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Analysis for Regulatory Elements in Yeast *MGMT* Gene Transcription

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The *Saccharomyces cerevisiae MGMT* gene encodes a O^6-methylguanine DNA methyltransferase that protects cells from mutation or death by DNA alkylating agents. Using an *in vitro* transcription system, we analyzed its promoter region to find regulatory elements for transcription initiation. DNase I footprinting and a transcription assay showed that a functional TATA box, 5'-TGATATAGCA-3', is located in the region spanning from -25 to -34. We also found one upstream repressing sequence (URS), -333 to -213, by promoter deletion and competition analysis. Gel mobility shift assays and Southwestern blot analysis using URS region indicate specific complex formations. These results indicate that several *cis*-acting and *trans*-acting elements might be involved in the transcriptional regulation of the *S. cerevisiae MGMT* gene.

The enzyme O^6-methylguanine-DNA methyltransferase (MGMT) is widely distributed in organisms and plays an important role in maintaining genetic information. This enzyme catalyzes the transfer of a methyl group from O^6-methylguanine and O^4-methylthymine moieties of DNA to the enzyme itself (Sekiguchi and Nakabeppu, 1987; Lindahl et al., 1988; Koike et al., 1990). This methyltransferase activity has been found in several species of bacteria (Jung et al., 1985; Lindahl et al., 1988), fish cells (Nakatsuru et al., 1987), insects (Green and Deutsch, 1983), yeast (Sassanfar and Samson, 1990) and filamentous fungus (Baker et al., 1992).

Methyltransferase activity, however, is dependent on the type of tissue and varies with species and developmental stage (Pegg, 1990; Fritz et al., 1991). In addition, it was reported that the rat hepatoma methyltransferase mRNA could be induced (2.5 to 5 fold) by various stress treatments, which ultimately caused the DNA breaks (Fritz and Kaina, 1992). This strongly suggests that a number of factors, including tissue, cell type, age and cell cycle, appear to be involved in the regulation of expression level. The promoter activity of human methyltransferase gene has been tested by transient expression of the bacterial chloramphenicol acetyltransferase (CAT) gene linked to this promoter (Harris et al., 1991). Though researchers have proposed that regulatory elements are present, none of them are well characterized to date.

It has been believed that the *S. cerevisiae* does not have an O^6-methylguanine-DNA methyltransferase because of its cell wall barrier and recombinational repair ability (Maga and McEntee, 1985). However, Sassanfar and Samson (1990) identified its presence and biochemically characterized the O^6-methylguanine-DNA methyltransferase enzyme activity in yeast. The enzyme recognizes both O^6-methylguanine and O^4-methylthymine *in vitro*. The molecular mass of the enzyme is approximately 25 kD and it accepts methyl groups from O^6-methylguanine to form S-methylcysteine. This methyl acceptor protein has a weak affinity with O^4-methylthymine. A yeast cDNA fragment containing suppression abilities to alkylation-induced killing and mutation in *E. coli ada- ogt-* mutant was cloned. The cloned yeast DNA fragment was mapped to chromosome IV (Xiao et al., 1991).

We have previously cloned the full genomic DNA of yeast *MGMT* gene and examined its expression (Joo et al., 1995). Its expression decreased when cells reached stationary phase and could not be induced by the pretreatment with alkylating agents MMS (methylmethane sulfonate) or MNNG (N'-methyl-N'-nitro-N-nitrosoguanidine). The transcription initiation site was determined by primer extension and the authentic start codon was the ATG at position +32 from transcription initiation site.

Here, we show analysis of 5'-flanking region of the yeast *MGMT* gene and identify regulatory elements required for its expression. To analyze the structure of its promoter, a series of the deletion mutants along the 5'-flanking region of the yeast *MGMT* gene were constructed, and an *in vitro* transcription system from yeast was employed.

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Finally, in order to determine whether cell-specific *trans*-acting factors bind to these *cis*-acting elements, DNase I footprinting, competition assay of *in vitro* transcription activity, gel mobility shift assay, and Southwestern blot analyses were performed. These studies provide the basis for eventual elucidation of the precise interactions between the yeast *MGMT* gene promoter and its cognate transcription factors.

Materials and Methods

Strains and plasmids

The *E. coli* strain, DH5α (*supE44, ΔlacU169, φ80lacZ M15), hsdR17A, recA1, gyrA96 thi1 rel1*) was used for transformation and DNA manipulation. The protease-deficient *S. cerevisiae* BJ2168 (*leu2, trp1, ura3-52, prb1-1122, pep4-3, prc1-407, MATa*) was used for the preparation of whole cell extract. pF0.88 was the parent plasmid for all constructs. It contains the yeast *MGMT* gene promoter isolated as a *Spe*I-*Hin*dIII fragment (-742 to +145) from pF5.4 (Joo et al., 1995).

Serial deletion of 5'-flanking region

To obtain a nested set of deletion in the 5'-flanking region of *MGMT* gene, exonuclease III was employed. The recombinant plasmid pF0.88 (10 μg) was digested with *Sac*I and *Sph*I. The digested DNA was dissolved in Exo III buffer (66 mM Tris-HCl, pH 8.0, 0.66 mM $MgCl_2$) and 500 units of Exo III (Promega) were added at 30℃. Each 5 μl sample was taken from the mixture at 15 sec intervals and put into a microcentrifuge tube containing 7.5 μl of S1 buffer (300 mM potassium acetate, pH 4.6, 250 mM NaCl, 60 units of S1 nuclease) on ice. After all samples were taken, they were incubated at 37℃ for 30 minutes and the S1 stop buffer (300 mM Tris-base, 50 mM EDTA) was added. After heating to 70℃ for 10 min to inactivate the S1 nuclease, samples from each time point were analyzed on 1% agarose gel to determine the extent of digestion. Each sample was ligated and transformed in *E. coli* DH5α cells.

Preparation of transcription extract

S. cerevisiae 100,000×g supernatants (S-100) were prepared according to Schultz et al. (1991) from the protease-deficient strain BJ2168 cells grown in YEPD (1% yeast extract, 2% Bactopeptone, 2% glucose, pH5.5). The cells were cooled by pouring over crushed ice and then spun at 4,000 rpm for 4 minutes in a Sorvall GS-3 rotor. After determinging wet weight of the pellet, the cells were washed successively in ice-cold distilled water (2 vol/g of cells) and in 1.3 volume of extraction buffer (100 mM Hepes-KOH, pH 7.9, 245 mM KCl, 5 mM EGTA, 1 mM EDTA, 2.5 mM DTT). The cell pellet was then resuspended in a 1.3 volume of extraction buffer supplemented with protease inhibitors (0.2 mM phenylmethylfulfonyl fluoride, 10 mM benzamide hydrochloride, 25 μg/ml l-chloro-3-(4-tosylamido)-4-phenyl-2-butanone, 3.5 μg/ml pepstatin A, 5 μg/ml leupeptin, 10 μg/ml aprotinin, 100 μg/ml chymostatin) and pelleted as above. The thick cell paste was loaded into a syringe, extruded into liquid nitrogen, and then stored at -70℃.

For most experiments, frozen cells were broken by extensive manual grinding of 2 g lots under liquid nitrogen using a ceramic mortar and pestle. Cell breakage was monitored by assaying soluble protein in the 100,000×g supernatants. After grinding, all procedures were performed at 4℃. The powder of broken cells was transferred to an ice-cold beaker, and a 1.3 volume of extraction buffer with protease inhibitors was added. Thawing of the powder was speeded up by slight warming. The final suspension of broken cells was briefly mixed by pipetting and then spun at 100,000×g for 2 h. The entire supernatant (minus the lipid pellicle) was collected by tube puncture and dialyzed for at least 4 h against 50 volume of YDB buffer (20 mM Hepes-KOH, pH 7.9, 50 mM KCl, 5 mM EGTA, 0.05 mM EDTA, 2.5 mM DTT, 20% glycerol, 0.2 mM phenylmethyl-sulfonyl fluoride, 0.5 μg/ml leupeptin). This supernatant was used as the transcription extract.

RNA polymerase II transcription reaction

Transcription reactions were performed in a final volume of 20 μl essentially as described (Schultz et al., 1992). Fourteen μl of reaction mixture contained 10 μl of S-100 (77 μg of protein) and 25 mM KCl, 10 mM magnesium acetate, 50 mM potassium glutamate, 5 mM EGTA, 0.05 mM EDTA, 2.5 mM DTT, 10% glycerol, and 5 units of RNasin (Promega). The pre-incubation with nonspecific DNA (20 μg/ml pGEM3EX) was performed for 5 minutes and then followed by a 15 min incubation with a specific template. Nucleotides were added and transcription was stopped after 40 min by adding 20 volumes of stop buffer (10 mM Tris-HCl, pH 7.5, 1% SDS, 1 mM EDTA). After extraction with phenol/chloroform and chloroform, the products were precipitated with the probe and 10 μg of *E. coli* tRNA. The hybridization reaction was performed at 70℃.

S1 nuclease protection assay

Specific products were detected by S1 nuclease protection analysis using a single-stranded RNA probe spanning from -222 to +145 of *MGMT* DNA and additional 46 bases of pBluescript SK(-) behind the *MGMT* DNA. The S1 nuclease reactions were performed at 37℃. This procedure yielded a 191-nucleotide labeled fragment for correctly initiated transcripts.

DNase I footprinting analysis

The 3'-ends of the *Hin*dIII digested plasmids were

filled in with Klenow enzyme in the presence of dNTP and [α-^{32}P]-dCTP (3,000 Ci/mmol), followed by digestion with *Eco*RI. The specific activity of the gel purified DNA fragment was greater than 2,000 cpm/fmole.

Radiolabeled DNA fragment (5 nM, 200,000 cpm/ng) was incubated at room temperature for 30 min with 45 or 90 nM recombinant yeast TBP (kindly provided by Byung Jae Lee, IMBG, SNU, Seoul, Korea) in a 20 μl reaction mixture containing 20 mM Hepes-KOH, pH 7.9, 60 mM KCl, 5 mM $MgCl_2$, 4% glycerol, 100 ng of poly [dG-dC] and 100 μg/ml of BSA. After incubation, 0.6 U of DNase I (Promega) was added and incubated on ice for 5 min. The reactions were terminated by the addition of 78 μl of stop buffer (20 mM Tris-HCl, pH 8.0, 20 mM EDTA, 250 mM NaCl, and 0.5% SDS). After extraction with phenol/chloroform and chloroform, the reaction products were precipitated with ethanol and analyzed on 6% polyacrylamide-urea sequencing gel. The standard Maxam-Gilbert C+T, C, A>C sequencing reactions of the fragments were used as markers.

Gel mobility shift assay

The gel retardation assay was carried out with minor modification of the method of Staudt et al. (1988). Binding reactions were similar to those described above for the *in vitro* transcription reaction. All reactions contained 4 μg of S-100 extract, 2 μg of poly (dA-dT), and 1 × binding buffer (25 mM KCl, 10 mM magnesium acetate, 50 mM potassium glutamate, 5 mM EGTA, 0.05 mM EDTA, 2.5 mM DTT, 10% glycerol). After 10 min preincubation with or without competitor DNA, the mixtures were further incubated for 30 min with 1 ng of labeled probe. S-100 extract was prepared from BJ2168 cells as described before. All reactions were performed at room temperature. Reaction products were electrophoresed on 4% non-denaturing polyacrylamide gel, and the gel was dried and autoradiographed.

Southwestern blot analysis

Southwestern blotting was performed following the procedure of Silva et al. (1987) with slight modification. Yeast S-100 extracts were fractionated on 10% SDS-PAGE with a 4% stacking gel. After electrophoresis, the gels were incubated in renaturation buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 2 mM EDTA, 0.1 mM DTT and 4 M urea) with gentle agitation. This procedure was repeated three times. Then the proteins were transferred onto nitrocellulose filters by electroblotting. To prevent non-specific binding, the filters were blocked for 2 h by a gentle shaking in blocking renaturation buffer (50 mM Hepes-KOH, pH 7.9, 65 mM KCl, 2 mM EDTA, 2 mM 2-mercaptoethanol, 0.1% Nonidet P-40, and 5% nonfat dry milk) and then incubated at room temperature with the binding solution used for the gel mobility shift assay containing non-specific competitor. The binding buffer was replaced with the same buffer containing 2×10^6 cpm of DNA probe per ml with or without 100-fold excess specific competitor. Binding was carried out for 3 h at room temperature in a sealed plastic bag with gentle agitation. The filters were briefly rinsed three times with binding buffer. DNA-protein complexes were visualized by autoradiography.

Results

Consensus sequences in MGMT gene promoter

The *S. cerevisiae MGMT* genomic DNA was initially cloned in pBluescript SK(-) plasmid (Joo et al., 1995), and the *Spe*I-*Hin*dIII fragment (887 bp) was subcloned and used for promoter analysis in this paper. This new plasmid, pF0.88, is made up of 773 nucleotides upstream from the *MGMT* translation start codon and the first 114 nucleotides of MGMT protein ORF. Computer analysis suggested that three potential TATA sequences and several characteristic *cis*-acting like elements (Fig. 1) were possibly located in this promoter sequence. (i) Three potential TATA boxes were located at -157 to -150, -123 to -115, and -34 to -25. (ii) A putative URS (upstream repressing sequence) element was located at -233 to -224 (5'-GGTGGAGGCC-3') with homology (9 of 10) to the decamer consensus sequence (5'-CGA/TGGA/TNGA/CA/C-3') which was found in promoters of several *S. cerevisiae* genes involved in DNA repair and nucleotide metabolism (Xiao et al., 1993). (iii) A putative RAP1 binding site at position -245 to -233 (5'-AAACCCAGTTCAG-3') with homology (9 of 13) to the RAP1 consensus binding site (5'-A/GA/CACCCAN-NCAT/CT/C-3') (Buchman et al., 1988a; Graham and Chambers, 1994). (iv) Two putative GCR1 binding sites at position -637 to -633 and -166 to -162 which are identical to the GCR1 consensus binding site (5'-CT/ATCC-3') (Baker, 1991; Sinclair et al., 1994).

Transcription activity from 5' promoter deletion mutants

To examine the functional significance of upstream promoter sequences, nested deletion constructs were made by digestion using exonuclease III as described in Materials and Methods. After incubation each promoter deletion mutant with S-100 yeast cell extract, the transcription activity was analyzed by S1 nuclease

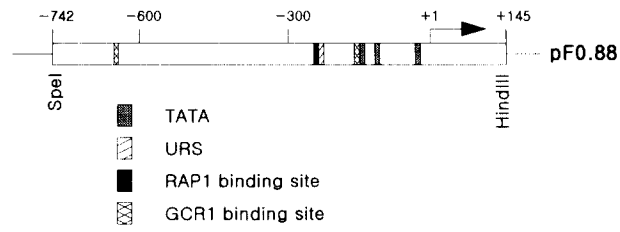

Fig. 1. The upstream region of *MGMT* gene. The numbers are designated from the transcription initiation site. The open reading frame of MGMT protein begins from +32 base after the transcription initiation site. Potential TATA boxes and several *cis*-acting like elements (decamer consensus sequence homologue, putative binding site for RAP1, and CA/TTCC motives for GCR1 binding) are represented in the figure.

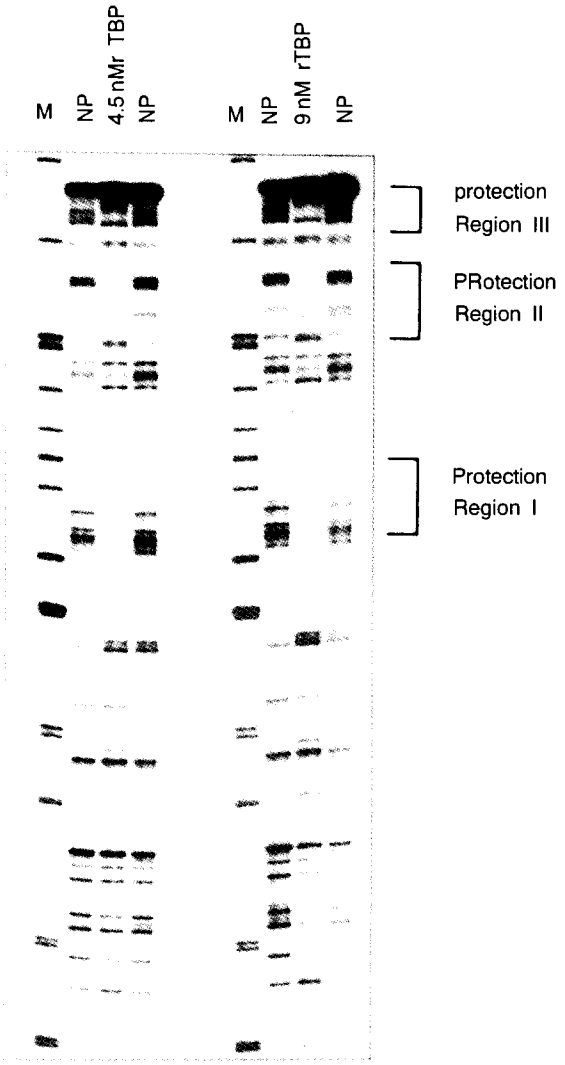

Fig. 2. DNase I footprinting analysis of the *MGMT* gene. To determine the functional TATA element, a DNase I footprinting assay was performed. A uniquely end-labeled DNA fragment was preincubated without TBP (NP), or 4.5 nM or 9 nM of purified yeast recombinant TBP (rTBP). As shown in the figure, there are three regions which are protected by rTBP. The positions of these regions are matched well to the putative TATA sequences according to computer analysis (Region I: -34 to -25, Region II: -123 to -115, Region III: -157 to -150).

mapping. For many genes, the first step in transcription initiation is the binding of TATA-binding protein (TBP) to a TATA element located upstream of the transcription start site. To determine which of them acts as a functional TATA box, DNase I footprinting analysis using purified recombinant yeast TBP (rTBP) was performed. We found that rTBP was able to protect above all three regions from DNase I digestion (Fig. 2). The protections of region III (-157 to -150) and region II (-123 to -115), however, seemed to be caused by nonspecific binding with rTBP. Deletion mutant Del-TATA3 (region III was deleted, Fig. 3) gave the same transcription activity as wild type promoter.

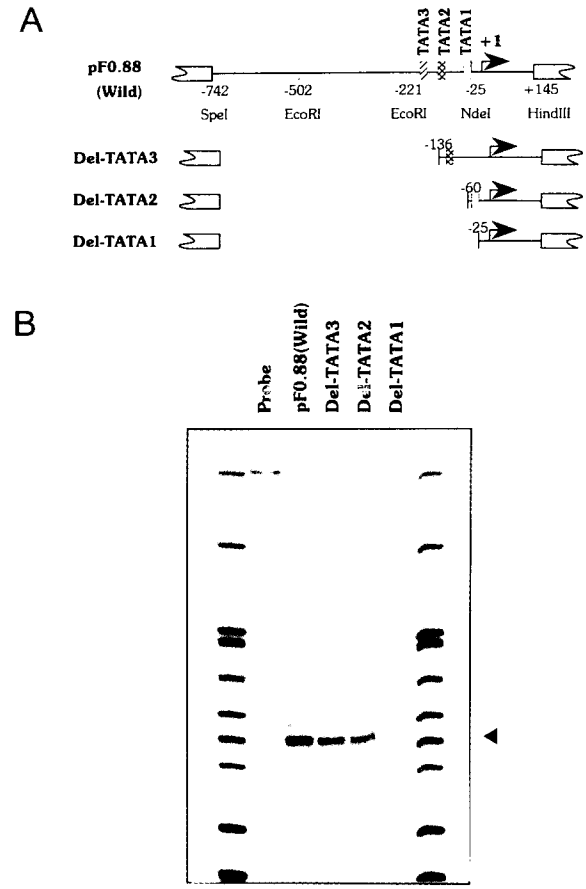

Fig. 3. *In vitro* assay of putative TATA box deletion mutants. Promoter constructs (A) were transcribed in S-100 extracts and the specific initiation (B) was confirmed by S1 nuclease protection assay using a single-stranded RNA probe spanning -222 to +191. Transcription activity of deletion mutant up to region I (Del-TATA1) completely disappeared, and this result strongly suggested that the region I (-34 to -25: 5'-TGATATAGCA-3') should be a functional TATA box.

This means that the TATA sequence in region III is not the real TATA box. The transcription activity from the other promoter deletion mutant, Del-TATA2 (region III and II were deleted), is a little lower than wild type promoter, but the transcription signal was still observed at a significant level. This strongly suggests that region II would not be the main TATA box. However, transcription activity from deletion mutants up to region I (Del-TATA1) completely disappeared, and we could conclude that the region I (-34 to -25: 5'-TGATATAGCA-3') should be a real TATA box.

Various deletion mutants (Fig. 4) up to the site of -392 showed the same transcription activities as the wild type construct (pF0.88). However, further deletion to -213 enhanced the transcription activity about five fold. In addition, internal deletion mutant, ΔEco (-502/-221) also showed increased transcription activity. These results strongly suggest the presence of an upstream repressing sequence (URS) in the region spanning from -392 to -221. Further deletions to -136 or -73 reduced the enhanced activity to the same

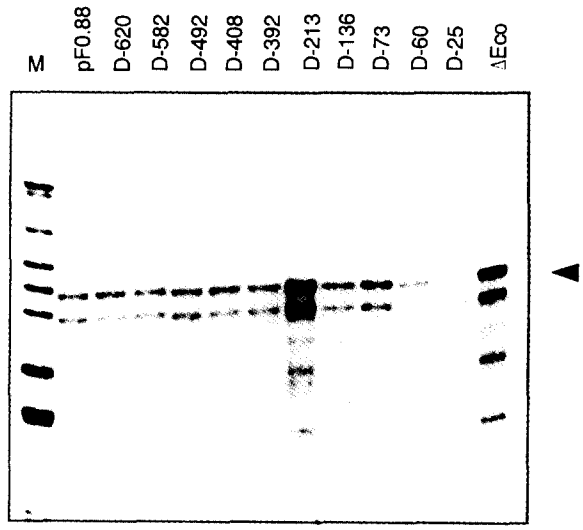

Fig. 4. *In vitro* assay of 5'-deletion and internal deletion promoter mutants. Transcription activity assay was performed as in the Materials and Methods. End points of 5'-deletions are indicated at the top of this figure. ΔEco is an internal deletion mutant from -502 to -221. Two constructs (D-213 and ΔEco) show the significantly increased transcription activities. These results imply the presence of an upstream repressing sequence (URS) in the region from -392 to -213, which is already expected from the computer analysis data (see Fig. 1).

level of wild type promoter. These results indicate that the minimal promoter was contained in region from -212 to +145, and was divided by essential domains, one near the 5' boundary (position -212 to -73) and a second domain (position -72 to +145).

Competition effect of -333/-213 fragment on transcription

In order to find out the proteins that interact with promoter elements defined above, competition analysis was employed. The plasmid pF0.88 was used as template DNA for *in vitro* transcription reaction, and various sized DNA fragments of *MGMT* promoter were employed as competitors (Fig. 5A). Each DNA fragment was prepared by PCR and added to the *in vitro* transcription reaction mixture by 100-fold molar excess than template. After a 10 min incubation of reaction mixture with competitor DNA, the template DNA and nucleotides were added. When the fragments of -492/-408 or -408/-392 were employed as competitor, any significant differences in transcription activity were not observed (Fig. 5A). On the other hand, the DNA fragment of -392/-213 remarkably enhanced the transcription activity. This strongly suggests the presence of an URS element between -392 and -213. To delineate the location of this URS element, the fragment was divided into two pieces of -392/-333 and -333/-213, and each fragment was used again as competitor against the promoter. Among these two fragments, the posterior fragment spanning from -333 to -213 only enhanced the transcription activity whereas the anterior fragment from -392 to -333 did not. This suggests that

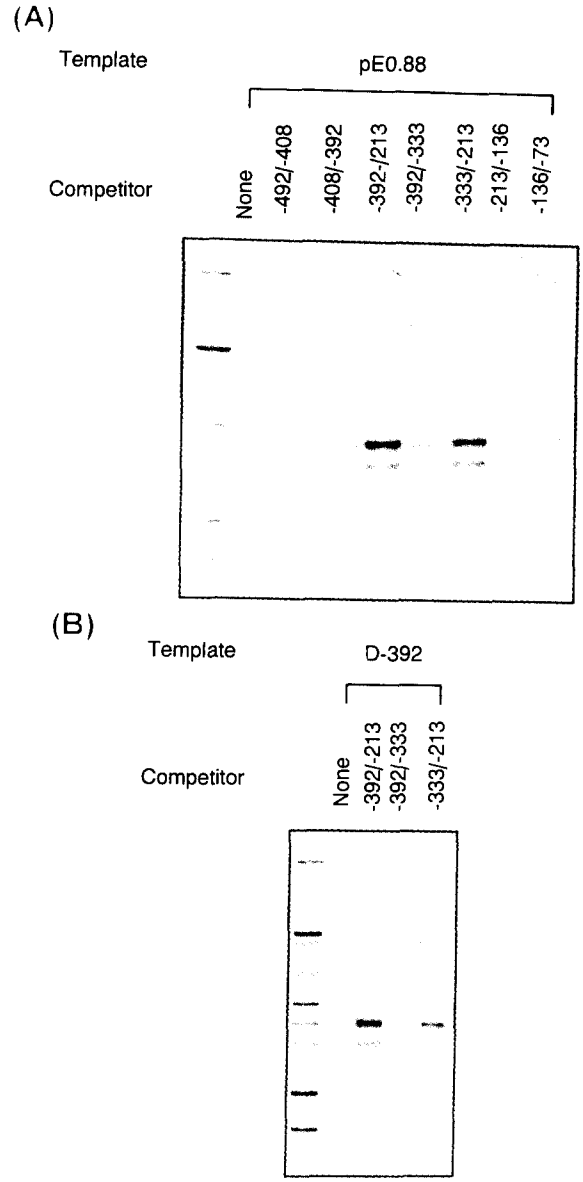

Fig. 5. *In vitro* transcription assay of *MGMT* gene promoter in the presence of various competitor DNAs. The template DNA (A: pF0.88, B: D-392) was *in vitro* transcribed and analyzed by S1 nuclease protection assay. After preincubation of S-100 extracts with various competitors prepared by PCR, the transcription activities of these mixture were analyzed. The template and competitor DNAs used in these experiments are shown at the top of figures.

the URS element is located in posterior region (-333/-213). In addition, the same results were observed when D-392 was used as template DNA (Fig. 5B). Preincubation with -392 to -213 DNA also increased the transcription activity from D-392, and this enhancement was due to the posterior part (-333/-213) of this fragment. Taken together, this transcription enhancement could be caused by the depletion of repressor proteins that interact with URS sequence on template DNA.

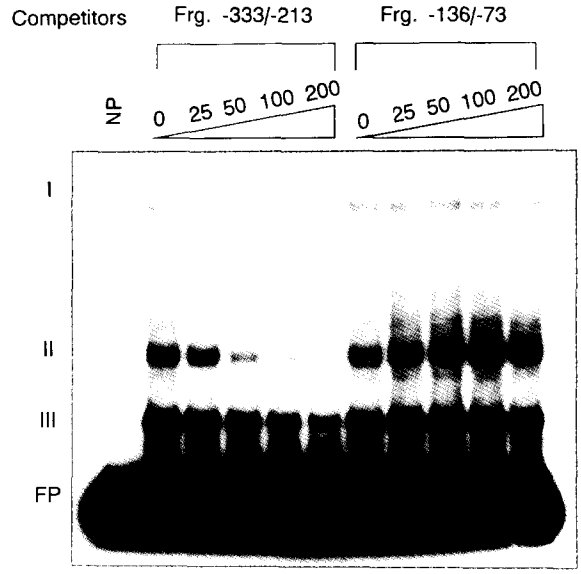

Fig. 6. Gel mobility shift assay using the DNA fragment containing URS (-333/-213). Cold -333/-213 and -136/-73 DNAs were used as specific and nonspecific competitor DNA, respectively. Probe and competitor DNAs were obtained using PCR. Each reaction mixture contained 2 μg of poly (dA-dT), 4 μg of crude yeast cell extracts (S-100), and 1 ng of labeled probe DNA. The probe DNA was labeled with [-^{32}P]-dCTP by PCR. The amounts of competitor DNAs used in this assay are indicated above the figure. FP, free probe-I, II and III, protein-DNA complexes.

Since URS elements are expected to bind one or more proteins, gel mobility shift assays were performed to identify URS binding proteins. At least three major retarded bands were detected when a double-strand DNA, -333 to -213, was used as a probe (Fig. 6). The addition of 25~200-fold molar excess of unlabeled DNA showed the disappearance of all three bands (I, II and III). The specificity of this interaction was confirmed by the addition of an equivalent molar excess of an irrelevant DNA fragment (-136 to -73). The formation of above complexes was not hindered by the presence of nonspecific DNA.

The existence of specific binding proteins to URS sequence in *MGMT* promoter was further investigated by Southwestern blot analysis (Fig. 7). The *MGMT* URS DNA (-333/-213) gave two bands on the electrophoresis gel of yeast whole-cell extracts. The addition of a 100-fold molar excess of unlabeled URS fragment could eliminate the detected bands, and this strongly suggested that binding of *MGMT* URS sequence to the proteins was very specific.

Discussion

The expression of *S. cerevisiae MGMT* gene cannot be induced by the treatment with DNA damaging agents, but its expression might be variable, depending on the growth stage (Joo et al., 1995) as in many housekeeping genes in yeast. This study was performed to search for *cis*- and *trans*-acting elements involved in the regulation of *MGMT* gene expression.

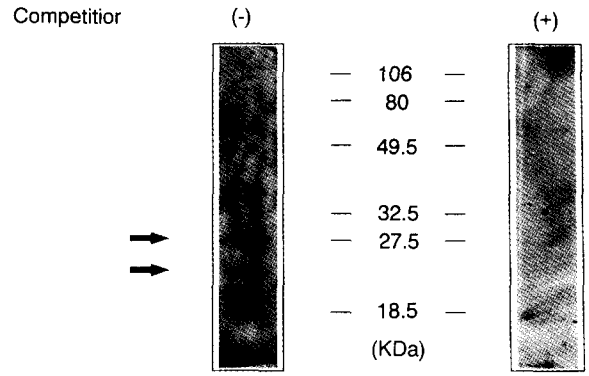

Fig. 7. Southwestern blot analysis with URS probe. Yeast S-100 extracts were separated by SDS-polyacrylamide (12%) gel electrophoresis and transferred to a nitrocellulose membrane. The membrane-bound proteins were renatured and incubated with the labeled -333/-213 DNA probe in the presence (+) or absence (-) of the unlabeled -333/-213 DNA.

For this purpose, artificially altered genes were used for *in vitro* transcription analysis.

In vivo or transfection analysis is particularly well suited for studies concerning the *cis*-acting elements of transcription and can only provide marginal information about the *trans*-acting elements involved. *In vitro* transcription extracts, however, capable of supporting specific transcription initiation appears to provide a powerful tool for studying *cis*- and *trans*-acting elements. The main advantage of *in vitro* experiment is its ease and that specific initiation activity can be reliably measured early in the procedure. In fact, in the case of the *MGMT* gene, primer extension, RPA (RNase protection assay) and S1 mapping experiments showed that the yeast *MGMT* gene gave a major transcript initiating at a cytosine residue (Joo et al., 1995). The protected band in S1 mapping analysis after *in vitro* transcription was 191 bases long which was consistent with the previous results (Fig. 3). Thus, the *in vitro* transcription system in this work supported active and specific transcription initiation from the *MGMT* promoter.

Formation of transcription complexes capable of initiating the mRNA synthesis requires several factors in addition to RNA polymerase II. These general factors include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, TFIIH, and TFIIJ (Sawadogo and Sentenac, 1990; Gileadi et al., 1992; Lu et al., 1992; Zawel and Reinberg, 1992). For many genes, the first step of transcription complex formation involves the binding of TFIID to a TATA element (Van Dyke et al., 1988; Buratowski et al., 1991; Cortes et al., 1992). In yeast, the TFIID fraction contains a monomeric TBP of ~27 kD which did not appear to be tightly complexed with other proteins. The binding of yeast TBP to TATA sequence was found to be relatively slow, but its interaction became stable after binding (Hahn et al., 1989). Therefore, the binding of TBP to DNA is likely to be a point at which assembly of transcription

complex is regulated. Evidence has been accumulating which suggests that some transcription factors exert their positive effects either directly or indirectly on the complex formation by interaction with TBP (Sawadogo and Roeder, 1985; Abmayr et al., 1988; Horikoshi et al., 1988 a, b, 1991; Pugh and Tjian, 1990; Stringer et al., 1990; Lee et al., 1991).

The upstream sequence of yeast *MGMT* gene is shown to contain potential TATA elements (Fig. 1). DNase I footprinting analysis showed that three regions were protected by yeast rTBP (Fig. 2). *In vitro* transcription using proposed TATA box deletion mutants showed that the region I (from -34 to -25: 5'-TGATATAGCA-3') was only a real TATA element of yeast *MGMT* gene (Fig. 3).

The transcription activity analysis of *S. cerevisiae MGMT* gene which has serial deletions at 5' flanking region in its promoter demonstrated the presence of one URS (Fig. 4). The region between -333 and -213 functions as the transcription repressing sequence. When this region was deleted, the expression level was increased more than three-fold. A gel mobility shift assay using this DNA as a probe showed three shifted bands, and Southwestern blot analysis showed that two proteins likely bind to this region specifically. In this region, two distinctive elements were found: 10-bp sequence (5'-GGTGGAGGCC-3') at -233 to -224 and the 13-bp RAP1-like motif (5'-AAACCCAGTTCAG-3') at -245 to -233. The 10-bp sequence at -233 to -224 conforms to decamer consensus sequences in the promoter of DNA repair genes (*MAG, RAD1, RAD2, RAD4, RAD10, RAD16, RAD51, DDR48, PHR1*) and in the nucleotide metabolism genes (*RNR2, RNR3*) from *S. cerevisiae* (Xiao et al., 1993). This decamer appeared as the best candidate for the functional URS element. For 5 cases out of the 11 genes listed above, *in vivo* evidence has confirmed that this sequence did indeed influence on transcription (the others are not tested). In the promoter of *RAD51, RNR2* and *MAG* genes, the decamer sequence showed URS activity (Elledge and Davis, 1989; Hurds and Roberts, 1989; Xiao et al., 1993), while it acted as UAS (upstream activating sequence) in *RAD2* and *PHR1* genes (Siede et al., 1992).

Another candidate for URS element was found in RAP1 (repressor/activator protein 1) binding consensus motif (Graham and Chambers, 1994). This motif lies in URS region at -245 to -233 and overlaps 1 bp with the decamer consensus sequence. The RAP1 protein (Shore and Nasmyth, 1987), also known as TUF (translation upstream factor, Huet et al., 1985) or GRF1 (general regulatory factor 1, Buchman et al., 1988b), is a multifunctional, sequence-specific DNA binding protein that binds to a conserved sequence found in the upstream of many yeast genes. It is known to play a role in transcription activation of many genes which encode glycolytic enzymes, components of translational machinery, and other general housekeeping proteins (Vignais et al., 1987; Chambers et al., 1988; Capieaux et al., 1989). In addition to its transcription activation function, it was found that RAP1 is also involved in transcription silencing. It was reported that the silencer elements, 1 kb apart from *HML* and *HMR*, participated in the repressing the mating-type gene promoters (Kurts and Shore, 1991; Sussel and Shore, 1991). Since RAP1 is also known to activate *MAT* (Kurts and Shore, 1991), it seems likely that the protein can act as either an activator or a repressor depending on its chromosome location and its binding site context even in the same set of genes.

Protein-protein interactions also appeared to be involved in transcription activation by RAP1. The GCR1 binds CT/ATCC sequences found in many glycolytic genes (Baker, 1991; Sinclair et al., 1994). However, the RAP1-GCR1 interaction, together with at least two other proteins GCR2 and GAL11 (Nishizawa et al., 1990; Uemura and Jigami, 1992), can boost RAP1- mediated expression almost 10-fold (Buchman et al., 1988b). Several CT/ATCC sequences are found at -664 to -660, -637 to -633, -455 to -451, and -52 to -48 on the *MGMT* promoter in either direct or inverted orientation, implying that GCR1 may play some roles in the regulation of *MGMT* expression. Recently, protein kinase A (PKA) was found to mediate the growth-regulated expression of ribosomal protein genes by modulating RAP1 transcriptional activity (Klein and Struhl, 1994). Therefore, the possibility remains to be tested that the interactions among decamer binding protein, RAP1, and GCR1 may regulate the growth phase dependent *MGMT* gene expression.

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