Environmental Mutagens & Carcinogens 10-1 : 1-10 (1990)

Regulatory Expression of DNA Repair Genes Involved in Adaptive Response

Soo Young Choe[1], Hee Won Lee, and Sang Dai Park[2]

Department of Zoology, College of Natural Sciences
Seoul National University,
Seoul 151-742, Republic of Korea
[1] *Department of Biology, Chungbuk National Univeristy*
Cheongju 360-763, Korea
[2] *To whom all correspondences should be addressed*

(Received January 7, 1990)

The regulation of DNA repair genes expression was investigated using fused genes, in which the promoter of repair genes was hybridized with the *lacZ* structural gene. The activities of β-galactosidase expressed from the fused genes were highly increased when the host cells were exposed to methylating agents, such as methyl methanesulfonate (MMS), N-methyl-N'-nitro-nitrosoguanidine (MNNG) and methyl nitrosourea (MNU). On the other hand, the enzyme activities from the fused genes were not induced when the cells were treated with ethylating or nonalkylating agents, such as ethyl methanesulfonate (EMS), 4-nitroquinoline-1-oxide (4NQO), Bleomycin, and Benzo(a)pyrene (BP). In *ada* mutant cells the inducibility of the β-galactosidase enzyme activities from the fused genes could not observed even after exposure to the methylating agents. These results strongly indicate that the expression of the repair genes involved in adaptive response absolutely requires the Ada protein.

INTRODUCTION

Most monofunctional alkylating agents are electrophilic compounds and have a single reactive group which covalently interacts with a single nucleophilic center in DNA. One of the major alkylated products in cells exposed to MMS and MNNG is 3-methyladenine. This modified base is known to be rapidly excised by two types of 3-methyladenine-DNA glycosylases, glycosylase I and II, which are encoded from *tag* and *alkA* genes, respectively (Karran *et al.*, 1982; Evensen and Seeberg, 1982). These two enzymes have different substrate specificities. The former removes 3-methyladenine and the latter appears to have a broader substrate specificity, releasing 3-methyl-

* This work was supported by the grants provided from the Ministry of Education and the Korea Science and Engineering Foundation, The Republic of Korea.

guanine, 7-methylguanine, 0^2-methylcytosine and 0^2-methylthymine, in addition to 3-methyladenine from alkylated DNA (McCarthy *et al.*, 1984).

The product of *alkA* gene, 3-methyladenine-DNA glycosylase II, represents only 5-10% of the total glycosylase activities in *E. coli* cells. However, when the cells are adapted with low doses of alkylating agents, the enzyme activity is elevated by 20 fold. This induction seems to be the part of a specific response to alkylating agents, and this so-called "adaptive response" leads to a marked increase in resistance of cells to both mutagenic and lethal effects to alkylating agents (Karran *et al.*, 1982; McCarthy *et al.*, 1984).

The product of *ada* gene, 0^6-methylguanine-DNA methyltransferase, has been proved to have a single polypeptide with a molecular weight of 39,000 dalton after cloning of the gene and purification of the gene product from an expression vector system (Sedgwick, 1983; Teo *et al.*, 1984; LeMotte and Walker, 1985; Demple *et al.*, 1985; Nakabeppu *et al.*, 1985). The level of both of the O^6-methylguanine-DNA methyltransferase and 3-methyladenine-DNA glycosylase II increased by a plasmid carrying *ada* gene. These results indicated that the Ada protein would be a positive regulator of the adaptive response. However, the molecular mechanism of the regulation by the Ada protein has not been fully established. The simplest model was that the Ada protein became activated by transferring a methyl grop to itself. LeMotte and Walker (1985) showed that the ability of the Ada protein to stimulate transcription resided in the amino terminus. This research was focused on the inducible pattern of the expression of *alkA* or *ada* genes to various alkylating agents. The plasmids carrying *PalkA-lacZ* or *Pada-lacZ* fused genes was constructed by *in vitro* recombination, and the β-galactosidase activities were measured. By using these fused genes, it would be expected that the regulatory expression of *alkA* or *ada* genes could be deduced from the β-galactosidase activities.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

E. coli K12 strain (*thr-1, leu-6, thi-1, lacY1, supE44, galK2, ara-14, xyl-5, mtl-1, his-4, argE3, strA31, tsx033*) and its derivatives, MS23 (as AB1157 but with additional *alkA1, his*$^+$), PJ1 (as AB1157 but with *ada-1*) cells were used throughout the present experiments. DH1 (*recA1, endA1, hyrA96, thi-1, hsdR17* (r_K^-, r_K^+), *supE44, relA1?*, λ^-)was used as host cell for DNA amplification. CHS26 (*ara*, Δ(*lac pro*), *thi*) cells were used for β-galactosidase assay.

Plasmids containing the *ada* gene (pJCP702), which have been made in this laboratory (Jung *et al.*, 1985), were used in this study. The plasmid pYN1000, which contains the *alkA* gene, was kindly supplied by Dr. M. Sekiguchi of Kyushu University, Fukuoka, Japan. Transformation of *E. coli* with these plasmids and manipulations of DNA were carried out as described by Maniatis *et al.*(1982).

Chemicals

N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), methyl methanesulfonate (MMS),

ethyl methanesulfonate (EMS), methylnitrosourea (MNU), 5-bromo-4-chloro-3-indolyl-β-galactopyranoside (X-gal), and ampicillin were purchased from Sigma Chemical Co. (St. Louis, MO 63178, USA).

DNA Recombination

Restriction enzymes were used as recommended by the supplier. The DNA fragments were analyzed on 0.7% agarose gels containing ethidium bromide. Transformation of *E. coli* cells with plasmids was carried out by the calcium chloride/rubidium chloride method (Kushner, 1978). Restriction map of the plasmids was constructed depending on the results of gel electrophoresis patterns after double-digestion with various restriction endonucleases.

β-galactosidase Assay

Overnight cultures were prepared in minimal A medium (10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, sodium citrate. $2H_2O$ per liter) supplemented with thiamine HCl, $MgSO_4$ and glucose. By innoculating the culture to a fresh medium, cells were grown with aeration at 37°C until reached to 2-5 X 10^7 cells/ml. The β-galactosidase assay was performed according to the procedure of Miller (1972).

One or two drops of 0.1% SDS and chloroform were added to each ml of assay mixture containing aliquots of the culture and assay medium which is consisted of 60 mM $Na_2HPO_4.7H_2O$, 40 mM $NaH_2PO_4.H_20$, 10 mM KCl, 1 mM $MgSO_4.7H_20$, and 50 mM β-mercaptoethanol. The mixture were placed in a water bath for 5 min at 28°C. The reaction was started by adding 0.2 ml of ONPG (4 mg/ml) to each tube. The reaction was stopped by adding 0.5 ml of 1 M Na_2CO_3 solution after sufficient yellow color have developed. The units of β-galactosidase activity were measured by using the following formula:

$$\text{Units} = 1000 \text{ X } \frac{OD_{420} - 1.75 \text{ x } OD_{550}}{T \text{ x } V \text{ x } OD_{600}}$$

T : time of the reaction in min
V : volume of culture used in the assay, in ml

RESULTS

In order to investigate the regulation of the *alkA* and *ada* gene expression, the structural region of the *lacZ* gene was placed under the control of the *alkA* or *ada* promoter. The strategy of these DNA recombination are described in figure 1. In case of *alkA* gene promoter recombination (Fig. 1A), 0.84 kb EcoRI/HincII fragment of pYN1000 plasmid containing *alkA* promoter and beginning region of *alkA* gene were inserted into pMC1403 plasmid. The target plasmid, pMC1403, carried only the structural gene of *lac* operon (Casadaban *et al.*, 1980). The resulting plasmid was designated as pS206. The other recombinant plasmid, which carries the *Pada-lacZ* fused gene (Fig. 1B), was constructed as follows. The protruding ends of the HindIII/EcoRI fragment (0.18 kb) of pJCP702 plasmid containing the promoter and beginning region of *ada* gene, were converted into the blunt ends with Klenow fragment of DNA polymerase I. This fragment was then

inserted into pMC1403 just before the *lacZ* structural region.

Figure 2 shows that the correct translational reading frames were truly conserved in *lacZ* gene. It was expected that the CSH26 cells harboring the fused gene might appear to be Lac$^+$ phenotypes owing to the nature of the repair gene promoters. The clones harboring the recombinant plasmids carrying the fused genes could be selected by its blue color on the plates containing X-gal.

Using these recombinant plasmids, regulation of expression of *alkA* and *ada* genes were analyzed. The cells were cultured overnight in minimal A medium containing 0.2% casamino acid and ampicillin, and various mutagens were exposed to the cells. The aliquots were removed periodically and the β-galactosidase activities in the culture were determined. Figure 3 shows the kinetics of the induction of β-galactosidase activities by various alkylating agents (MMS, MNNG, MNU, and EMS). Cells harboring the recombinant plasmids produced about 100 units of β-galactosidase at normal conditions,

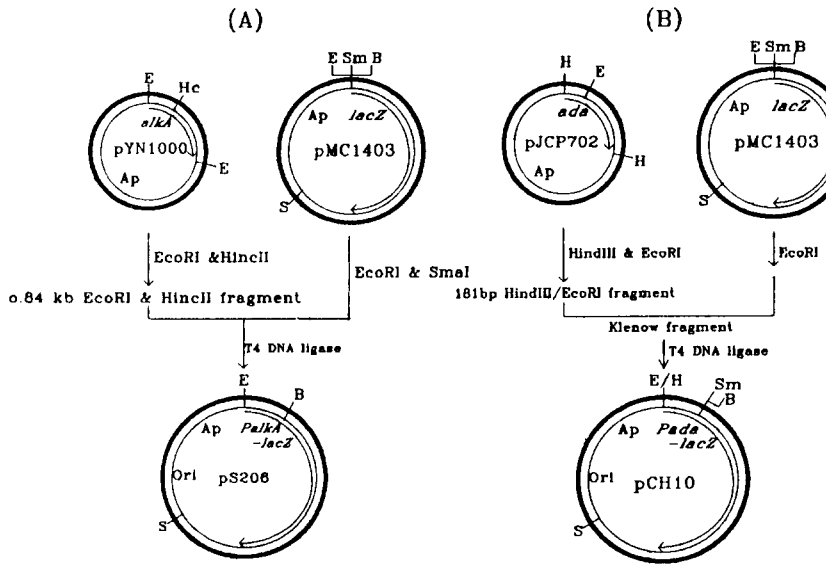

Fig. 1. **Strategy for gene fusion of the *alkA* and *ada* promoter region to the face of *lacZ* structural region in pMC1403 plasmid.**

(A) The double-digested DNA fragment of pYN1000 with EcoRI and HincII was substituted with the small DNA fragment of pMC1403 double-digested with EcoRI and SmaI. The expression of *lacZ* gene was made to be under the control of *alkA* promoter in pS206.

(B) The double-digested DNA fragment of pJCP702 with HindIII and EcoRI was inserted into pMC1403 plasmid digested with EcoRI. The expression of *lacZ* gene was made to be under the control of *ada* promoter in pCH10.

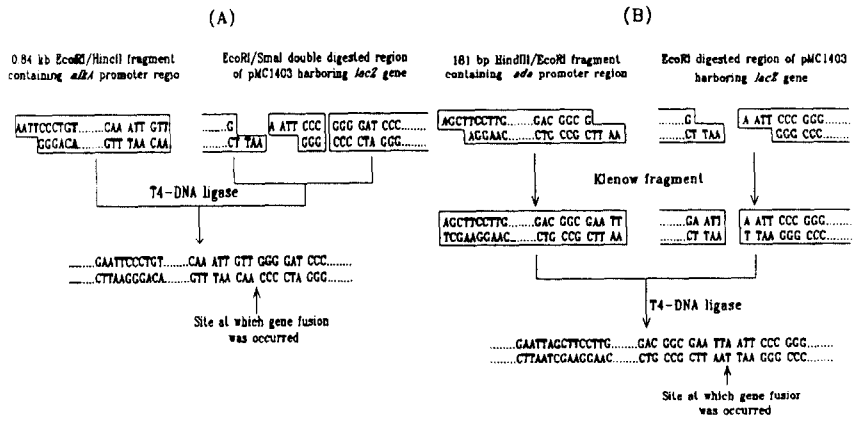

Fig. 2. Nucleotide sequences of the region in which the DNA recombination was occured.

(A) Joining the *alkA* promoter with the *lacZ* structural gene does not change the reading frame of the fused gene. The fused protein would be expressed from this gene.

(B) The HindIII/EcoRI fragment containing *ada* promoter was inserted in the EcoRI site of the pMC1403 plasmid after end filling with Klenow fragment of DNA polymerase I.

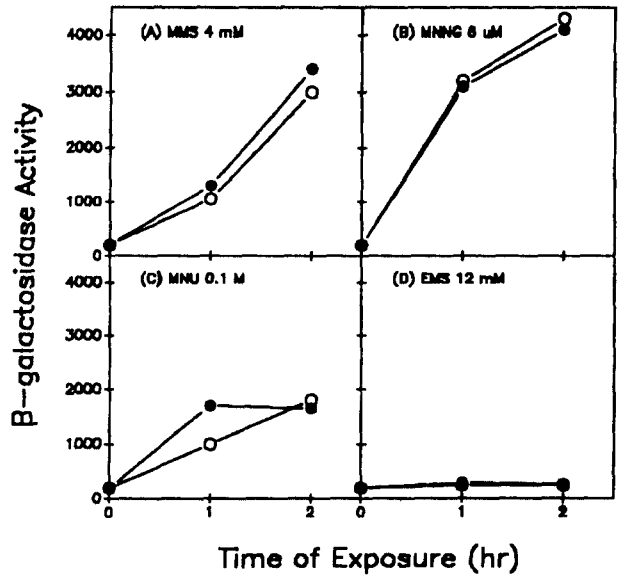

Fig. 3. β-galactosidase activities from the fused genes in CSH26 cells were determined after treatment with various alkylating agents for desired times. Open symbols indicate the enzyme activities from pS206 plasmid, and closed symbols those from pCH10.

(A) MMS (4 mM) (B) MNNG (6 μM)
(C) MNU (0.1 M) (D) EMS (12 mM)

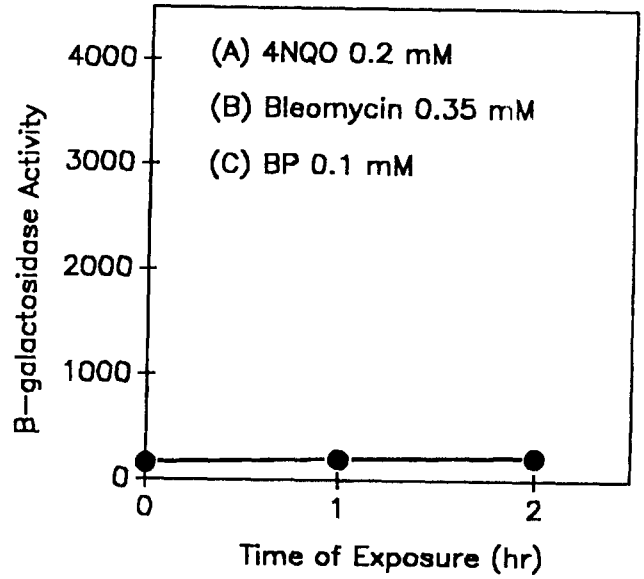

Fig. 4. β-galactosidase activities from the fused genes in CSH26 cells after treatment of nonalkylating agents. Symbols are used as the same in figure 3. In this figure all the symbols are superimposed.
(A) 4NQO (0.2 mM) (B) Bleomycin (0.35 mM)
(C) Benzo (a) pyrene (0.1 mM)

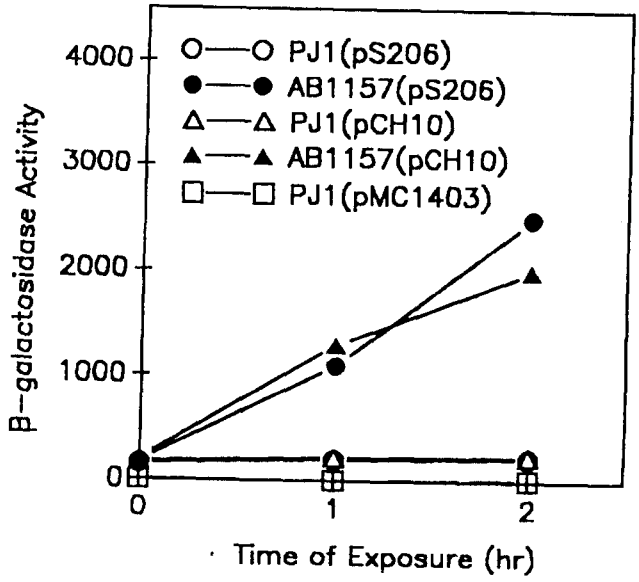

Fig. 5. Expression of the fused genes in cells which lack or contain the Ada protein. Effects of *ada* gene product on the expression of the fused genes were assayed during exposure to MMS.

but they showed a 20-30 fold increase in activity (about 3,000-4,000 units) to the exposure of methylating agents, such as MMS, MNNG or MNU. Induction by MMS, MNNG and MNU were effective at the concentration of 4 mM, 6 μM and 100 mM, respectively. Except for the extent of induction, the kinetics in the case of MNU were essentially similar to those of MMS and MNNG. An ethylating agent, EMS, was not effective in inducing the β-galctosidase activities. It was also found that 4NQO, beleomycin and BP were not effective in inducing the *alkA* or *ada* gene expression (Fig. 4). These results strongly suggested that the inducibility of the promoters would be controlled by the methylating agents.

The control pathways of *alkA* or *ada* gene expression were investigated. The fused genes were introduced into the wild type AB1157 and *ada* mutant cells, and the β-galactosidase activities were determined (Fig. 5). In AB1157 cells the enzyme activities were highly increased when adapted with MMS, but the induction of the enzyme activities was not observed in PJ1 mutant cells. From these results, it was clearly suggested that the operation of *alkA* or *ada* promoter was affected by the Ada protein, the product of *ada* gene. This implies that the Ada protein can act as a positive regulator which controls the expression of *alkA* or *ada* genes.

DISCUSSION

The adaptive response is a controlling system of DNA repair processes in *E. coli,* which was first suggested by Samson and Cairns (1977). At least two different types of DNA reapir enzymes associated with adaptive response have been introduced. One is 3-methyladenine-DNA glycosylase II and the other is 0^6-methylguanine-DNA methyltransferase (Karran *et al.,* 1982; Evensen and Seeberg, 1982; Thomas *et al.,* 1982; Demple *et al.,* 1983).

Methyltransferase is an enzyme that is induced as a result of adaptive response and are able to directly remove particular methyl groups from DNA molecules (Karran *et al.,* 1979; Cairns, 1980). The *ada* locus was first identified by isolating a mutant strain that blocks the induction of the adaptive response (Jeggo, 1979). The 3-methyladenine-DNA glycosylase II activity is increased approximately 20-fold during the adaptive response and initiates excision repair of methylated bases.

In the present study, the regulation of the gene expression was investigated using the gene fusion. The promoters of *alkA* and *ada* genes was hybridized with the *lacZ* structural region by *in vitro* recombination. The β-galactosidase activities from the fused genes would be increased if the promoters was activated by regulator. Figure 3 shows that the enzyme activities expressed from the fused genes in CSH26 cells exposed to alkylating agents such as MMS, MNNG, MNU, and EMS. When the cells were treated with methylating agents, induction of enzyme activities were observed. However, the induction could not be determined in the case of EMS treatment. This result strongly

suggested that the Ada protein, which was known to be a regulator for the induction, could be activated only by the methylation. There remains two possibilities for the no induced expression of the fused genes by the exposure to EMS. One is that the Ada protein cannot recognize and accept the ethyl adducts in DNA, and the other is that the Ada protein cannot be activated, even though it acts as a acceptor of the ethyl group from DNA. It still remains as a question.

The activities of β-galactosidase was not elevated in CSH26 cells harboring the fused genes in the case of the exposure to nonalkylating agents, such as 4NQO, bleomycin, and BP (Fig. 4). These results suggest that the Ada protein does not interact with the adducts caused by the above chemicals.

On purposed of the investigation for the roles of Ada protein, the inducibilities of the fused gene expression was examined in *ada* mutant, PJ1 cells. Figure 5 shows the enzyme activities in wild-type and *ada* mutant cells exposed to MMS. In wild-type cells the induction was normally observed, but the induction could not observed in *ada* mutants. These results indicate that the Ada protein plays as the positive regulator for the induced expression of the *alkA* and *ada* genes. The proposed regulatory mechanisms of gene expression involved in adaptive response indicates that Ada protein is activated by binding with methyl group and the activated Ada protein can bind with some common region on the promoters of *alkA* and *ada* genes.

REFERENCES

1. Cairns, J., (1980): Efficiency of the adaptive response of *Escherichia coli* to alkylating agents. *Nature* 286: 176-178.

2. Casadaban, M.J., J. Chou, and S.N. Cohen, (1980): *In vitro* gene fusions that join an enzymatically active β-galactosidase segment to aminoterminal fragments of exogeneous proteins; *E. coli* plasmid vectors for the detection and cloning of transcriptional initiation signals. *J. Bacteriol.* 143: 971-980.

3. Demple B., B. Sedgwick, P. Robins, N. Torry, M.D. Waterfield, and T. Lindahl, (1985): Active site and complete sequence of the suicidal methyltransferase that counters alkylation mutagenesis. *Proc. Natl. Acad. Sci.* (USA) 82: 2688-2692.

4. Evensen G. and E. Seeberg, (1982): Adaptation to alkylation resistance involves the induction of a DNA glycosylase. *Nature* 296: 773-776.

5. Jeggo, P., (1979): Isolation and characterization of *Escherichia coli* K12 mutants unable to induce the adaptive response to simple alkylating agents. *J. Bacteriol.* 139: 783-791.

6. Jung Y.K., S.Y. Choe, and S.D. Park, (1985): Molecular cloning of *ada* gene required for the adaptive response to alkylating agents in *E coli. Korean J. Gentics* 7: 193-201.

7. Karran, P., T. Lindahl, and B. Griffin, (1979): Adaptive response to alkylating agents involves alteration *in situ* of 0^6-methylguanine residues in DNA. *Nature* 280: 76-77.

8. Karran P., T. Hjelmgren, and T. Lindahl, (1982): Induction of a DNA glycosylase for N-methylated purines is part of the adaptive response to alkylating agents. *Nature* 296: 770-773.

9. Kushner, S.R., (1978): An improved method for transformation of *Escherichia coli* with ColE1-derived plasmids. *In:* Genetic engineering (H.B. Boyer and S. Nicosia ed.). Elsevier/North-Holland, Amsterdam, p. 17.

10. LeMotte P.K. and G.C. Walker, (1985): Induction and autoregulation of *ada*, a positively acting element regulating the response of *Escherichia coli* K12 to methylating agents. *J. Bacteriol.* 161: 888-895.

11. McCarthy T.V., P. Karran, and T. Lindahl, (1984): Inducible repair of 0-alkylated DNA purimidines in *Escherichia coli. EMBO J.* 3: 545-550.

12. Maniatis T., E.F. Fritsch, and J. Sambrook, (1982): *In:* Molecular cloning. A laboratory manual. Cold Spring Harbor Lab., New York.

13. Miller, J.H., (1972): *In:* Experiment in molecular genetics. Cold Spring Harbor Lab., New York.

14. Nakabeppu Y., H. Kondo, S. Kawabata, S. Iwanaga, and M. Sekiguchi (1985): Purification and structure of the intact Ada regulatory protein of *Escherichia coli* K-12, 0^6-methylguanine-DNA methyltransferase. *J. Biol. Chem.* 260: 7281-7288.

15. Samson L. and J. Cairns, (1977): A new pathway for DNA repair in *Escherichia coli. Nature* 267: 281-282.

16. Sedgwick B., (1983): Molecular cloning of a gene which regulates the adaptive response to alkylating agents in *Escherichia coli. Mol. Gen. Genet.* 191: 466-472.

17. Teo I., B. Sedgwick, B. Demple, B. Li, and T. Lindahl, (1984): Induction of resistance to alkylating agents in *E coli:* the ada^+ gene product serves both as a regulatory protein and as an enzyme for repair of mutagenic damage. *EMBO J.* 3: 2151-2157.

18. Thomas L., C.H. Yang, and D.A. Goldthwait, (1982): Two DNA glycosylases in *Escherichia coli* which release primarily 3-methyladenine. *Biochemistry* 221: 1162-1169.

적응반응 관련 DNA 회복유전자의 발현조절에 관한 연구

최수영[1], **이희원**, **박상대**[2]

충북대학교 생물학과[1]
서울대하교 동물학과[2]

Iac Z 구조유전자에 DNA 회복 유전자인 ada 유전자의 promoter를 붙인 융합유전자를 사용하여 DNA 회복유전자의 발현에 대한 조절기작을 연구하였다. MMS, MNNG, MNU와 같은 메칠화제로 처리하였을 경우 β -galactosidase의 활성은 증가하지만 EMS, 4NQO, Bleomycin, BP와 같은 에칠화제나 비알킬화제로 처리하면 활성의 증가를 보이지 않았다. 그러나 ada 돌연변이세포에서는 메칠화제를 처리하여도 융합유전자의 β -galactosidase 효소의 활성증가를 보이지 않았다. 이러한 결과는 적응반응에 관련된 회복유전자의 발현은 Ada단백질에 의해 조절됨을 시사한다.