Purification and Properties of the Alkylation Repair DNA Glycosylase Encoded by the *MAG* Gene from *Saccharomyces cerevisiae*†

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ABSTRACT: The *MAG* gene of *Saccharomyces cerevisiae* encodes an alkylation repair DNA glycosylase whose sequence is homologous to the AlkA DNA glycosylase from *Escherichia coli*. To investigate the biochemical properties of MAG in comparison to AlkA, MAG was expressed in *E. coli* and purified to electrophoretic homogeneity. N-Terminal sequencing of the purified protein identified the translational start site which corresponded to that predicted previously from the nucleotide sequence. Polyclonal antibodies raised against MAG inhibited the enzymatic activity of MAG, but not that of AlkA, and vice versa, implying that the structures of the active site regions of these enzymes are antigenic, but sufficiently different to have different epitopes. Kinetic analysis of base excision from DNA exposed to [^{3}H]methyl-*N*-nitrosourea and [^{3}H]dimethyl sulfate showed that MAG was as effective as AlkA in removing 3-methyladenine, 7-methylguanine, and 7-methyladenine. However, the purified MAG enzyme did not catalyze the excision of O^2-methylthymine, which is a major substrate for AlkA. Furthermore, 3-methylguanine was excised 20−40 times more slowly by MAG than by AlkA. The kinetics of 3-methylguanine excision by MAG were found to be similar to the low rate of 3-methylguanine excision catalyzed by 3-methyladenine DNA glycosylase I (Tag) of *E. coli*. Expression of MAG in *alkA* mutant cells did not effectively restore alkylation resistance of the mutant as did AlkA itself. It thus appears that MAG is a less versatile enzyme than AlkA in spite of the sequence relationship and may have a similar function in yeast as the nonhomologous Tag enzyme in *E. coli*.

DNA glycosylases for the removal of alkylated bases were first detected in *Escherichia coli* (Lindahl, 1976; Laval, 1977) and have subsequently been found in all other organisms investigated, including yeast, mammalian, and plant cells [for a review, see Sakumi and Sekiguchi (1990)]. The physiological significance of such enzymes in protecting cells from alkylation exposure was first shown by isolation and characterization of *E. coli* mutants deficient in 3-methyladenine DNA glycosylase activity (Karran et al., 1980; Evensen & Seeberg, 1982; Clarke et al., 1984). These mutants proved to be extremely sensitive toward simple methylating agents such as methyl methanesulfonate and *N*-methyl-N′-nitro-*N*-nitrosoguanidine. Further characterization of the mutants revealed the presence of two enzymes of this type in *E. coli*, one being constitutively expressed and encoded by *tag*, and the other being inducible and part of the adaptive response to alkylation damage (Evensen & Seeberg, 1982; Karran et al., 1982). The latter enzyme was identified as the product of the *alkA* gene (Evensen & Seeberg, 1982) which was originally identified by mutant analysis as a gene locus required for alkylation resistance (Yamamoto et al., 1978).

The isolation of *E. coli* double mutants lacking both Tag and AlkA has also allowed the cloning and characterization of the corresponding gene functions in other organisms. The double mutant has up to 7 orders of magnitude lower plating efficiency on agar medium containing methyl methanesulfonate (MMS)[1] than wild-type cells. This phenotypic property has been exploited to select for gene sequences from any source expressing such enzymes in *E. coli* simply by functional complementation of the alkylation-sensitive phenotype of the mutants. This approach was first applied in the cloning of the endogenous genes *tag* and *alkA* from the *E. coli* genome (Clark et al., 1984), but has since been used to select alkylbase DNA glycosylase genes from *Micrococcus luteus* (Pierre & Laval, 1986), *Saccharomyces cerevisiae* (Chen et al., 1989, 1990; Berdal et al., 1990), *Schizosaccharomyces pombe* (Bjørås et al., in preparation), rat (O'Connor & Laval, 1990), mouse (Engelward et al., 1993), human cells (Chakravarti et al., 1991; O'Connor & Laval, 1991; Samson et al., 1991), and quite recently also from *Arabidopsis thaliana* (Santerre & Britt, 1994). From sequence comparisons, it appears that the glycosylase encoded by the *S. cerevisiae* gene *MAG* is homologous to the *E. coli* AlkA enzyme (Berdal et al., 1990; Chen et al., 1990) whereas the mammalian and plant enzymes are similar with little or no relevant sequence homology to the yeast or bacterial sequences (Santerre & Britt, 1994).

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[1] Abbreviations: MMS, methyl methanesulfonate; DMS, dimethyl sulfate; NMU, *N*-nitro-*N*-methylnitrosourea; m^{3}A, 3-methyladenine; m^{3}G, 3-methylguanine; m^{7}G, 7-methylguanine; m^{7}A, 7-methyladenine; m^{2}T, O^2-methylthymine; AP, apurinic or apyrimidinic.

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In the present study, we have expressed the yeast MAG DNA glycosylase in *E. coli*, purified the enzyme to homogeneity, and compared its properties to those of the homologous AlkA enzyme of *E. coli*. Significant differences exist between the functional role of these two enzymes in spite of the sequence similarity.

MATERIALS AND METHODS

Plasmid Vectors and Bacterial Strains. The *MAG* gene bacterial expression vector pBKY143 was constructed by cloning the 2.2 kb *Eco*RI fragment of pBKY142 (Berdal et al., 1990) into the *Eco*RI site of pKK223-3 (Pharmacia) which carries the strong hybrid *tac* promoter. The translational start site in this vector is located 182 bp from the transcriptional start site. Expression of MAG in strain JM103 from the *tac* promoter is induced 2-fold by induction with IPTG. Plasmid pBKY141 contains the 2.2 kb *MAG* gene fragment in opposite orientation relative to the *tac* promoter in pKK223-3. The relative expression level of MAG obtained from the three plasmids pBK141, pBK142, and pBK143 is 1:5:10, respectively, and the 3-methyladenine DNA glycosylase activity of MAG from pBK143 is comparable to that of AlkA obtained from plasmid pBK161 (Kaasen et al., 1986). Bacterial strains used were BK2118 (*tag-2, alkA1*) and MS23 (*alkA1*; Yamamoto et al., 1978).

Purification of MAG Protein. Cells (BK2118 transformed by pBKY143) were grown in K-medium at 37 °C in 8 L batches until a density of 8×10^8/mL, and extracts were prepared by a combination of plasmolysis and lysozyme treatment as previously described (Seeberg, 1978). The cell extract (fraction I) was applied to an Affigel-blue (Bio-Rad) column (1 × 10 cm) equilibrated with buffer A [50 mM MOPS (pH 7.5)/1 mM EDTA/10 mM mercaptoethanol/100 mM KCl/20% glycerol] and eluted with a linear gradient of 0.1−1.0 M KCl in buffer A. Active fractions (eluting at 0.35 M KCl) were pooled (fraction II), desalted on Sephadex G-25M (Column PD-10, Pharmacia) equilibrated with buffer B [50 mM MES (pH 6.5)/1 mM EDTA/10 mM mercaptoethanol/100 mM KCl/20% glycerol], and applied to a MonoS column (HR5/5; Pharmacia) equilibrated with buffer B. The column was eluted with a linear gradient of 0.1−0.8 M NaCl in buffer B, and active fractions eluting at 0.35 M NaCl (fraction III) were pooled and diluted with 2 volumes of buffer C [100 mM Tris (pH 8.5)/1 mM EDTA/10 mM mercaptoethanol/100 mM KCl/20% glycerol]. Fraction III was applied to a MonoQ column (HR 5/5; Pharmacia) equilibrated with buffer C. The MAG glycosylase was eluted at 0.25 M KCl with a linear gradient from 0.1 to 0.5 M KCl (fraction IV). Active fractions diluted with 2 volumes buffer B were applied to MonoS and eluted as described above for the first MonoS chromatography (fraction V).

DNA Substrates. Calf thymus DNA (Sigma) was purified by equilibrium gradient centrifugation in $CsCl_2$ (Bjelland & Seeberg, 1987) and exposed to [^{3}H]dimethylsulfate (1.2 Ci/mmol) or [^{3}H]-*N*-nitroso-*N*-methylurea (16.4 Ci/mmol) in 10 mM NaCacodylate, pH 7.0 for 1 h at 37 °C. After exposure, the DNA was purified as described (Bjelland & Seeberg, 1987), and the specific activities were 6−12 000 dpm/μg and 8000 dpm/μg, respectively.

Enzyme Assays. AP endonuclease activity was assayed as described (Bjelland & Seeberg, 1987) using acid-depurinated DNA as substrate. Methyltransferase assays were performed as described by Margison et al. (1985). Alkylbase DNA glycosylase activity was measured by the method of Riazuddin and Lindahl (1978) as slightly modified (Bjelland & Seeberg, 1987). Standard reaction mixtures contained 0.3 μg of DNA [5 μL in 0.01 M Tris·HCl (pH 8.0)/1 mM EDTA], 70 mM MOPS (pH 7.5)/1 mM EDTA/1 mM dithiothreitol/5% glycerol, and enzyme in a total volume of 50 μL. One unit is defined as the amount of enzyme required to release 1 pmol of methylated base from dimethyl sulfate-treated DNA in 30 min at 37 °C. The AlkA enzyme was purified as described (Bjelland et al., 1994).

HPLC Separation of Methylated Bases. Excision of the various methylated bases released from DMS- or MNU-treated DNA was analyzed on reverse-phase HPLC. After ethanol precipitation, the supernatant containing the excised methylated bases was concentrated by evaporation and applied to a Varian micropack column (MCH-10). The methylated bases were eluted (1 mL/min) by a linear gradient of 0−10% methanol versus 0.1 M triethylammonium acetate, pH 6.2, for 15 min followed by 10−100% methanol versus 0.1 M triethylammonium acetate for 25 min. In this system, the retention times were 13 min for m^3G, 17 min for m^7G, 18 min for m^2T, 21 min for m^7A, and 23 min for m^3A. To further resolve m^7A from contaminating m^3A, the m^7A fraction was rechromatographed on the reverse-phase column using the same conditions. The small m^2T fraction, contaminated by both m^7G and m^7A, was rechromatographed by anion-exchange HPLC (Particil-10 SCX) developed with 0.02 M ammonium formate, pH 4.0, in 6% methanol to 0.2 M ammonium formate, pH 4.0, in 8% methanol in 25 min at a flow rate of 2 mL/min. In this system, the retention times were 10 min for m^2T, 13 min for m^7G, and 18 min for m^7A.

Polyclonal Antibodies against MAG and AlkA. Purified MAG and AlkA proteins were used for immmunization of rabbits by standard procedures. The IgG fraction was concentrated from the serum supernatant by $(NH_4)_2SO_4$ precipitation with a cutoff of 30−50% saturation. The antibodies were further purified by the Pharmacia HiTrap protein A affinity column system using elution conditions of 0.1 M citric acid, pH 4.0. Finally, the antibodies were again precipitated with 50% $(NH_4)_2SO_4$, dialyzed, and dissolved in phosphate-buffered saline. For the enzyme inhibition experiments, the antibody was preincubated with the enzyme for 5 min at 4 °C to allow protein−antibody interaction prior to the enzymatic reaction.

Protein Sequencing. The amino acid sequence of the N-terminal end was analyzed on a 477A sequencer from Applied Biosystems equipped with an on-line 120A PTH analyzer.

Cell Survival Measurement. MMS sensitivity was monitored by plating appropriate dilutions of bacterial overnight cultures on LB agar containing different amounts of MMS as indicated (Kaasen et al., 1986).

RESULTS

Purification of the MAG Enzyme. The MAG protein was purified by a five-step procedure as indicated in Table 1. The protein was expressed from plasmid pBKY143 in *E. coli* strain BK2118 (*tag alkA*) to avoid any interference of endogenous *E. coli* alkylbase DNA glycosylase activity during purification. The enzyme was purified 2000-fold in 6% yield relative to the specific activity and enzyme amount

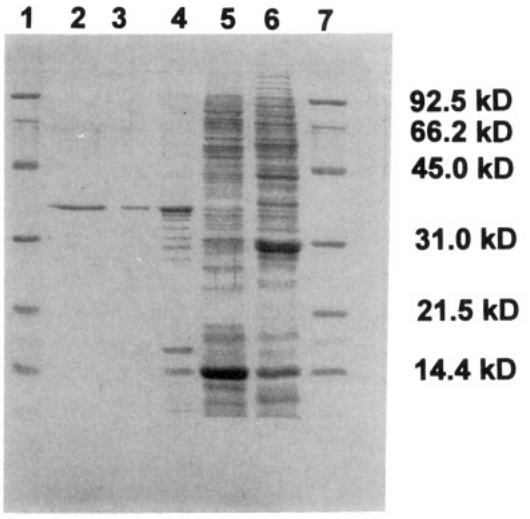

FIGURE 1: SDS−polyacrylamide (15%) gel electrophoresis of the different fractions obtained during purification of the MAG protein. Lanes: (1) molecular mass markers; (2) fraction V (Mono S); (3) fraction IV (Mono Q); (4) fraction III (Mono S); (5) fraction II (Affigel blue); (6) fraction I (extract); (7) molecular mass markers. Marker proteins were lyzosyme (14 kDa), soybean trypsin inhibitor (21.5 kDa), carbonic anhydrase (31 kDa), ovalbumin (45 kDa), bovine serum albumin (66.2 kDa), and phosphorylase B (92.5 kDa). The gel was stained with Coomassie blue.

Table 1: Purification of the *S. cerevisiae* MAG DNA Glycosylase

fraction	volume (mL)	protein (mg)	specific purification activity (units/mg)	recovery *x*-fold	recovery %
(I) protein extract	1220	990	14	1	100
(II) Affigel blue	98	60	180	13	80
(III) Mono S	3.5	0.3	18000	1300	43
(IV) Mono Q	2.5	0.1	20000	1400	15
(V) Mono S	1.5	0.03	28000	2000	6

recovered in the extract. Figure 1 shows SDS−polyacrylamide gel electrophoresis of the various fractions obtained during purification. The purified enzyme migrates as a protein of 34 kDa consistent with the molecular mass calculated from the sequence (Berdal et al., 1990; Chen et al., 1990). From the purification scheme, it appears that the MAG protein constitutes only about 0.05% of the protein in the cell extract (Table 1).

N-Terminal Sequence of the Purified Protein. The N-terminal amino acid sequence of the purified protein was analyzed in order to identify the translational start site of the protein. The sequence obtained was Met-Lys-Leu-Lys-X-Glu-Tyr-X-X-Leu-Ile, which compares to the sequence Met-Lys-Leu-Lys-Arg-Glu-Tyr-Asp-Glu-Leu-Ile deduced from the start site previously suggested from sequence analysis and deletion studies (Berdal et al., 1990; Chen et al., 1990).

General Properties of the MAG Enzyme. The purified MAG protein did not show any associated AP-endonuclease activity when assayed with acid-depurinated plasmid DNA or alkyltransferase activity when tested on DNA treated with *N*-nitroso-*N*-methylurea (data not shown). Addition of the divalent cations Mg^{2+}, Ca^{2+}, or Mn^{2+} at 2 mM concentration did not stimulate or inhibit the glycosylase activity, whereas 30% and 50% inhibition was observed with 2 mM $ZnSO_4$ and $FeCl_3$, respectively (data not shown). The AlkA enzyme was not affected by the addition of Zn^{2+} or Fe^{3+}. The sulfhydryl blocking agents *p*-(hydroxymercuri)benzoate and *N*-methylmaleimide caused 70% and 50% inhibition at 1 mM concentration as compared to 50% and 30% inhibition of AlkA. No effect on the enzymatic activity was observed by the addition of 5 mM m^3A or m^3G for either enzyme.

Enzyme Inhibition by Polyclonal Antibodies. Polyclonal antibodies were raised against the purified MAG and the purified AlkA proteins. No cross-reaction was observed for the two antibodies as judged from Western blots (data not shown) or enzyme inhibition experiments (Figure 2). However, the polyclonal antibodies inhibited the glycosylase activity of the enzyme toward which the antibody was raised, indicating that the active site regions have antigenic epitopes which, however, are sufficiently different in structure to prevent cross-reaction (Figure 2). In the case of the MAG enzyme, 30% of enzyme activity remained even at saturating antibody concentrations. The remaining activity was similar to the activity of the noninhibited enzyme with respect to the excision of both m^3A and m^7G (data not shown). The reason for the remaining activity is unclear but might reflect an equilibrium of the antibody/enzyme binding which is not influenced by the amount of antibody above a certain concentration.

Substrate Specificity of MAG. The AlkA enzyme of *E. coli* has been shown to react with a wide variety of different methylated bases in DNA (Karran et al., 1982; McCarthy et al., 1984). We have examined the MAG enzyme in the same respect using either DNA exposed to DMS (Figure 3) or DNA exposed to NMU (Figure 4) as a substrate. Removal of N-alkylated purines was investigated with the DMS-treated DNA since DMS is an SN2 reacting agent which produces almost only N-alkylations in DNA (Beranek et al., 1980). Different amounts of different *N*-methylpurines are present in such DNA, and the relative affinities for different bases are best analyzed by determining the rate of removal of all bases simultaneously with different amounts of enzyme added to the reaction. From such measurements, it appears that MAG is similar to AlkA in its affinity for m^3A, m^7G, and m^7A (Figure 3). However, whereas m^3G is the methylpurine most rapidly removed by AlkA, the reaction of MAG with m^3G proceeds 20−40 times more slowly. The rate of m^3G removal by MAG is similar to that observed for the Tag enzyme from *E. coli* (Figure 3; Bjelland et al., 1993).

In another set of experiments, we have investigated the possible reactions of MAG with m^2T, using as a substrate DNA exposed to NMU since NMU produces a high proportion of O-alkylations. Still, m^2T is produced at very low levels, and two subsequent HPLC steps are required to single out m^2T release from the background of methylpurines and other components. It was confirmed that m^2T is a good substrate for AlkA (McCarthy et al., 1984); however, no release of m^2T by MAG could be detected.

Survival of AlkA Mutant Cells Transformed by MAG-Expressing Plasmids. The *MAG* gene was cloned on the basis of its ability to complement the extreme alkylation sensitivity of the *tag alkA* and the *tag ada* double mutants (Chen et al., 1989; Berdal et al., 1990). In view of the sequence relationship between MAG and AlkA, we have investigated the effect of MAG expression on the alkylation sensitivity displayed by the *alkA* single mutant (Figure 5). Transformation of the *alkA* mutant by plasmids which produce alkylbase DNA glycosylase activity similar to that of AlkA in adapted wild-type *E. coli* affects only a slight increase of MMS exposure survival, and this is not further

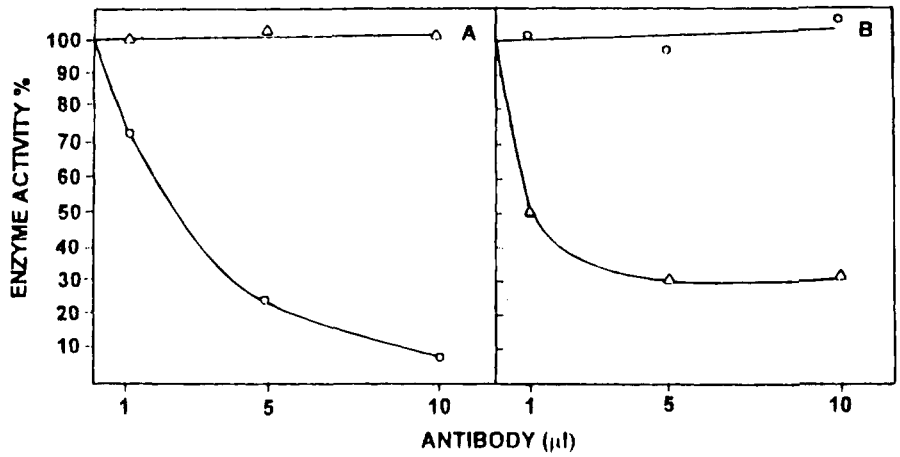

FIGURE 2: Antibody inhibition of alkylbase DNA glycosylase activity. IgG-purified polyclonal antibodies toward: MAG (△) or AlkA (○) were added to the enzyme reaction on ice 5 min before the start of incubation for 30 min at 37 °C. Left panel: Assay of AlkA activity. Right panel: Assay of MAG activity. The concentration of antibodies was 3 μg of protein/μL for both preparations.

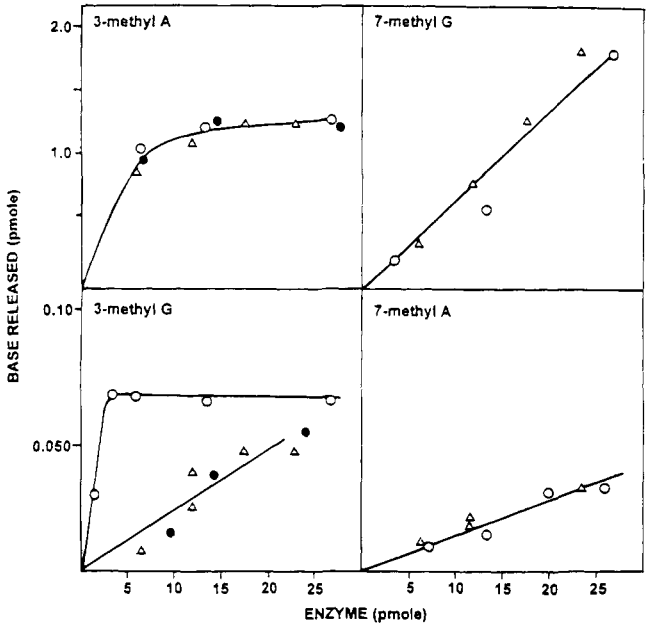

FIGURE 3: Release of N-alkylated purines from DMS-treated DNA by increasing amounts of MAG and AlkA enzymes. Different amounts of AlkA (○) or MAG (△) were incubated with DNA exposed to DMS, and the release of various N-alkylated purines was analyzed by reverse-phase HPLC. Upper left panel, m^3A excision; upper right panel, m^7G excision; lower left panel, m^3G excision; lower right panel, m^7A excision. Comparative analyses of excision by the Tag DNA glycosylase from *E. coli* (●) were performed with the same substrate in the case of m^3A and m^3G excision.

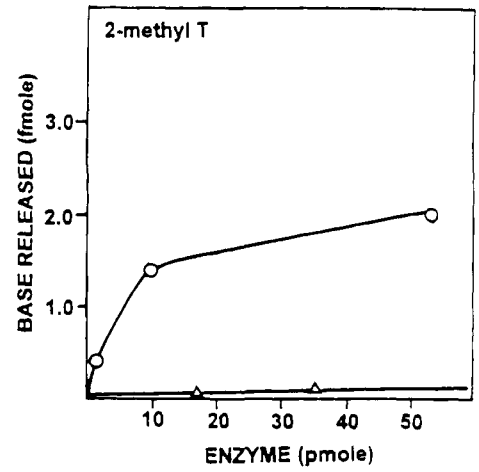

FIGURE 4: Release of 2-methylthymine from NMU-treated DNA by increasing amounts of MAG and AlkA enzymes. Different amounts of AlkA (○) and MAG (△) were incubated with alkylated DNA exposed to NMU, and the release of m^2T was analyzed by successive reverse-phase and anion-exchange HPLC.

increased by transformation by a plasmid with 10-fold higher expression. Full rescue is obtained by transformation with a plasmid that expresses similar amounts of AlkA (Figure 5; Kaasen et al., 1986). It thus appears that MAG cannot replace the function of AlkA which is missing in *alkA* mutant cells.

DISCUSSION

In this paper, we have conducted a biochemical investigation of the MAG DNA glycosylase from *S. cerevisiae* as purified after expression in *E. coli*. The translational start site was identified by N-terminal sequencing of the protein, and the substrate specificity of the enzyme for methylated bases was examined and compared to that of the homologous AlkA protein of *E. coli*. The MAG glycosylase appears to be quite inefficient in the removal of m^3G, which has been shown to be a very good substrate for AlkA (Karran et al., 1982). Another important difference is that MAG cannot excise O^2-methylated pyrimidines. It thus appears that in spite of the sequence relationship that MAG does not possess all the functions carried by AlkA. Structural differences between the two enzymes could be anticipated from differences observed in the primary structure and are substantiated here by the specificity in enzyme inhibition caused by the polyclonal antibodies produced against the two enzymes. The most prominent difference in primary structure is the presence of a sequence motif in the MAG enzyme with a very high density of positively charged residues (10 of 15 from residues 232−246) which becomes quite apparent when an alignment is made between the sequences of the AlkA enzymes from *E. coli* (Nakabeppu et al., 1984) and *B. subtilis* (Morohoshi et al., 1993), and the MAG sequence (Berdal et al., 1990; Chen et al., 1990; Figure 6). This region, which is specific for the eukaryotic MAG enzyme, most probably represents a nuclear localization signal. The high density of positively charged residues may cause high affinity for DNA even in the absence of any alkylated base residues.

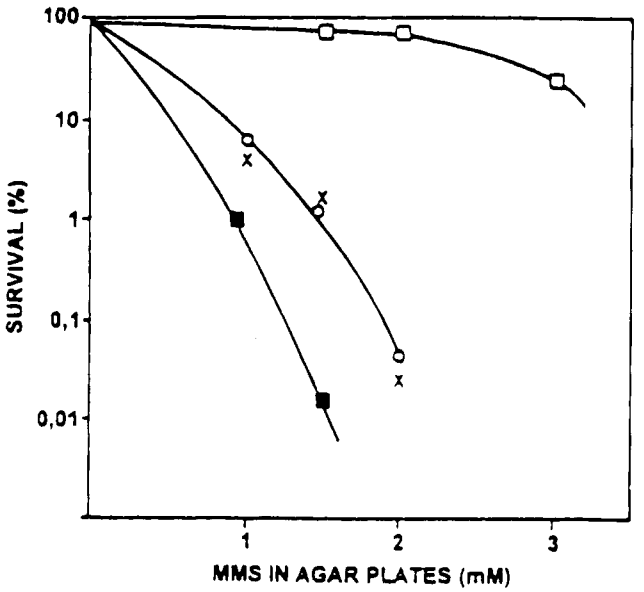

FIGURE 5: MMS survival of *alkA* mutant cells transformed by plasmids expressing AlkA and MAG. Symbols: (■) MS23 (*alkA1*) transformed by pKK223-3; (×) MS23 transformed by pBK142 (*MAG*$^+$); (○) MS23 transformed by pBK143 (*MAG*$^+$), (□) MS23 transformed by pBK161 (*alkA*$^+$).

S. cer.	GIGPWSAKMFLISGLKRMDVFAPED	209
E. col.	GIGRWTANYFALRGWQAKDVFLPDD	238
B. sub.	GIGPWTANYVLMRCLRFPTAFPIDD	258
S. cer.	LGIARGFSKYLSDKPELEKELMRER	234
E. col.	YLIKQRFPGM----------	248
B. sub.	VGLIHSIKILRNM----------	271
S. cer.	KVVKKSKIKHKKYNWKIYDDDIMEK	259
E. col.	----------TPAQIR-R	255
B. sub.	----------NRKPTKDEILE-	282
S. cer.	CSETFSPYRSVFMFILWRLASTNTD	284
E. col.	YAERWKPWRSYALLHIWYTEGWQPD	280
B. sub.	ISVPWKEWQSYATFYLWRVLY	303
S. cer.	AMMKAEENFVKS	296
E. col.	EA	282

FIGURE 6: Amino acid sequence alignment of the C-terminal parts of *S. cerevisiae* MAG, *E. coli* AlkA, and *B. subtilis* AlkA proteins.

E. coli mutants carrying *alkA* were originally isolated on the basis of their sensitivity to MMS. The mutants appeared normal in 3-methyladenine DNA glycosylase activity, and it was only when *tag* mutants were isolated that the glycosylase function of the *alkA* gene was discovered (Evensen & Seeberg, 1982). It was further shown that AlkA is very efficient in removing m^{3}G from DNA (Karran et al., 1982), and it was concluded that *alkA* mutant cells are sensitive to MMS exposure because of deficiency in m^{3}G removal. The data presented here show that expression of MAG in *alkA* mutant cells does not restore alkylation resistance to any large extent (Figure 5), which supports this conclusion since MAG only very slowly removes m^{3}G from alkylated DNA (Figure 4). It was found previously that very high expression of Tag complements the MMS sensitivity of *alkA* mutant cells (Kaasen et al., 1986; Sakumi et al., 1986). However, this was recently shown to be caused by a low m^{3}G-removing activity inherent of Tag (Figure 4) which becomes significant at very high expression levels (Bjelland et al., 1993). The expression of MAG effected by the plasmids used in this study is much lower and too low for the m^{3}G-removing activity to become significant.

Recently, additional substrates for the AlkA and MAG enzymes have been identified. The AlkA enzyme was shown to recognize oxidized pyrimidine residues in DNA, i.e., 5-formyluracil and 5-hydroxymethyluracil (Bjelland et al., 1994), and also hypoxanthine (Saparbaev & Laval, 1994). We have not found any glycosylase activity associated with MAG for the removal of oxidized pyrimidines. However, MAG was reported to remove hypoxanthine, although the reaction rate was found to be low, in fact 1 order of magnitude lower than that observed for alkA (Saparbaev & Laval, 1994). The MAG enzyme has also been found to have biologically significant activities for removal of 7-hydroxyethyl- and 7-chloroethylguanine from DNA (Matijasevic et al., 1993). It may be inferred that the N-7-modified guanines, and perhaps hypoxanthine, resemble structures like 7-methylpurines and 3-methyladenine whereas oxidized pyrimidines may be similar to the O^2-methylated pyrimidines (Bjelland et al., 1994).

It has become more and more evident from recent studies that endogenous chemical methylations play a significant role during normal cell metabolism and that alkylation repair enzymes are essential for normal cell growth (Lindahl, 1993). In support of this is the demonstration that *S. cerevisiae* alkyltransferase mutants show higher spontaneous mutation frequencies than wild-type cells (Xiao & Samson, 1994). It is also evident that MAG recognizes spontaneously formed 7-alkylguanine and 3-alkyladenine, since MAG overproduction increases the spontaneous mutation frequency in an AP-endonuclease-deficient strain through the accumulation of premutagenic AP-sites. Nevertheless, *MAG* mutant cells are viable, although very sensitive to alkylation exposure (Chen et al., 1990). However, in view of the limited ability of MAG to remove lesions such as m^{3}G and m^{2}T, it would be reasonable to propose that a second enzyme of this type also could exist in *S. cerevisiae* and that the *MAG* gene function for alkylation protection serves a similar function in yeast as the *tag* gene function in *Escherichia coli*.

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