

# DNA repair activity of *Schizosaccharomyces pombe* Mag1p and Mag2p for alkylation damage in *Escherichia coli* and *Saccharomyces cerevisiae*

Shogo IKEDA, Keigo OKAMOTO, Kayo TANOUE, Ikumi SHIONO,  
and Shinji KAWANO

Department of Biochemistry, Faculty of Science, Okayama University of Science,  
1-1 Ridai-cho, kita-ku, Okayama 700-0005, Japan.

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3-Methyladenine (3MeA) DNA glycosylase removes some forms of alkylated DNA bases and initiates the base excision repair pathway to maintain genome integrity. *Schizosaccharomyces pombe* has two paralogues of 3MeA DNA glycosylase, Mag1p and Mag2p, which share homology with *Escherichia coli* AlkA and *Saccharomyces cerevisiae* Mag1p. A previous genetic study showed that both Mag1p and Mag2p are involved in the repair of damage caused by methyl methanesulfonate (MMS). To clarify the biological function of these redundant enzymes, we performed complementation tests using *E. coli alkA*Δ and *S. cerevisiae MAG1*Δ cells. *S. pombe* Mag1p rendered these recipient cells resistant to MMS to the same level as wild type cells. In contrast, Mag2p did not affect the sensitivity of *alkA*Δ and *MAG1*Δ cells to MMS. Mag2p may have evolved to function only in *S. pombe* cells separately from the known base excision repair pathway.

**Keywords:** alkylation damage, base excision repair, 3-methyladenine DNA glycosylase, *Schizosaccharomyces pombe*.

## 1. Introduction

Cellular DNA is continually damaged by endogenous and environmental alkylating agents which generate cytotoxic and mutagenic methylated bases (Friedberg et al., 2006; Sedgwick et al., 2007). The abundant toxic lesion 3-methyladenine (3MeA) in cells is removed by a specific DNA glycosylase to leave an apurinic/apyrimidinic (AP) site, which initiates the base excision repair (BER) pathway to maintain genome integrity. Most eukaryotic cells possess 3MeA DNA glycosylase that shares homology with *Escherichia coli* AlkA, which is the prototype enzyme (Wyatt et al., 1999; Friedberg et al., 2006). 3MeA DNA glycosylase of *Saccharomyces cerevisiae* was cloned after rescuing *E. coli alkA*Δ from alkylating agents such as methyl methanesulfonate (MMS), and deletion of the gene from yeast cells resulted in hypersensitiveness to alkylating agents (Chen et al., 1989). A fission yeast *Schizosaccharomyces pombe* has two paralogues of 3MeA DNA glycosylases, Mag1p and Mag2p, which have

considerable homology with AlkA and *S. cerevisiae* Mag1p (Memisoglu and Samson, 1996; Kanamitsu et al., 2007; Adhikary et al., 2013). The amino acid sequences of *S. pombe* Mag1p and Mag2p have 44.8% identity with each other. The *mag1* and *mag2* single mutants as well as the double mutant showed no obvious MMS sensitivity (Kanamitsu et al., 2007; Dalhus et al., 2013). However, a genetic study using nucleotide excision repair-deficient cells revealed that *mag1* and *mag2* certainly function in the repair of MMS damage (Kanamitsu et al., 2007). Genetic interactions of *mag1* and *mag2* with the AP lyase gene *nth1* indicated that both enzymes are implicated in manipulation of the AP site (Kanamitsu et al., 2007). The genetic redundancy of these enzymes in repairing alkylation DNA damage remains to be elucidated. In this study, we expressed Mag1p and Mag2p in *alkA*Δ and *S. cerevisiae MAG1*Δ cells and measured the functional complementation of these enzymes in a bid to repair MMS damage in recipient cells.

**Table 1.** Bacterial and yeast strains used in this study.

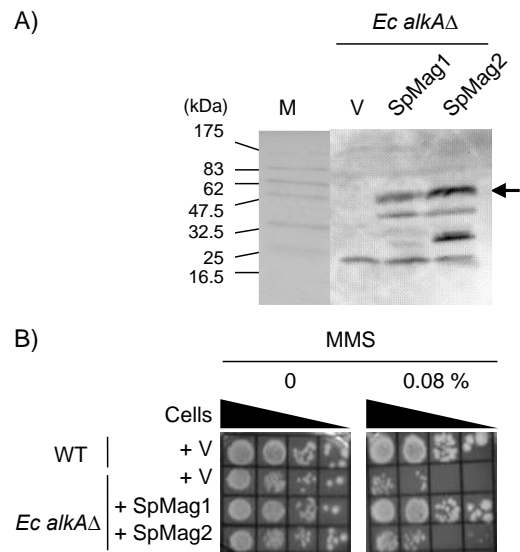
Organism	Strain	Genotype	Source
<i>E. coli</i>	MV1161	<i>thr1 ara14 leuB6 DEL(gpt-proA)62 lacY1 tsx33 supE44 galK2 hisG rfbD1 mgl51 rpsL31 kdgK51 xyl5 mtl1 argE3 thi1 rfa550</i>	Volkert <i>et al.</i> , 1986
<i>E. coli</i>	MV1571	MV1161 with <i>alkA51::Mu-dl(Amp<sup>R</sup> lac)</i>	Volkert <i>et al.</i> , 1986
<i>S. cerevisiae</i>	BY4743	MATa <i>his3Δ1 leu2Δ0 met15Δ0 ura3Δ0</i> / MATα <i>his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0</i>	Thermo Scientific
<i>S. cerevisiae</i>	YER142C	BY4743 with <i>mag1::kanMX</i> (homozygous diploid)	Thermo Scientific Clone ID 36140

## 2. Materials and Methods

### 2.1. Plasmids for expression of *S. pombe* Mag1p and Mag2p.

The bacterial and yeast strains used in this study are listed in Table 1. A low copy number plasmid pMW219 (NIPPON GENE Co., Ltd.) was used for the expression of Mag1p and Mag2p in *E. coli* cells. The *mag1* and *mag2* genes fused in frame behind the glutathione *S*-transferase (GST) gene were amplified by PCR from pESP1-*mag1* and pESP1-*mag2* plasmids (Kanamitsu *et al.*, 2007) and subcloned into pMW219 using *SphI* and *SacI* sites. The PCR primers used are as follows: GST(+7)*SphI*-Fw (5'-GTC GGC ATG CCC CTA TAC TAG GTT ATT G-3'), *mag1*(+687)*SacI*-Rv (5'-GGA GCT CTG AGT GTT TCT TCG GCC-3'), and *mag2*(+642)*SacI*-Rv (5'-GGA GCT CTT ACT GGC CTT TAG GAT GG-3'). *E. coli alkAΔ* strains with the recombinant pMW219 plasmid were selected on LB medium (1% tryptone, 0.5% yeast extract and 1% NaCl) containing ampicillin and kanamycin. An expression plasmid pYES2 (Life Technologies-Invitrogen) that includes the *GAL1* promoter, the 2 $\mu$  origin for high-copy maintenance, and *URA3* for selection was used to express Mag1p and Mag2p in *S. cerevisiae*. *GST-mag1* and *GST-mag2* genes were amplified by PCR, and subcloned into pYES2 using *KpnI* and *SphI* sites. The PCR primers used are as follows: GST(+8168)*KpnI*-Fw (5'-GCG GTA CCA TGG CCC CTA TAC TAG GTT ATT-3'), *mag1*(+687)*SphI*-Rv (5'-GGA TAG CAT GCT CAG TGT TTC TTC GGC C-3'), and *mag2*(+642)*SphI*-Rv (5'-GGA TAG CAT GCT TAC TGG CCT TTA CGA TGG-3'). Recombinant plasmids were introduced to *S. cerevisiae MAG1Δ* strain with lithium acetate (Moreno *et al.*, 1991). Transformants were selected on SD medium (0.67% yeast nitrogen base w/o amino acids

[Becton, Dickinson and Co.] and 2% glucose) supplemented with 20  $\mu$ g/mL histidine and 40  $\mu$ g/mL leucine.



**Fig. 1.** Repair activity of *S. pombe* Mag1p and Mag2p in *E. coli alkAΔ* cells for MMS-induced damage. (A) Expression of GST-fused Mag1p and Mag2p in *E. coli*. M; molecular weight standard, V; empty vector (pMW219), SpMag1; expression plasmid for GST-Mag1p, SpMag2; expression plasmid for GST-Mag2p. Arrow indicates intact GST-Mag1p or GST-Mag2p. (B) Survival assay to MMS damage. *E. coli* cells were grown at 37°C in LB medium until the OD<sub>660</sub> of the culture reached near 0.5. After adjusting the *E. coli* cell concentration to OD<sub>660</sub> = 0.01 in LB medium, the cells were diluted 10<sup>-2</sup>-, 10<sup>-4</sup>-, 10<sup>-6</sup>-, and 10<sup>-8</sup>-fold in LB medium. The diluents (10  $\mu$ L) were then spotted onto LB agar plates containing MMS (Sigma-Aldrich). Photographs of colonies were taken after growth overnight at 37°C. WT; wild-type cells (MV1161), *Ec alkAΔ*; *E. coli alkAΔ* cells (MV1571).

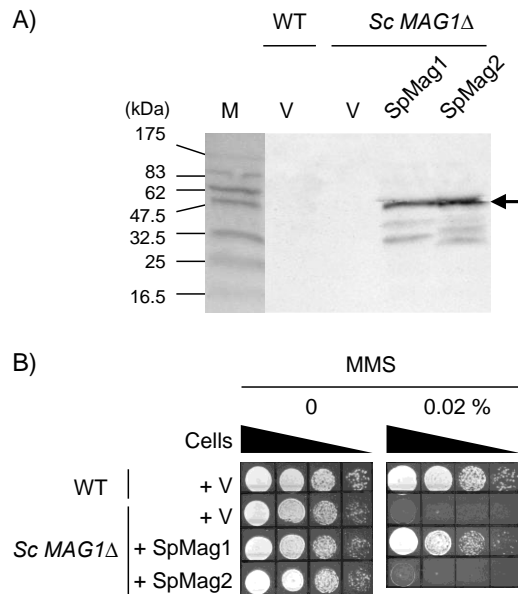


Fig. 2. Repair activity of *S. pombe* Mag1p and Mag2p in *S. cerevisiae* *MAG1* $\Delta$  cells for MMS-induced damage. (A) Expression of GST-fused Mag1p and Mag2p in *S. cerevisiae*. M; molecular weight standard, V; empty vector (pYES2), SpMag1; expression plasmid for GST-Mag1p, SpMag2; expression plasmid for GST-Mag2p. Arrow indicates intact GST-Mag1p or GST-Mag2p. (B) Survival assay to MMS damage. *S. cerevisiae* cells were grown overnight at 28°C in YP medium containing 2% raffinose. The diluted cells ( $1 \times 10^2$  to  $1 \times 10^5$ ) were spotted onto YP + raffinose agar plates containing MMS. Photographs of colonies were taken after 2 days of growth at 28°C. WT; wild-type cells (BY4743), *Sc MAG1* $\Delta$ ; *S. cerevisiae* *MAG1* $\Delta$  cells (YER142C).

## 2.2. Expression and detection of GST-fused Mag1p and Mag2p.

GST-fused Mag1p and Mag2p expressed in *E. coli* and *S. cerevisiae* cells was detected by western blotting using anti-GST antibody. *E. coli* cells carrying the expression plasmid were cultured in LB medium and whole cell extracts were prepared by boiling the cells in SDS-PAGE sampling buffer. *S. cerevisiae* cells harboring the expression plasmid were grown in YP medium (1% yeast extract and 2% polypeptone) containing 2% raffinose at 28°C until the OD<sub>600</sub> of the culture reached near 1.0. After adding galactose to a final concentration of 2%, the culture was grown overnight at 28°C. Whole cell extracts were prepared by disrupting cells using glass beads in a sampling buffer for SDS-PAGE (Adams et al., 1998). Proteins in extracts were fractionated by SDS-PAGE (12.5%

polyacrylamide), and transferred onto a Hybond-P blotting membrane (GE Healthcare). The GST-fusion proteins were visualized using horseradish peroxidase-labeled anti-GST antibody (GE Healthcare, 1:5,000) and the ECL Western Blotting Detection Reagents Kit (GE Healthcare). Imaging of the chemiluminescence was performed with ImageQuant LAS-4000mini (GE Healthcare).

## 3. Results and Discussion

*S. pombe* Mag1p and Mag2p fused to GST were expressed in *E. coli* *alkA* $\Delta$  cells, and their repair activity of MMS damage was estimated by a survival assay (Fig. 1). A low copy number plasmid was used for this complementation tests, because of the deleterious effect of high level expression of 3MeA DNA glycosylases (Posnick and Samson, 1999; Troll et al., 2014). GST-Mag1p and GST-Mag2p with expected molecular masses were observed by an anti-GST antibody. Some degradation products were also produced. *E. coli* *alkA* $\Delta$  cells carrying an empty vector exhibited severe sensitivity to MMS. Expression of Mag1p rendered the *alkA* $\Delta$  cells resistant to MMS damage to the same level as wild type cells. In contrast to Mag1p, Mag2p did not affect the sensitivity of *alkA* $\Delta$  cells to MMS.

To estimate the repair activity of *S. pombe* Mag1p and Mag2p in eukaryotic cells, the proteins were expressed in *S. cerevisiae* *MAG1* $\Delta$  cells (Fig. 2). GST-Mag1p and GST-Mag2p with expected molecular masses were observed in *S. cerevisiae* cells by western blotting. The *MAG1* $\Delta$  cells exhibited severe sensitivity to MMS, and expression of Mag1p rendered the cells resistant to MMS damage to the same level as wild type cells. In agreement with the test using *E. coli*, Mag2p did not affect the sensitivity of the yeast *MAG1* $\Delta$  cells to MMS.

The repair activity of Mag1p in heterologous cells showed that the enzyme removed alkylated bases by its DNA glycosylase activity and initiated the BER pathway to completely repair MMS-induced damage. The results agree well with a previous biochemical study in which purified Mag1p was able to excise 1,N<sup>6</sup>-ethenoadenine, 3MeA, 3-methylguanine, and 7-methylguanine, as substrates (Alseth et al., 2005; Adhikary and Eichman, 2011; Troll et al., 2014). Although Mag2p shears highly conserved amino acid sequence among other members of the 3MeA DNA glycosylase family, the protein does not have or has very weak activity to repair MMS damage in

heterologous cells. In biochemical experiments, purified Mag2p did not excise 1,*N*<sup>6</sup>-ethenoadenine and other deaminated and oxidized bases (Dalhus et al., 2013; Adhikary et al., 2013). *mag2* genetically interacts with the AP lyase gene *nth1* and the nucleotide excision repair gene *rad16* (Kanamitsu et al., 2007), so Mag2p has possibly evolved to function only in *S. pombe* cells. More recently, the three-dimensional structure of *S. pombe* Mag2p was resolved by X-ray crystallography (Dalhus et al., 2013; Adhikary et al., 2013). Unlike other DNA glycosylases, Mag2p does not flip the abasic moiety into the active site (Adhikary et al., 2013). Mag2p is localized in nuclei and is inducible by treatment with H<sub>2</sub>O<sub>2</sub> and MMS (Chen et al., 2003; Dalhus et al., 2013). Specific binding of Mag2p to the AP site *in vitro* suggests the possible function of damage sensing separately from the known BER pathway (Dalhus et al., 2013).

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