Characterization of UV-Inducible Gene(*UVI-155*) in *Schizosaccharomyces pombe*

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The present study intends to characterize the DNA damage-inducible responses in yeast. The fission yeast, *Schizosaccharomyces pombe* was used in this study as a model system for higher eukaryotes. To study UV-inducible responses in *S. pombe*, five UV-inducible cDNA clones were isolated from *S. pombe* by using subtration hybridization method. To investigate the expression of isolated genes, *UVI-155*, the cellular levels of the transcripts were determined by Northern blot analysis after UV-irradiation. The transcripts of isolated gene (*UVI-155*) increased rapidly and reached maximum accumulation after UV-irradiation. Compared to the message levels of control, the levels of maximal increase were approximately 5 fold to UV-irradiation. In order to investigation whether the increase of *UVI-155* trascripts was a specific results of UV-irradiation, *UVI-155* transcript levels were examined after treating the cells to mthylmethane sulfonate (MMS). The transcripts of *UVI-155* were not induced by treatment of 0.25% MMS. These results implied that the effects of damaging agents are complex and different regulatory pathways exist for the induction of these genes. To characterize the *UVI-155* gene, gene deletion experiments were analyzed. The deleted strain was not well grown. This result indicated that the *UVI-155* gene is essential for cell viability.

Key words – *UVI-155*, UV-inducible gene, DNA repair, cell viability, MMS

Organisms have a potential to enhance resistance to DNA damage. The DNA damage-tolerance mechanisms involve the cellular responses to damage at or near replication forks. These mechanisms, which do not result on the removal of damage from the genome, include a variety of recombinational and mutagenic processes that are not necessarily unique to the processing of damaged DNA[1,2]. In addition, some damage tolerance mechanisms are associated with a significant increase in mutation frequency, thus providing the potential for genetic diversity within a population of affected cells[6,7]. The molecular mechanisms of DNA repair and tolerance responses are still unclear in eukaryotic cells.

One of the most interesting aspects of cellular response to DNA damage may be the regulation of its activity. In *E. coli*, four major regulatory systems that control the expression of several genes induced by DNA damages of environmental stresses have been identified: the SOS response, the adaptive response to alkylation damage, the response to oxidative damage, and the heat-shock response[6,9,10]. The SOS response which plays multiple roles in DNA repair, recombination, and mutagenesis provides a molecular model of coordinate gene regulation. A number of DNA damage-inducible genes were recently identified and characterized in eukaryotes. However, available data do not indicate any obvious similarity to inducible responses in prokaryotes.

In higher eukaryotic cells, several cDNA clones showing the inducibility by damaging agents were isolated[2,4]. Although the functions of most genes are not yet defined, these studies elicited several interesting general insights. Firstly, multiple and diverse DNA-damaging agents can induce expression of several genes, and damage-inducible genes may be ubiquitous[1,11,12]. Secondly, constitutive expression and inducibility of genes can be influenced by the DNA repair capacity of cells. Thirdly, heat shock treatment induces the expression of some genes which are induced by DNA-damaging agents[19,21].

Among these genes, *DINI* was identified as a gene encoding a regulatory subunit of ribonucleotide reductase (*RNR3*)[20]. Many of yeast genes with known functions are also inducible by DNA damage. These include *RNR2*, a gene encoding the small subunit of ribonucleotide reductase[17]; *CDC8* encodes thymidylate kinase; *UBI4*, which encodes polyubiquitin ; *POLI*, which encodes DNA polymerase α; *CDC9*, the gene for DNA ligase[12,13]. Among these, *RNR2* and *CDC8* could play indirect roles in DNA repair by providing precursors for repair synthesis. The

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CDC8, *CDC9*, and *POL1* genes are also cell-cycle regulated. The enhanced expression of *CDC9* following exposure to UV-irradiation has been demonstrated in non-cycling stationary phase cultures. Therefore, the induction of this gene is the direct response to UV-irradiation, rather than simple synchronization of cell cycle. The *UBI4* gene which is required for the degradation of proteins is increased in stationary phase and meiosis[15,16]. The induction of this gene by DNA damaging agents suggests that some genes could be induced in response to aberrant proteins generated by the treatment of DNA damaging agents.

The present study intends to characterize the DNA damage-inducible responses in eukaryotic cells. The fission yeast, *S. pombe*, which displays efficient DNA repair systems, was used in this study as a model system for higher eukaryotes. To study UV-inducible responses in *S. pombe*, five UV-inducible cDNA clones were isolated from *S. pombe* by using subtration hybridization method. To investigate the expression of isolated genes, the cellular levels of the transcripts of these genes were determined by Northern blot analysis after UV-irradiation.

Materials and Methods

Strains, cell culture, and genetic methods

E. coli strain DH5*a* (F- endA1, hsd17, (r-, mk-), supE44, thi-1, recA1, gyrA96, relA1, lacIqZ-M15) and yeast *S. pombe* strain JY741 (h- ade6- M210 leu1-32 ura4-D18) was used for this study. *E. coli* strains were grown on LB media (1% tryptone, 1% sodium chloride, 0.5% yeast extract). Yeast strain was grown in YE (2% glucose, 0.5% yeast extract) medium supplemented with appropriate amino acids. Standard molecular biology techniques were employed as described[18]. *S. pombe* chromosomal DNAs were prepared according to the methods of Choi[3].

Preparation of DNA probe by random priming

The DNA fragment was labeled with [a-^{32}P] dCTP(3,000 Ci/mmole) by random primed DNA labeling method[5]. The labeling reaction was carried out in 20 *ul* of the standard random priming buffer containing 50 *ng* of DNA, 30 *uCi* of [a-^{32}P] dCTP, dATP, dGTP,dTTP and 2 unit of Kenow enzyme for 1 hr at 37^{0}C.

Subtraction Hybridization

For subtraction hybridization, 15 μg of biotinylated DNA were mixed with 3 μg of single stranded DNA from UV-induced cDNA library and resuspended in 1 X hybridization buffer(100 mM Tris-HCl, pH 7.5, 0.3 M NaCl, 1mM EDTA, 0.1% SDS, 1 μg/ml poly(A)). The mixture was boiled for 1 minute and then incubated at 68℃ for 36hours. After hybridization, the biotinylated DNA was removed by the addition of streptavidin and extraction with phenol/chloroform. Remaining DNA was converted to double stranded DNA using T7 primer and klenow fragment. Ten μl of DNA solution was mixed with reaction buffer to final concentrations of 40 mM potassium phosphate, pH 7.5, 6.6 mM MgCl2, 500 μM dNTP, 20 ng T7 primer, 5 unit klenow fragment, 100 unit of T4 DNA ligase, and then incubated at 16℃ for 6 hours. This mixture was used directly for transformation into *E. coli* cells.

Treatment of DNA damaging agent and northern blot analysis

One hundred milliliters of cells grown to mid exponential stage were harvested, washed and then resuspended in 10 ml of distilled water. The cell suspension was evenly spreaded onto 150mm petri dish and then exposed to 200 J/m^2 of ultraviolet (UV)-light or 0.25% MMS treatment. The treated cells were inoculated into fresh YE medium, incubated at 30^{0}C in the dark, and collected at indicated times. Total RNA was prepared according to Jang *et al.*[8]. RNA was denatured and electrophoresed in 1.2% agarose containing formaldehyde and transferred onto nitrocellulose filters. The membrane was hybridized with probe DNA fragment at 68^{0}C. After hybridization, the membrane was washed twice in 2X SSPE, 0.1% SDS and 0.2X SSC, 0.01% at 55^{0}C. After the final rince, the membrane was wrapped with plastic wrap, and exposed onto X-ray film (X-Omat, Kodak) for 12 hrs or more.

Results and Discussion

Isolation of UV inducible gene

DNA damage triggers complex cellular responses in *E. coli* that include induction of several genes involved in repair, recombination and mutagenesis. The SOS response is an example of a stress response, where an environmental stress condition activates transcription of a group of genes [14,15,16]. In these kinds of responses to environmental stress, heat shock response has been found and well characterized in both prokaryotic and eukaryotic organ-

isms[9,13]. However, there is no direct evidence for an SOS-like response in eukaryotic cells. For the characterization of UV-inducible response in eukaryotic cells, UV-inducible genes were isolated from *S. pombe* cells by subtraction hybridization methods.

The isolation of UV-inducible genes from *S. pombe* was accomplished using subtraction hybridization method. For the induction of UV-inducible transcripts, *S. pombe* cells in mid-log phase were irradiated with 200 J/m^2 of UV-light and incubated for 3 hours. Poly(A)$^+$ RNA isolation from UV-induced or normal cells were used for templates for cDNA synthesis. For the enrichment of UV-inducible cDNA fragments, substraction hybridization was performed. About 10 μg of single stranded DNA obtained from normal RNA was hybridization with UV-induced RNA. The unhybridized UV-induced RNA was made cDNA and then ligated into TA cloning vector. After transformation into XL1 blue cells, about 50 clones were obtained as substracted library. To confirm UV-inducibility of these clones, dot blot analysis was performed (Fig. 1). Among these cDNA clone, one clone showed higher intensity with UV-induced. This isolated gene was designated as *UVI-155* (**UV**-inducible). To determine whether the inducibility of the isolated UV-inducible genes by UV-irradiation, total RNAs isolated from *S. pombe* cells were analyzed by Northern blotting using with *in vitro* transcribed RNA probes. To confirm UV-inducibility of the isolated gene, northern blot analysis was performed (Fig. 2). This result was indicated that the *UVI-155* gene was induced by UV-irradiation. *ACT1* gene was used as an internal control.

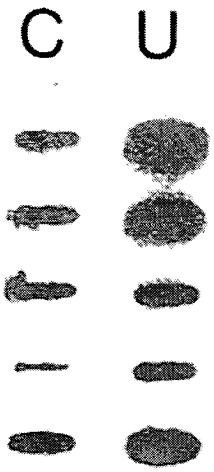

Fig. 1. Screening of substraction hybridization. Equal amount of DNA was transferred onto two sheets of NC filters using slot blot kit and hybridized with ^{32}P-labelled first strand cDNA probes prepared from normal or UV-irradiated *S. pombe* cells. Lane C, probes from normal cells; U, probes from UV-irradiated cells.

Figure 2 shows the kinetics of UV-inducibility by UV-irradiation. The transcripts of *UVI-155* increased rapidly and reached maximum accumulation at 1 hrs after UV-irradiation. Compared to the message levels of control, the levels of maximal increase were approximately 5 folds to UV-irradiation. In order to investigation whether the increase of *UVI-155* trascripts was a specific results of UV-irradiation, *UVI-155* transcript levels were examined after treating the cells to methylmethane sulfonate (MMS). As shown in figure 3, the transcripts of *UVI-155* iduced by treatment of 0.25% MMS for 30 minutes. These results implied that the effects of damaging agents are complex and

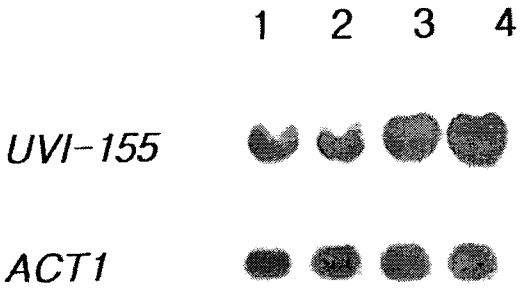

Fig. 2. Increase of *UVI-155* transcript levels by UV-irradiation. Exponentially growing *S. pombe* cells were irradiated with 180 J/m^2 of UV-light and incubated at 30°C for 0, 2, 4, or 6 hours. At the times indicated, total RNA were isolated and the transcript levels of UVI-155 were determined by Northern blot analysis. *ACT1* gene used as an internal control. Lane 1, 2, 3, and 4; post-irradiation incubation for 0, 2, 4, and 6 hours, respectively.

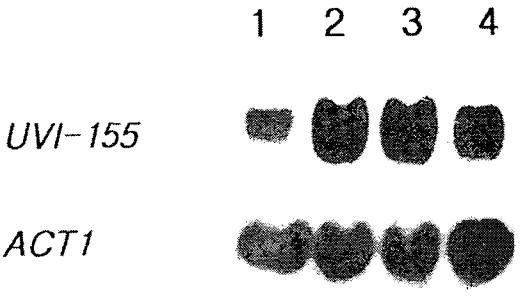

Fig. 3. The transcript levels of *UVI-155* gene in MMS teated cells. Exponentially growing *S. pombe* cells were treated with 0.25% MMS for 30 min, and incubated at 30°C. After incubating, RNA was isolated and northern blot analysis was performed with *in vitro* transcribed *UVI-155* probes. Lane 1, 2, 3, and 4, post-treated incubation for 0, 2, 4, and 6 hours, respectively.

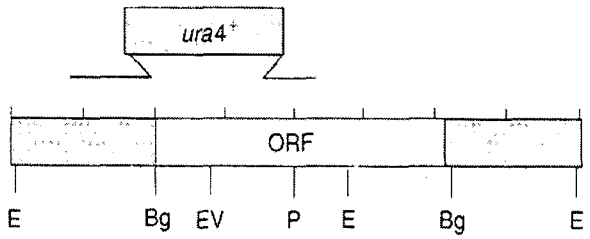

Fig. 4. Construction of *UVI-155* gene deletion mutant. The 2.5 kb *Bgl*II-*Bgl*II of *UVI-155* gene was replasced with 1.5 kb *Ura4+* fragment to create deletion murants (*UVI-155::Ura4+*).

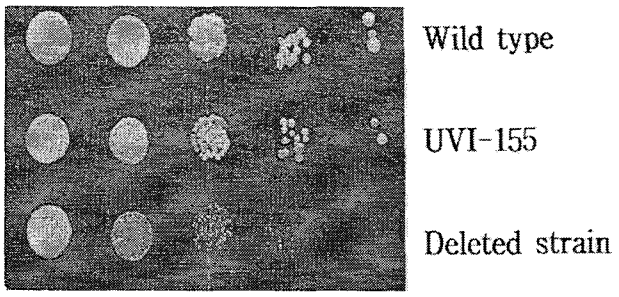

Fig. 5. The *UVI-155* gene restores cell viability and DNA repair. The *UVI-155* gene is an essential gene for cell growth. Cells from a single colony were cultured, assayed by spotting serially diluted cells onto YE medium, and incubated at 30°C for 3 days. wild-type, JY741; deleted strain, *UVI-155* gene replaced by Ura4+; *UVI-155*, isolated UV-inducible gene.

different regulatory pathways exist for the induction of these genes. These results imply that *UVI-155* gene product might be involved in specific cellular response such as DNA repair, recombination or mutagenesis.

Gene deletion of *UVI-155*

To determine the *UVI-155* gene is required for cell viability and DNA repair, *UVI-155* gene deleted strain was constructed. The construction was made in which a 2.5 kb *Bgl*II-*Bgl*II fragment, containing the majority of the ORF including the ATG, was replaced by the *Ura4+* gene (Fig. 4). The generation of gene mutations was verified by Southern blot analysis. The viability of deleted strain was assayed by spotting serially diluted cells onto plates. This assay showed that the *UVI-155* deleted cells has a strongly reduced growth (Fig. 5). This result indicated that the *UVI-155* gene is essential for cell viability.

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초록 : 효모 *Schizosaccharomyces pombe*에서 자외선 유도유전자 *UVI-155*의 분리 및 특성 연구

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본 연구는 DNA 상해유도기작을 규명하기 위하여 하등 진핵생물인 분열형 효모 *Schizosaccharomyces pombe*로부터 subtraction hybridization 방법을 이용하여 자외선 유도 유전자인 *UVI-155*을 분리하고 그 유전자 구조와 발현양상을 조사하였다. UVI-155 유전자의 발현양상을 Northern hybridization 방법으로 살펴본 결과 자외선(ultraviolet-light) 조사 1시간 후에 최대의 발현 증가를 나타내었다. 반면 알킬화제인 MMS (methyl methanesulfonate) 처리에 의해서도 발현이 증가되었다. 이 결과 다른 UV-inducible 유전자와는 다르게 *UVI-155* 유전자는 UV와 MMS 등의 DNA 상해에 모두 발현이 증가됨을 알 수 있었다. 또 한 유전자의 기능을 알기 위하여 null-mutant 세포 주를 제조하여 그 특성을 살펴본 결과 이 유전자는 세포의 성장에 필수적인 유전자임을 알 수 있었다.