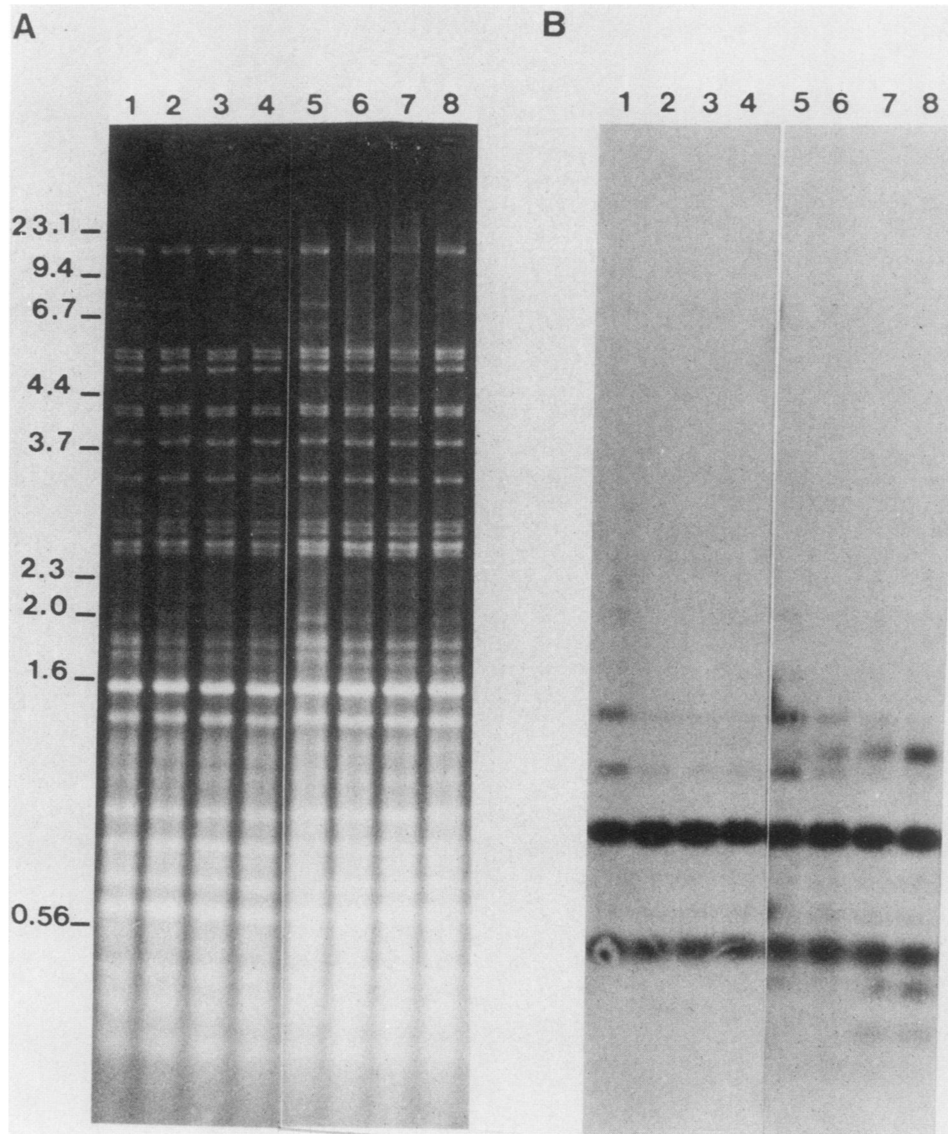

FIG. 3. Illustration that methylated and nonmethylated yeast DNA is susceptible to *Sau3A*I digestion. Quantities of DNA, enzyme, sampling times, and size standards are as in Fig. 2. (A) Ethidium bromide-stained gel. (B) Southern blot probed by *TRP1* fragment. Lanes 1 to 4 contain parental DNA; lanes 5 to 8 contain DNA from pMFH1-transformed cells.

at several loci in yeast haploids containing the *dam* gene have been measured (Table 4). The haploids were obtained from the dissection of MH16 as discussed above. Two independent haploids which contained pMFH1 were examined. The result at one locus (*met13*) indicates that reversion is stimulated by adenine methylation at GATC. Two loci (*lys2* and *CAN1*) demonstrate a small increase in forward mutation frequency, whereas two other loci (*his7* and *leu1*) exhibit a decrease in reversion frequency. If we correct the data for the fraction of cells which contain plasmid (as done for the recombination frequency), these differences become more pronounced.

DISCUSSION

We have confirmed the report that the *E. coli dam* gene can be expressed in yeast cells and that the adenine methylase produced is capable of methylating GATC sequences (3). A GGCC-specific cytosine methylase gene has previously been transformed into yeasts, and it was expressed and methylated yeast DNA (8). Not all susceptible sequences appear to be fully methylated, because either insufficient enzyme is present, certain GATC sequences are inaccessible to the methylase, or N6 methyl adenine is efficiently removed by yeast repair systems. We favor the second explanation because of the discrete band patterns in the higher-molecular-weight sizes (2.3 to 21 kb) observed in the *Dpn*I digest (Fig. 4). If the methylase recognized GATC sites randomly, we would not have expected to see discrete bands in fragments so large. In yeast DNA, which is 40% guanine plus cytosine (8), GATC sequences should appear randomly every 278 bp. A 10-kb fragment could potentially contain 36 *Dpn*I sites, and random methylation of these would generate a variety of fragments rather than the discrete bands observed. The complete digest of untransformed yeast DNA by *Mbo*I (Fig. 2A) revealed no fragments

FIG. 4. Evidence that *dam*-transformed yeast cells are susceptible to *Dpn*I cleavage. Reaction conditions and size standards are as in Fig. 2. Sampling times were extended to 4 (lanes 5 and 11) and 8 (lanes 6 and 12) h. (A) Ethidium bromide-stained gel. (B) is the corresponding Southern blot probed by *TRP1* DNA. Lanes 1 to 6 contain *Dpn*I-digested untransformed parental DNA. Lanes 7 to 12 contain *Dpn*I-digested, pMFH1-transformed DNA.

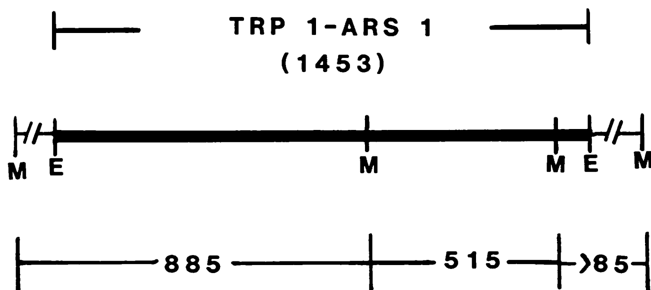

FIG. 5. Restriction map of *TRP1* 1.4-kb EcoRI fragment. Schematic diagram representing the 1.45-kb *TRP1* region from YRp7 (42) used as a probe for Fig. 2B, 3B, and 4B. E is *Eco*RI and M is *Mbo*I.

larger than approximately 2.3 kb. This indicates that any yeast DNA fragment larger than 2.3 kb in the *Dpn*I digest contains at least one GATC sequence that was not cleaved. That the *dam* methylase might preferentially methylate specific GATC sequences in yeast chromatin suggests that the higher-order structure of chromosomes may determine what DNA sequences are accessible. The *dam* gene may therefore serve as a useful in vivo probe for comparing chromatin structure before and during such cellular events as transcription and replication.

The fragment contains two internal GATC sites at positions 852 and 1357 as defined in reference 42. The dark line represents the *Eco*RI fragment cloned into YRp7; the fine line represents yeast chromosomal sequences.

TABLE 2. Effect of in vivo adenine methylation on mitotic recombination[a]

Strain	No. of cultures	Recombination frequency (10^6)							
		Intragenic						Intergenic	
		lys2-1/*lys2-2*	*tryl-1*/*tryl-2*	*his7-1*/*his7-2*	*met13-c*/*met13-d*	*trp5-c*/*trp5-2*	*leul-c*/*leul-12*	*canl*[R]/*CANl*[s]	*cyh2*[R]/*CYH*[s]
MH16	4	1.2	2.2	4.1	9.1	13.1	40.4	314	246
MH16 [pMFH1]	8	4.1	3.5	2.6	17.5	21.4	72.1	395	475
Relative increase		3.4	1.6	0.6	1.9	1.5	1.8	1.3	1.9
Corrected MH16 [pMFH1]	8	5.0	4.0	3.4	19.3	25.3	86.3	408	942
Corrected relative increase		4.2	1.8	0.8	2.1	1.8	2.1	1.3	3.8

[a] Values given are a geometric mean frequencies. The relative increase indicates the ratio of geometric means at a given locus, for strains containing pMFH1, over the untransformed control. The corrected values are geometric means calculated after correcting for plasmid loss. The corrected values are determined from the following formula: total number of recombinants = (number of cells without plasmid × MH16 frequency) + (number of cells with plasmid × corrected frequency).

Although *Sau3A*I digests of the parental and transformant DNA were identical, the limit digests contained many specific bands at sizes larger than 2.3 kb. We have used several different preparations of *Sau3A*I from different commercial sources. All enzymes gave similar results. The complete *Mbo*I digest of untransformed DNA confirms that the large *Sau3A*I fragments must contain GATC sites (see argument above). One possible reason that certain GATC sites are not cleaved by *Sau3A*I is that *Sau3A*I is known to be sensitive to methylation of the cytosine in the GATC sequence (30, 41). Most eucaryotic cytosine methylation occurs at CG sequences; therefore GATCG sequences, where the C were methylated, would be resistant to cleavage by *Sau3A*I. The estimates for cytosine methylation in yeasts range from approximately 1% (17) to <0.03% (35). If the latter value were correct, there are potentially 2,000 5-methylcytosine residues in a diploid genome. Our data are consistent with the existence of small amounts of 5-methylcytosine in yeasts. It is important to note that the digestion pattern of DNA fragments smaller than 2.3 kb is indistinguishable for both *Sau3A*I and *Mbo*I (Fig. 2 and 3). We conclude that whatever is preventing *Sau3A*I from completely digesting yeast DNA is not present in all GATC sequences in the population because the limit digest is often reached.

The central role of 6-methyladenine in recombination and mismatch repair in *E. coli* led us to determine the response of a eucaryotic cell to this modified base. Exogenous alkylating agents, such as methyl methanesulfonate, stimulate recombination and mutation in yeasts, but they do not primarily methylate the N6 position of adenine. Methylation of adenine in GATC sequences had relatively little effect on meiotic recombination in yeast cells. Meiotic cells containing the *dam* clone demonstrate no significant effect on map distances; we conclude that adenine methylation does not affect meiotic crossing-over. We have not yet proven that meiotic gene conversion is unaffected, but we consider it probable that there will be little effect. Fogel et al. (10) have shown that meiotic gene conversion and crossing-over are correlated; they may reflect alternative aspects of a single recombination process. Since meiotic crossing-over shows little change in *dam* transformants, we feel it likely that meiotic gene conversion will respond similarly.

In the presence of the *dam* gene mitotic recombination does show a 2.2-fold average increase after correction for plasmid loss. This observation extends to gene conversion at five of six loci examined and crossing-over at two loci. We suspect that these effects are nonspecific rather than due to GATC sequences being directly involved in yeast recombination per se. In other words, the stimulation of recombination comes from the addition of a methyl group to adenine, rather than the modification of a specific sequence (see below). The increase in mitotic recombination might result from double-stranded breaks generated by overlapping excision tracts on opposite DNA strands (43). (In contrast, *E. coli* strains with decreased amounts of methylation [i.e., *dam*$^-$] are hyperrecombinogenic [37].) Alternatively, single-stranded gaps created by repair of 6-methyladenine might themselves stimulate mitotic recombination. To test whether heteroduplex formation and correction is stimulated by in vivo 6-methyladenine, it would be useful to look at recombination frequencies in strains deficient in heteroduplex correction (such as *cor* mutations [9]) or excision repair (e.g., *radl*, *rad3*) to determine if frequencies were still elevated.

Mutation frequencies were increased 1.5-, 1.5-, and 25-fold at three loci in the transformants containing the *dam* clone; two other loci exhibited very slight decreases. These effects are substantially less than the 10- to 300-fold increase shown in *E. coli* strains which overproduce *dam* (19). Two explanations of the slight mutagenic effect of 6-methylade-

TABLE 3. Effect of in vivo adenine methylation on meiotic recombination[a]

Map interval	*DAM*-containing tetrads					Total tetrads					Standard map distance	
	P	N	T	Xp	Xe	P	N	T	Xp	Xe	Xp	Xe
lys2–tyrl	9	0	10	26.3	26.5	19	2	20	39.0	40.8	37.3	38.7
tyrl–his7	7	0	12	31.6	32.2	15	0	26	31.7	32.4	44.2	47.2
ade5–met13	3	2	13	69.4	97.1	10	4	25	62.8	77.4	68.7	94.4
met13–cyh2	9	1	7	38.2	39.8	22	1	14	27.0	27.3	15.0	14.8
cyh2–trp5	2	0	16	44.4	47.4	7	2	30	53.8	60.6	40.4	42.4
trp5–leul	7	0	3	15.0	14.8	15	0	8	17.4	17.2	17.6	17.4

[a] P, N, and T refer to parental, nonparental, and tetra-type tetrads, respectively. Xp is the map distance calculated by Perkins' (33) formula. Xe is the map distance calculated via Ma and Mortimer (23). Standard map distances are from Mortimer and Schild (32). The *DAM*-containing tetrads are those that segregated 4+:0−, 3+:1−, and 2+:2− for pMFH1, whereas the total tetrads include the 1+:3− and 0+:4− tetrads.

TABLE 4. Effect of in vivo adenine methylation on mutation[a]

Strain	No. of cultures	Mutation frequency (10^8)				
		Reversion				Forward
		MET13	*LYS2*	*LEU1*	*HIS7*	($canl^R$)
+	3	0.89	83	2.9	2.4	220
+ [pMFH1]	6	8.0	120	2.3	1.6	330
Relative increase		9.0	1.4	0.8	0.7	1.5
Corrected + [pMFH1]	6	21.8	124	2.3	1.4	340
Corrected relative increase		24.5	1.5	0.8	0.6	1.5

[a] Values are geometric mean frequencies. Reversion frequencies represent auxotrophic revertants of the loci in Table 1; forward mutation is a measurement of $CAN1^s \rightarrow can1^R$. For the method used to correct for plasmid loss, refer to footnote *a*, Table 2.

nine in yeast cells are that it mispairs frequently or is recognized and acted upon by error-prone repair. Methylation at the N-1, N-3, and N-7 positions of adenine is potentially mutagenic (in both procaryotic and eucaryotic cells) because either base pairing is directly affected or specific glycosylases remove the alkylated base followed by error-prone repair (39). Whereas methylation at the 6 position of adenine is presumably not mutagenic in *E. coli*, there is a possibility that it might be in eucaryotes. The electrophilic nature of the methyl group affects the keto-enol equilibrium of adenine and could thereby affect the frequency of mispairing during replication. If true, mutations found in yeast strains containing *dam* should preferentially occur at GATC sequences (i.e., in a targeted fashion). If, on the other hand, 6-methyladenine stimulates error-prone repair, mutations might occur anywhere, depending on the number of nucleotides degraded and resynthesized.

The effects of methylation at the N6 position of adenine in yeast GATC sequences are substantially less severe than the response to other adenine-alkylating mutagens such as methyl methanesulfonate. This may be due to the relative frequency of GATC sequences compared to the probability of methylating random adenines and other bases. Alternatively, it may reflect that 6-methyladenine is less recombinogenic and mutagenic than 3-methyladenine, the primary adduct formed by methyl methanesulfonate (39). We tend to favor the former explanation because it more easily explains the discrepancy in the observed effects on mutation. The *met13* locus, for example, displayed a 25-fold increase in reversion, whereas reversion at the *his7* locus was decreased by 1.7-fold. This would be understandable if the number of susceptible GATC sequences varied from locus to locus.

We are currently testing the effects of mutations in the various yeast repair epistasis groups (18) on the responses to in vivo *dam* adenine methylation. It has been proposed that nucleotide excision is the major DNA metabolic pathway by which alkylation adducts in DNA are eliminated (16). To examine this, an integrating plasmid has been constructed to avoid the complications of plasmid loss. Strains containing an expressed, integrated *dam* gene will allow us to easily control for copy number and should permit an examination of the effects of heterologous *dam* methylation in cells deficient in the ability to repair DNA damages.

ACKNOWLEDGMENTS

These experiments were supported by Public Health Service grant R01-GM 29172 from the National Institutes of Health and a career development award from the Schweppe Foundation of Chicago to R.E.M.

We thank Rich Kostriken for kindly supplying the *dam*-containing plasmid pRK99 and Bob Blumenthal for helpful discussions during the course of this work and for careful criticism of early versions of this manuscript. L. Dorsey, R. Sherman, and V. Thiel provided expert typographical skills.

LITERATURE CITED

1. **Adams, R. L. P., and R. H. Burdon.** 1983. DNA methylases, p. 119–144. *In* S. T. Jacob (ed.), Enzymes of nucleic synthesis and modification, vol. 1. DNA enzymes. CRC Press, Cleveland.
2. **Bale, A., M. d'Alarco, and M. G. Marinus.** 1979. Characterization of DNA adenine methylation mutants of *Escherichia coli* K 12. Mutat. Res. **59:**157–165.
3. **Brooks, J. E., R. M. Blumenthal, and T. R. Gingeras.** 1983. The isolation and characterization of the *Escherichia coli* DNA adenine methylase (*dam*) gene. Nucleic Acids Res. **11:**837–851.
4. **Doerfler, W.** 1983. DNA methylation and gene activity. Annu. Rev. Biochem. **52:**93–124.
5. **Esposito, M. S., and R. E. Esposito.** 1969. The genetic control of sporulation in *Saccharomyces*. I. The isolation of temperature-sensitive sporulation-deficient mutants. Genetics **61:**79–89.
6. **Esposito, M. S., and J. E. Wagstaff.** 1981. Mechanisms of mitotic recombination, p. 341–370. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
7. **Fangman, W. L., and V. A. Zakian.** 1981. Genome structure and replication, p. 27–58. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
8. **Feher, Z., A. Kiss, and P. Venetiano.** 1983. Expression of a modification methylase gene in yeast. Nature (London) **302:**266–268.
9. **Fogel, S., R. K. Mortimer, and K. Lusnak.** 1981. Mechanisms of meiotic gene conversion, or "wanderings on a foreign strand," p. 289–340. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
10. **Fogel, S., R. K. Mortimer, K. Lusnak, and F. Tavares.** 1979. Meiotic gene conversion: a signal of the basic recombination event in yeast. Cold Spring Harbor Symp. Quant. Biol. **43:**1325–1342.
11. **Gafner, J., E. M. De Robertis, and P. Philippen.** 1983. Delta-sequences in the 5′ non-coding region of yeast transfer RNA genes. EMBO J. **2:**583–591.
12. **Gelinas, R. E., P. A. Myers, and R. J. Roberts.** 1977. Two sequence specific endonucleases from *Moraxella bovis*. J. Mol. Biol. **114:**169–179.
13. **Glickman, B. W., P. van der Elsen, and M. Radman.** 1978. Induced mutagenesis in *dam*⁻ mutants of *Escherichia coli*: a role for 6-methyladenine residues in mutation avoidance. Mol. Gen. Genet. **163:**307–312.
14. **Golin, J. E., and M. S. Esposito.** 1977. Evidence for joint genic control of spontaneous mutation and genetic recombination during mitosis in Saccharomyces. Mol. Gen. Genet. **150:**127–135.
15. **Grier, G. E., and P. Modrich.** 1979. Recognition sequence of the *dam* methylase of *Escherichia coli* K12 and mode of cleavage of *Dpn* I endonuclease. J. Biol. Chem. **254:**1408–1413.
16. **Hanawalt, P. C., P. K. Cooper, A. K. Ganesan, and C. A. Smith.** 1979. DNA repair in bacteria and mammalian cells. Annu. Rev. Biochem. **48:**783–836.
17. **Hattman, S., C. Kenny, L. Berger, and K. Pratt.** 1978. Comparative study of DNA methylation in three unicellular eucaryotes. J. Bacteriol. **135:**1156–1157.
18. **Haynes, R. H., and B. A. Kunz.** 1981. DNA repair and mutagenesis in yeast, p. 371–414. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **Herman, G. E., and P. Modrich.** 1981. *Escherichia coli* K-12

clones that overproduce *dam* methylase are hypermutable. J. Bacteriol. **145**:644–646.
20. **Hinnen, A., J. B. Hicks, and G. R. Fink.** 1978. Transformation of yeast. Proc. Natl. Acad. Sci. U.S.A. **75**:1929–1933.
21. **Lacks, S., and B. Greenberg.** 1975. A deoxyribonuclease of *Diplococcus pneumoniae* specific for methylated DNA. J. Biol. Chem. **250**:4060–4066.
22. **Lacks, S., and B. Greenberg.** 1977. Complementary specificity of restriction endonucleases of *Diplococcus pneumoniae* with respect to DNA methylation. J. Mol. Biol. **114**:153–168.
23. **Ma, C., and R. K. Mortimer.** 1983. Empirical equation that can be used to determine genetic map distances from tetrad data. Mol. Cell. Biol. **3**:1886–1887.
24. **Malone, R. E., and M. F. Hoekstra.** 1984. Relationships between a hyper-rec mutation (*rem1*) and other recombination and repair genes in yeast. Genetics **107**:33–48.
25. **Malone, R. E., and D. Hyman.** 1983. Interactions between the *MAT* locus and the *rad 52-1* mutation in yeast. Curr. Genet. **7**:439–447.
26. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
27. **Marinus, M. G., and E. B. Konrad.** 1976. Hyper-recombination in *dam* mutants of *Escherichia coli* k12. Mol. Gen. Genet. **149**:273–277.
28. **Marinus, M. G., and N. R. Morris.** 1973. Isolation of deoxyribonucleic acid methylase mutants of *Escherichia coli* K-12. J. Bacteriol. **114**:1143–1150.
29. **Marinus, M. G., and N. R. Morris.** 1975. Pleiotrophic effects of a DNA adenine methylation mutation (*dam-3*) in *Escherichia* K12. Mutat. Res. **28**:15–26.
30. **Modrich, P., and R. J. Roberts.** 1982. Type-II restriction and modification enzymes, p. 109–154. *In* S. M. Linn and R. J. Roberts (ed.), Nucleases. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Mortimer, R. K., and D. Hawthorne.** 1969. Yeast genetics, p. 385. *In* D. H. Rose and J. S. Harrison (ed.), The Yeasts, vol. 1. Academic Press, Inc., New York.
32. **Mortimer, R. K., and D. Schild.** 1980. Genetic map of *Saccharomyces cerevisiae*. Microbiol. Rev. **44**:519–571.
33. **Perkins, D. D.** 1949. Biochemical mutants of the smut fungus *Ustilago maydis*. Genetics **34**:607–626.
34. **Plischke, M., R. Von Borstel, R. Mortimer, and W. Cohn.** 1976. Genetic markers and associated gene products in *S. cerevisiae*, p. 767–832. *In* G. Fasman (ed.), Handbook of biochemistry and molecular biology, CRC Press, Cleveland.
35. **Proffitt, J. H., J. R. Davie, D. Swinton, and S. Hattman.** 1984. 5-Methylcytosine is not detectable in *Saccharomyces cerevisiae* DNA. Mol. Cell. Biol. **4**:985–988.
36. **Pukkila, P. J., J. Peterson, G. Herman, and M. Meselson.** 1983. Effects of high levels of DNA adenine methylation on methyl-directed mismatch repair in *Escherichia coli*. Genetics **104**:571–582.
37. **Radman, M., R. E. Wagner, B. W. Glickman, and M. Meselson.** 1980. DNA methylation, mismatch correction and genetic stability, p. 121–130. *In* M. Alacevic (ed.), Progress in environmental mutagenisis. Elsevier, Amsterdam.
38. **Ratzkin, B., and J. Carbon.** 1977. Functional expression of cloned yeast DNA in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:487–491.
39. **Singer, B.** 1982. Mutagenesis from a chemical perspective. *In* J. F. Lemontt and W. M. Generoso (ed.), Molecular and cellular mechanisms of mutagenesis. Plenum Press, New York.
40. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by electrophoresis. J. Mol. Biol. **98**:503–517.
41. **Streek, R. E.** 1980. Single-strand and double-strand cleavage at half-modified and fully modified recognition sites for the restriction nucleases *Sau* 3A and *Taq* I. Gene **12**:267–275.
42. **Struhl, K., D. T. Stinchcomb, S. Scherer, and R. W. Davis.** 1976. High frequency transformation of yeast: autonomous replication of hybrid DNA molecules. Proc. Natl. Acad. Sci. U.S.A. **76**:1035–1039.
43. **Szostak, J., T. Orr-Weaver, R. Rothstein, and F. Stahl.** 1983. The double-strand break repair model for recombination. Cell **33**:24–35.
44. **Tschumper, G., and J. Carbon.** 1980. Sequence of a yeast DNA fragment containing a chromosomal replicator and the *TRP1* gene. Gene **10**:157–166.
45. **Wagner, R., and M. Meselson.** 1976. Repair tracts in mismatched DNA heteroduplexes. Proc. Natl. Acad. Sci. U.S.A. **73**:4135–4139.
46. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragment to diazobenyloxymethyl paper and rapid hybridization using dextran sulfate. Proc. Natl. Acad. Sci. U.S.A. **76**:3683–3687.