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Excision Repair Functions in *Saccharomyces cerevisiae* Recognize and Repair Methylation of Adenine by the *Escherichia coli dam* Gene

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Unlike the DNA of higher eucaryotes, the DNA of *Saccharomyces cerevisiae* (bakers' yeast) is not methylated. Introduction of the *Escherichia coli dam* gene into yeast cells results in methylation of the N-6 position of adenine. The UV excision repair system of yeast cells specifically responds to the methylation, suggesting that it is capable of recognizing modifications which do not lead to major helix distortion. The UV repair functions examined in this report are involved in the incision step of pyrimidine dimer repair. These observations may have relevance to the rearrangements and recombination events observed when yeast or higher eucaryotic cells are transformed or transfected with DNA grown in *E. coli*.

DNA methylation has profound and varied effects on cellular metabolism. Higher eucaryotes exhibit methylation of specific cytosine molecules during the regulation of gene expression (2, 9). The genomes of lower eucaryotes, such as *Drosophila melanogaster* (1), *Saccharomyces cerevisiae* (10, 13, 20), and various other fungi (6), have a low or undetectable 5-methylcytosine content, suggesting that DNA methylation may not be involved in gene control in these organisms. Procaryotes also use methylation, in restriction-modification systems (19) and in mismatch repair recognition (21). For example, the *Escherichia coli dam* gene product methylates the N-6 position of adenine residues in 5′-GATC-3′ sequences as part of the methyl-directed mismatch repair system (21). Insults by exogenous methylating agents lead to increased mutation and recombination rates and cell death in both eucaryotes and procaryotes (14). Considering the variety of cellular responses to DNA methylation, we have been interested in determining whether and how *S. cerevisiae* reacts to DNA methylation in vivo by the *E. coli dam* gene product. The *E. coli dam* product methylates at a position that is different from that of most exogenous agents, such as methyl methanesulfonate. Previously, we have shown that the yeast cell expresses a cloned *dam* gene and methylates its chromosomal DNA (15). Methylation causes a general twofold increase in mitotic recombination and a moderate increase in mutation frequencies at some loci. In this report, we demonstrate that *S. cerevisiae* actively removes *N*-6-methyladenine from its genome by using the excision repair epistasis group. We infer that the yeast excision repair pathway can respond not only to large DNA damage such as UV-induced cyclobutyl rings (5) or DNA strand cross-links (3) but also to a potentially non-helix-distorting adduct such as *N*-6-methyladenine. (This work has been submitted by M.F.H. in partial fulfillment of the Ph.D. degree requirements of Loyola University.)

Yeast cells have at least three repair groups for coping with damage to normal DNA structure (reviewed in reference 14). These groups are named by a prominent locus in each and include (i) the *RAD3* group, the members of which are defined by UV-sensitive mutations and participate in UV excision repair; (ii) the *RAD52* group, the members of which are defined by X-ray-sensitive mutations and participate in recombination repair, many loci of which are also involved in mitotic or meiotic recombination or both; and (iii) the *RAD6* group, the members of which affect both UV and X-ray sensitivity and participate in error-prone repair. To determine how the yeast cell responds to *dam*-produced in vivo adenine methylation, we integrated the *dam* gene into the yeast genome and examined cells containing the *dam* gene along with mutations in error-prone (*rad6-1*), recombination (*rad52-1*), or excision (*rad1-2* and *rad3-2*) repair. Yeast spheroplasts were transformed with YIpDAM (Fig. 1), and stable integrants were selected. Tetrad analysis demonstrated that the integrated *dam* gene was stable and expressed (Fig. 2). Spores from a hemizygous *dam* strain were dissected, and DNA from the resulting spore clones was digested by *Dpn*I (which cleaves at GATC only when the adenine molecule is methylated [16, 17]). In all cases examined (five tetrads), the *dam* gene cosegregated with the *URA*$^+$ gene. Specific methylation in a defined chromosomal region was examined by Southern blot analysis by using as a probe a 1.4-kilobase *Eco*RI fragment containing the *TRP1* gene (15). Similar to previous results for a high-copy-number episomal plasmid, not all potential GATC sites were digestible by *Dpn*I. If the *TRP1* region were completely methylated at GATC, fragments of 885, 515, and <84 base pairs would be expected. Larger fragments (>885 base pairs) must contain unmethylated GATC sites. This could be caused by inefficient functioning or low levels of the *E. coli* gene product in yeast cells resulting in incomplete methylation, by higher-order chromosomal structures protecting GATC sequences from the *dam* enzyme, or by the active removal of methyl groups by a yeast repair system or systems.

To test the latter possibility, diploids heterozygous for both *dam* and various *rad* mutations were constructed. Dissection of tetrads and subsequent analysis of segregants indicated that all double mutant combinations were viable (Table 1). Thus, *N*-6-methyl adducts in adenine are not lethal

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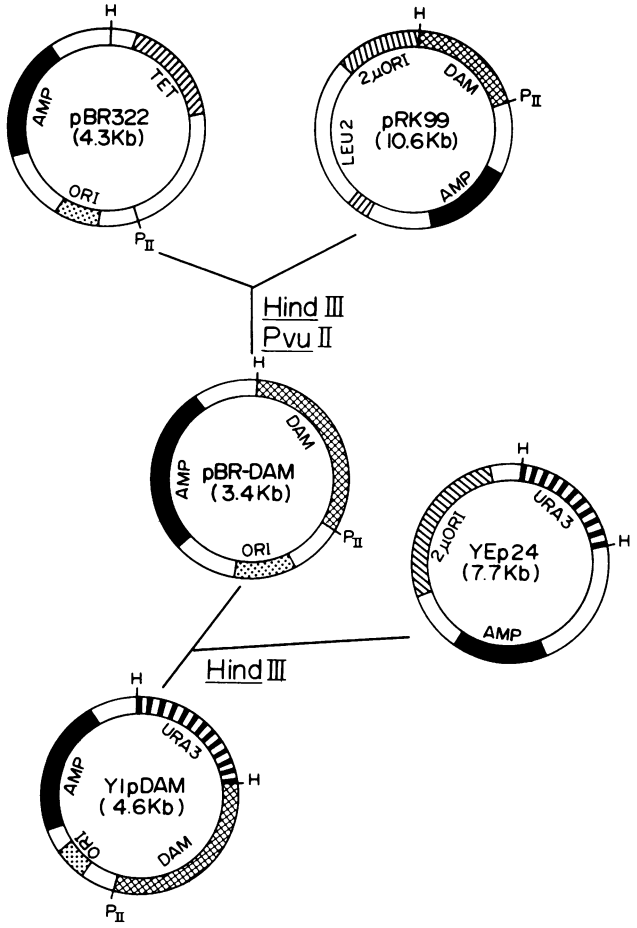

FIG. 1. Construction of YIpDAM. Plasmids pBR322 and pRK99 were each digested by *Hin*dIII and *Pvu*II; the 2.3-kb *Hin*dIII-*Pvu*II band from pBR322 and the 1.1-kb *dam*-containing fragment from pRK99 were extracted from low-melting-temperature agarose, ligated, and transformed into *E. coli* HB101. The 1.1-kb *URA3* *Hin*dIII fragment from YEp24 was introduced into the unique *Hin*dIII site of pBR-DAM, and ampicillin-resistant, uracil-independent transformants of MC1066 (11) were selected on minimal medium containing ampicillin.

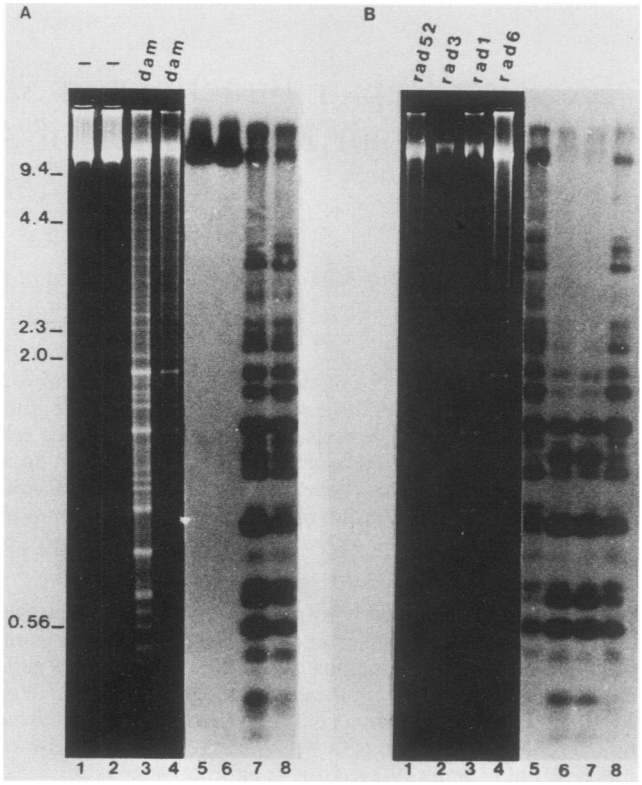

FIG. 2. Analysis of *dam* methylation in wild-type and repair deficient strains. (A) Lanes 1–4 represent total yeast DNA isolated from a wild-type strain heterozygous for the *dam* insert and digested with *Dpn*I. Four spores from a tetrad are shown: MH69-3A (1), MH69-3B (2), MH69-3C (3), MH69-3D (4). Lanes 5–8 represent the Southern blot analysis of the same DNAs. The probe was the *Eco*RI 1.4-kb *TRP1* fragment from YRp7. The relevant genotypes are shown above the lanes. (B) Lanes 1–4 represent total yeast DNA digested with *Dpn*I from four different *rad* mutants, all of which contained the *dam* gene. Lanes 5–8 represent the Southern blot analysis of the same DNAs. The probe was the *TRP1* fragment used in panel A. Relevant genotypes are shown above the lanes. Size standards (λ digested by *Hin*dIII) are shown on the left.

in haploid strains containing mutations in any of the three major yeast repair epistasis groups.

The *dam* gene is expressed and active in all of these strains (Fig. 2). DNA from all *dam rad* strain combinations is susceptible to *Dpn*I digestions, whereas DNA from strains lacking the *dam* gene is not digested by *Dpn*I. Southern blot analysis of the *TRP1* region in *rad6-1* and *rad52-1* strains reveals a hybridization pattern similar to that of the wild-type *dam*-containing haploid parent. However, DNA from *rad3* and *rad1* strains containing *dam* exhibits almost complete *Dpn*I susceptibility (Fig. 2). Although there is clearly some DNA present at sizes greater than 885 base pairs, indicating some unmethylated sites, there is a pronounced increase in the amount of digestion compared with wild-type strains. (The existence of the larger bands could be caused by some residual activity in the *rad1* or *rad3* mutants, by a low-level repair system, or by incomplete methylation.) This suggests not only that there is sufficient *dam* gene product present in yeast cells to methylate essentially all GATC sequences, but also that chromosome structure may not play a role in susceptibility to methylation. Analysis of complete tetrads from hemizygous *dam*, heterozygous *rad1* (or *rad3*) diploids by *Dpn*I digestion confirmed the observation that excision repair mutants were deficient in removing *N*-6-methyl groups. Tetratype tetrads with all four possible segregants were analyzed; DNA from *dam rad1* and *dam rad3* was always digested to a much greater extent by *Dpn*I than were Rad$^+$ *dam*-containing siblings (Fig. 3). Strains deficient in excision repair clearly allowed more 6-methyladenine to accumulate, indicating that wild-type cells are able to recognize and remove 6-methyladenine from chromosomal DNA by using excision repair functions previously known to act on damages such as UV-induced cyclobutyl dimers and psoralen cross-linking (14). Thus, at least part of the excision repair group appears to be involved in general repair in yeast cells and possibly involved in the initial recognition and reaction to many (and quite subtle) DNA structural changes.

For *E. coli*, adenine methylation is a normal occurrence, and the absence of *N*-6-methyladenine leads to hyper-recombination and hypermutation (4). In yeast cells, the opposite appears to be true. Introduction of the *dam* gene to *S. cerevisiae* modestly stimulates mitotic recombination and (at some loci) mutation (15). The yeast cell recognizes and

TABLE 1. Segregation analysis for $dam^+ \frac{RADX}{radX}$ diploids[a]

Diploid genotype	Segregant genotype (no.)			
	RADX dam$^+$	*RADX*	*radX dam*$^+$	*radX*
dam $\frac{+}{rad52\text{-}1}$	13	25	22	13
dam $\frac{+}{rad6\text{-}1}$	22	12	10	20
dam $\frac{+}{rad3\text{-}2}$	10	19	17	12
dam $\frac{+}{rad1\text{-}2}$	14	22	20	14

[a] Wild-type strains containing an integrated *dam* gene were mated with the various *rad* mutations listed. The resulting diploids were sporulated, and tetrads were dissected by standard procedures (15). Segregants were analyzed for the presence of *dam* and *rad* mutations. *radX* refers to the *rad* mutation listed under the diploid genotype for a given row.

responds to the methyl adduct by its excision repair system. Current evidence suggests that *rad1* and *rad3* mutants are blocked at a stage involved in the incision step of excision repair (22, 23). The observed increases in both mitotic recombination and mutation caused by *dam* methylation suggest that it is possible that the error-prone or recombination repair systems may act on the intermediate created by excision repair functions. This is directly testable by analyzing multiple mutants.

An interesting technical paradox is generated by this study with respect to the transfection of higher eucaryotic cells. Transformation studies of yeast cells and transfection studies of mouse, monkey, and human cells have recently demonstrated that the input DNA accumulates base substitutions and rearrangements (7, 8, 18, 24). High rates of recombination have also been reported for the transfected DNA (11, 12). A common feature of many of these experiments is that the introduced DNA was propagated in *E. coli* before transfection; the DNA therefore contained *dam*-produced methylation. If the methylated DNA is recognized and acted upon by excision repair, the nicks and gaps generated may stimulate recombination and mutation. Using a *dam*$^-$ *E. coli* strain as a host for plasmid preparation may seem to be a simple solution to this problem. However, these *E. coli* strains are hypermutable. This creates the problem of preexisting mutations being present in the plasmid population before introduction to eucaryotic cells.

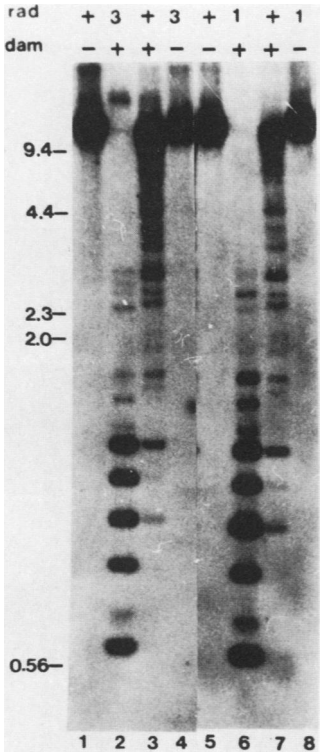

FIG. 3. Segregation analysis of *dam*-methylation in tetratype tetrads for *dam* and *rad1* or *rad3* strains. *Dpn*I-digested DNA was from the following strains: MH73-3A (1), MH73-3B (2), MH73-3C (3), MH73-3D (4), MH75-8A (5), MH75-8B (6), MH75-8C (7), MH75-8D (8). Relevant genotypes are given above each lane. DNA isolation, digestion, and Southern blot analysis were done as described in the text. The probe was the *TRP1* fragment described in the legend to Fig. 2.

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