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Isolation and Characterization of UV-inducible genes in Eukaryotic cells

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Abstract 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. The transcripts of isolated gene (UVI30) 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 UVI30 trascripts was a specific results of UV-irradiation, UVI30 transcript levels were examined after treating the cells to Methylmethane sulfonate (MMS). The transcripts of UVI30 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 structure of UVI30 gene, nucleotide sequences were analyzed. The nucleotide sequence of 1,340 nucleotide excluding poly(A) tail contains one open reading frame, which encodes a protein of 270 amino acids. The predicted amino acid sequences of UVI30 do not exhibit any significant similarity to other known sequences in the database.

Key words: UV-inducible gene, DNA repair, Subtration hybridization, DNA-damaging agents

Introduction

DNA repair is a cellular event involving the removal of damaged or mispaired bases from the genome of living cells [1,2]. The forms of DNA damage that can be repaired by direct reversal are apparently limited. The most general mode of DNA repair observed in nature is excision repair. The damaged or inappropriate bases removed by damage specific DNA incision and excision activity, and the missing nucleotides are replaced by the sequential actions of DNA polymerase and DNA ligase.

Organisms also 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 [3-5].

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 [6-8]. 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 [9]. The SOS response which plays multiple roles in DNA repair, recombination, and mutagenesis provides a molecular model of coordinate gene regulation [9]. 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 [10-12]. 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

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induce expression of several genes, and damage-inducible genes may be ubiquitous [13,14]. Secondly, constitutive expression and inducibility of genes can be influenced by the DNA repair capacity of cells [13]. Thirdly, heat shock treatment induces the expression of some genes which are induced by DNA-damaging agents [15,16].

Among these genes, DINI was identified as a gene encoding a regulatory subunit of ribonucleotide reductase (RNR3) [17]. 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 [18]; CDC8 encodes thymidylate kinase ; UBI4, which encodes polyubiquitin ; POLI, which encodes DNA polymerase α; CDC9, the gene for DNA ligase [19]. Among these, RNR2 and CDC8 could play indirect roles in DNA repair by providing precursors for repair synthesis. The 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 [20]. 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. 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.

Of many yeast genes involved in DNA repair, several genes (PHR1, RAD2, RAD6, RAD7, RAD18, RAD23, RAD51, RAD54) have been shown to be transcriptionally induced by DNA damaging agents. The expression of RAD2 gene required for damage specific incision of DNA was very well studied [21-23].

Analogies of induction patterns or promoter structures have not yet reveled any common regulatory networks or signalling systems. Clearly, there is still much to be learned about the cellular responses to DNA damage in eukaryotic cells. The inducibility of certain genes by DNA damage constitutes one of the most interesting parts in understanding the survival of the cells. Despite extensive studies in prokaryotic cells, especially in *E. coli*, little information about DNA damage inducible network is available in eukaryotic cells.

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

Bacterial, Yeast Strains and Plasmids

XL1-Blue cells were used as host cells for library construction. The recombinant plasmids constructed in this study were propagated in DH5 α cells. *E. coli* strains Y1090 or JM101 were used as host cells for bacteriophage λ gt11 or M13.

The phagemid pBluescript SK(+) and KS(+) were used as cloning vehicles of cDNA library. pUC18, 19 and M13 mp18, 19 were used for subcloning or nucleotide sequence analysis. The helper phage, M13KO7 was used to obtain single stranded phagemid DNA form cDNA libraries.

Culture of *E. coli* and Yeast Cells

E. coli cells were cultured in Luria-Bertani (1% Bacto-tryptone, 1% NaCl, 0.5% yeast extract) medium at 37℃ with vigorous shaking. When necessary, ampicillin and tetracycline were added to final concentrations of 50 $\mu g/m\ell$ and 100$\mu g/m\ell$, respectictively. XL1-Blue cells infected with M13KO7 were grown in 2 X YT (1.6% Bacto-tryptone, 1% yest extract, 0.5% NaCl) medium containing 70$\mu g/m\ell$ of kanamycin. Y1090 cells for infection of λ gt11 bacteriophage were cultured in NZCYM(1% NZ amine, 0.5% NaCl, 0.5% yeast extract, 0.1% casamino acids, 0.2% $MgSO_4$) medium supplemented with 0.4% maltose.

S. pombe cells were grown in YE(3 % glucose, 0.5% yeast extract), EMN (Edinburg minimal medium), or SD (synthetic dextrose; 2% glucose, 0.67% yeast nitrogen base) media supplement with appropriate amino acids. The medium was solidified by adding 1.5% Bacto-agar (Difco) for plate culture.

Southern blot analysis

Chromosomal DNA from *S. pombe* cells was digested to completion by various restriction enzymes, electrophoresed on a 0.7% agarose gel and transferred onto S&S Nytran membrane. The membrane was hybridized with ^{32}P-labelled DNA at 42^{0}C for 16 hours under 50% formamide condition.

Northern blot analysis

Isolated RNA was denatured, fractionated on 1.2% agarose gel containing 0.66 M formaldehyde, and transferred onto S&S Nytran membrane. The probe and the filter hybridization/washing conditions were as described in the instruction manual [25].

Nucleotide sequence analysis

All sequencing reactions were performed by the double-stranded dideoxy method using kits from Pharmacia LKB or US Biochemicals. Homology searches comparing the amino acid sequence were performed using FASTA.

Treatment of DNA Damaging Agents

S. pombe cells grown to mid-log phase (OD_{595}=0.5) were

harvested, washed, and resuspended in 50 mM potassium phosphate buffer, pH 7.0, to a final concentration of 5×10^7 cells/㎖. The cell suspension was spread onto petridish, and then exposed to 254 ㎚ UV-light from a mercury germicidal lamp at an incident dose of 1.42 J/㎡/sec. After treatment of DNA damaging agents, aliquots of cells were spread onto YES plates for survival test or cultured at 30℃ in dark place for RNA isolation [25].

Subtraction Hybridization

For subtraction hybridization, 15㎍ of biotinylated DNA were mixed with 3㎍ 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㎍/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 ㎕ of DNA solution was mixed with reaction buffer to final concentrations of 40 mM potassium phosphate, pH 7.5, 6.6 mM MgCl2, 500㎛M 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.

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 [26-28]. In these kinds of responses to environmental stress, heat shock response has been found and well characterized in both prokaryotic and eukaryotic organisms [29-31]. However, these 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/㎡ 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㎍ 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). Five cDNA clones were confirmed to be UV-induced. To determine 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. ACT1 gene was used as an internal control. Figure 2 shows the induction kinetics of UV-inducible genes by UV-irradiation. This isolated gene was designated as UVI30 (**UV-inducible**). The UVI30 transcripts increased rapidly and reached maximum accumulation at 3 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 UVI30 trascripts was a specific results of UV-irradiation, UVI30 transcript levels were examined after treating the cells to Methylmethane sulfonate (MMS). As shown in Figure 3, the transcripts of UVI30 was not induced by treatment of 0.25% MMS for 30 minutes. These results implied that the effects of damaging agents are complex and different regulatory pathways exist for the induction of these genes. These results imply that UVI30 gene product might be

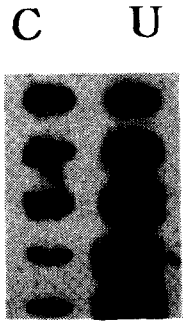

Fig. 1. Screening of subtration hybridization. Equal amount of DNA was transferred onto two sheets of NC fiters using slot blot kit and hybridized with ^{32}P-labeled first strand cDNA probes prepared from normal or UV-irradiated *S. pombe* cells. Lane C, probes from normal cells; U, probes from UV-irradiated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

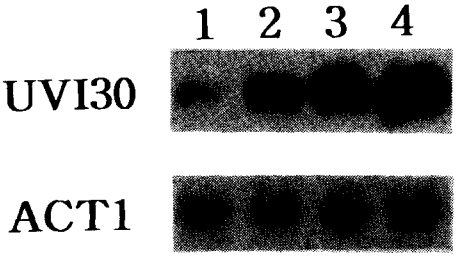

Fig. 2. Increase of UVI30 transcript levels by UV-irradiation. RNA preparation and Northern blot analysis were carried out. Lane 1, 2, 3, and 4, post-irradiation incubation for 1, 2, 4, and 6 hours, respectively.

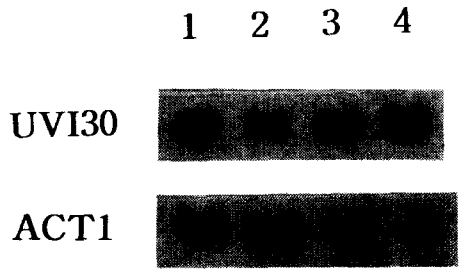

Fig. 3. The transcript levels of UVI30 gene in MMS teated cells. Exponentially growing *S. pombe* cells were treated with 0.2% MMS for 30 min, and incubated at 37℃. After incubating, RNA was isolation and northern blot analysis was performed with *in vitro* transcribed UVI30 probed. Lane 1, 2, 3, and 4, post-treated incubation for 1, 2, 3, and 4 hours, respectively.

involved in UV-specific cellular response such as DNA repair, recombination or mutagenesis.

Genetic Mapping of UVI30 gene

In order to confirm that *S. pombe* chromosome contains the same DNA, Southern blot analysis was performed. The probe hybridization with only one or two fragments for each digestion (Fig. 4), indicating that UVI30 gene locus existed as a single copy in *S. pombe* genome.

To characterize the structure of UVI30 gene, nucleotide sequences were analyzed. The nucleotide sequences of 1,340 nucleotide excluding poly(A) tail contain one open reading frame, which encodes a protein of 270 amino acids. The predicted amino acid sequences of UVI30 do not exhibit any significant similarity to other known sequences in the database.

Although the gene product of this gene is not characterized yet, data obtained suggest that UVI30 gene product is a stress protein such as heat shock proteins. Identification of the gene production and the induction of the protein by various damaging agents is in progress.

Acknowledgments

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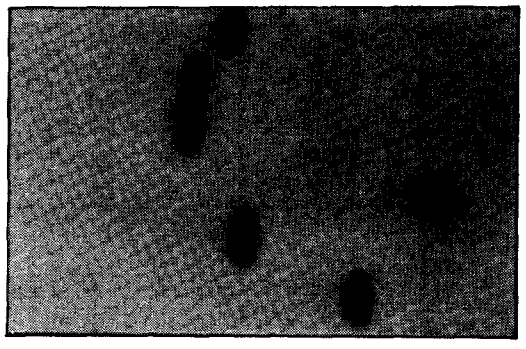

Fig. 4. Southern blot analysis of UVI30 gene. *S. pombe* genomic DNA isolation from JY741 cells were digested with various restriction enzymes. Restriction enzymes used were shown at the top of each Lane.

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ccagattccttgacaattccagaatccattacaatgaacctgtctgccaatttcattaat
gggttggtgtgtaattatcaaaactgtacaactcaagttttgaattgtttttctagaag
aaggcttgacttgctatctaaagcagacgtacattcatccaatattaaaagtctggggtt
ccgtagaagagctcttgcaaacgcaaggcgttggatctgaccaccggaaaaatttttgct
atgttcaagcaaaggagtatcacggccgttaggaaggttttgtattacctcagtcatgta
 M  F  K  Q  R  S  I  T  A  V  R  K  V  L  Y  Y  L  S  H  V
agttttgtcaagtgcatccataacagaattaatatctctgtcaacaccattttgcaaact
 S  F  V  K  C  I  H  N  R  I  N  I  S  V  N  T  I  F  Q  T
ctccaaaattgtagaacccaaaagatgaggtttttgatctacaatggcaacctttttaag
 L  Q  N  C  R  T  Q  K  M  R  F  L  I  Y  N  G  N  L  F  K
caaccaattagtatcgatgttagtctacggatacccatcaatgtaaatatcctctctagg
 Q  P  I  S  I  D  V  S  L  R  I  P  I  N  V  N  I  L  s  R
gtaagtctttctcaacacttctaccaaagtagattttccagatccagaaattccaacaat
 V  S  L  S  Q  H  F  Y  Q  S  R  F  S  R  S  R  N  S  N  N
tgcaaccttttctctagcttcaatagacaattttacattattcagcgcgaagtggttacg
 C  N  L  F  S  S  F  N  R  Q  F  Y  I  I  Q  R  E  V  V  T
ctcggagtctgggtatgcaaacgatactccatcgaatttctatttttcctacaagaggaaa
 L  G  V  W  V  C  K  R  Y  S  I  E  F  Y  F  S  Y  K  R  K
tttgaaatatccacttttatgaagatttcctggcttcaaaaaactgagtttcagaactct
 F  E  I  S  T  F  M  K  I  S  W  L  Q  K  T  E  F  Q  N  S
actagtcgcgattcggctagctcatacattgggcaaagacatgatgcaagtagaagccat
 T  S  R  D  S  A  S  S  Y  I  G  Q  R  H  D  A  S  R  S  H
caacaatgtaaaagtagccaaagacagcacctggaccatttgttcaacagtataaaattc
 Q  Q  C  K  S  S  Q  R  Q  H  L  D  H  L  F  N  S  I  K  F
tttggatataaatatggaagaacaataaaagagaagagctctgacaaaatacaaaagaga
 F  G  Y  K  Y  G  R  T  I  K  E  K  S  S  D  K  I  Q  K  R
gttgttaatagcaaacccaagactagtccaaaaggctcgccttttgaaagcttcccagct
 V  V  N  S  K  P  K  T  S  P  K  G  S  P  F  E  S  F  P  A
tttgcgcaaagaatttttatgattttctcgaaaaaaagacttcaccgaatatccttttaat
 F  A  Q  R  I  F  M  I  F  S  K  K  R  L  H  R  I  S  F  N
tgtatccaaattgacgatcgttttatgaaggatagaggtagtcgatattacaacatcttg
 C  I  Q  I  D  D  R  F  M  K  D  R  G  S  R  Y  Y  N  I  L
acacatccgctcagtgctgacatatatatagaaaaacattcaggaagtcagacaaagaat
 T  H  P  L  S  A  D  I  Y  I  E  K  H  S  G  S  Q  T  K  N
tgggcttactgcaaccagtacagcagccaatctccagccagttgcgaaagaccatgctat
 W  A  Y  C  N  Q  Y  S  S  Q  S  P  A  S  C  E  R  P  C  Y
tccaataatcgccatagtaaatgcaataaaaacctcctcaatgagagaactaatcatgtt
 S  N  N  R  H  S  K  C  N  K  N  L  L  N  E  R  T  N  H  V
tcgcatatcactgatattgttaacaagtattttagatgccacgagtggataattttcgat
 S  H  I  T  D  I  V  N  K  Y  F  R  C  H  E  W  I  I  F  D
ttgatcgaaccaagcttgatcctgagtgaaaagtactttaacagcgaacacccggtagtg

 L  I  E  P  S  L  I  L  S  E  K  Y  F  N  S  E  H  P  V  V
atcacaccaaatttagcagatataaaaaaaatataatgactaaaaaaatagggaagcccc
 I  T  P  N  F  S  R  Y  K  K  N  I  M  T  K  K  I  G  S  P
cgatccagcagcaacgaccaaaaccatgttagaccaaaatgctactccaatcgatggtaa
 R  S  S  S  N  D  Q  N  H  V  R  P  K  C  Y  S  N  R  W  *
aatttgcataaacaaatttaagcactttgaaataacgtaagcaaatataggaacactagc
accttgtatcaaagacgtgagcaaaccaagaagaataaaaccaccttagctttttgacttt
ccaaatactaataacccgatatgttaacgaacatgcataataaataaataaaaataaatg
gaaagtttggtctacattcgtttcttgaaaggattctttttccatacgcaacttccttttg
ctcaacaatttttcttcgcgacacaattgtaggagattctatatcggtgaatggagattc
taaactggtgaataatgactctctggataactgcgaaaaactttcattgtaattaatcca
atacttttcaagcagtgattcctcatttggtaaaataaacggattatcagcttcttcctt
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Fig. 5. The nucleotide sequence and deduced amino acids of the UVI30 gene. Corresponding predicted amino acid residues are shown in single letter code. Start and stop codons are indicated by asterisks. Two poly adenylation signals are underlined.

REFERENCES

1. Carr, A.M., K.S. Sheldrick, J.M. Murray, R. Al-Harithy, F.Z. Watts, and A.R. Lehmann, 1993. Evolutionary conservation of excision repair in *Schizosaccharomyces pombe*: evidence for a family of sequences related to the *Saccharomyces cerevisiae* RAD2 gene. *Nucleic Acid Res.* **21**, 1345-1349.
2. Feinberg, A.P., and B, Vogelstein, 1984. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**, 266-267.

3. Boothmann, D.A., M. Meyers, N. Fukunaga, and S.W. Lee, 1993. Isolation of X-ray-inducible transcripts from radioresistant human melanoma cells. *Proc. Natl. Acid. Sci. USA* **90**, 7200-7204.
4. Birkenbihl, R.P., and S. Subramani, 1992. Cloning and characterization of *rad21* an essential gene of *Schizosaccharomyces pombe* involved in DNA double strand break repair. *Nuclei Acid Res.* **20**, 6605-6611.
5. Fleer, R., C.M. Nicolet, G.A. Pure and E.C. Friedberg, 1987. *RAD4* gene of *Saccharomyces cerevisiae*: Molecular cloning and partial characterization of a gene that is inactivated in *Escherichia coli*. *Mol. Cell. Biol.* **7**, 1180-1192.
6. Fornace, A.J.Jr, D.W. Nebert, M.C. Hollander, J.D. Luethy, M. Papathanasiou, J. Fargnoli, and N.J. Holbrook, 1989. Mammalian genes coordinately regulated by growth arrest signal and DNA-damaging agents. *Mol. Cell. Biol.* **9**, 4196-4203.
7. Harosh, I, and P. Deschavanne, 1989. The *RAD3* gene is a member of the DEAH family RNA helicase-like protein. *Nucleic Acids Res.* **19**, 6331.
8. Elledge, S.J., and R.W. Davis, 1989. DNA damage induction of ribonucleotide reductase. *Mol. Cell. Biol.* **9**, 4932-4940.
9. Maga, J.A., T.A. McClanahan, and K. McEntee, 1986. Transcriptional regulation of DNA damage responsive (DDR) genes in different rad mutant strains of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **205**, 276-284.
10. Birkenbihl, R.P., and S. Subramani, 1992. Cloning and characterization of *rad21* an essential gene of *Schizosaccharomyces pombe* involved in DNA double strand break repair. *Nuclei Acid Res.* **20**, 6605-6611.
11. Fornace, A.J.Jr., I.Jr. Alamo, and C. Hollander, 1988. DNA Damage-inducible transcripts in mammalian cells. *Proc. Natl. Acad. Sci. USA* **85**, 8800-8804.
12. Elledge, S.J., and R.W. Davis, 1987. Identification and isolation of the gene encoding the small submit of ribonucleotide reductase from *Saccharomyces cerevisiae*: DNA damage-inducible gene required for mototic viability. *Mol. Cell. Biol.* **7**, 2783-2793.
13. McClanahan, T., and K. McEntee, 1986. DNA damage and heat shock dually regulated genes in *Saccharomyces cerevisiae*. *Mol Cell. Biol.* **6**, 90-95.
14. Morrison, A., E.J. Miller, and L. Prakash, 1988. Domain structure and functional analysis of the carbosyl-terminal polyacidic sequence of the *RAD6* protein of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **8**, 1179-1185.
15. Schild, D., B. Konfort, C. Perez, W. Gish, and R.K. Mortimer, 1983. Isolation and characterization of yeast DNA repair genes. I. Cloning of the *RAD52* gene. *Curr. Genet.* **7**, 85-92.
16. Weinert, T.A., and L.H. Hartwell, 1990. Characterization of *RAD9* of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. *Mol. Cell. Biol.* **10**, 6554-6564.
17. Sung, P., S. Prakash, and L. Prakash, 1988b. The *RAD6* protein of *Saccharomyces cerevisiae* polyubiquitinates histones, and its acidic domain mediated the specificity. *Genes. Dev.* **2**, 1476-1485.
18. Radman, M., G. Villani, S. Boiteux, A.R. Kinsella, B.W. Clickman, and S. Spadari, 1978. Replication fidelity : mechanisms of mutation avoidance and mutation fixation. *Cold Spring Harbor Symp. Quant, Biol.* **43**, 937.
19. Montelone, B.A., S. Prakash, and L. Prakash, 1981. Recombination and mutagenesis in *rad6* mutants of *Saccharomyces cerevisiae* : Evidence for multiple functions of the *RAD6* gene. *Mol. Gen. Genet.* **184**, 410-415.
20. Miller, R.D., L. Prakash, and S. Prakash, 1982. Defective excision of pyrimidine dimers and interstrand DNA cross-links in *rad7* and *rad23* mutant of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **188**, 235.
21. Sancar, G.B., F.W. Smith, and P.F. Heelis, 1987. Purification of the yeast *PHR1* photolyase from an *Escherichia coli* overproducing strain and characterization of the intrinsic chromophores ofthe enzyme. *J Biol Chem.* **262**, 15457.
22. Sung, P., S. Prakash, and L. Prakash, 1988b. The *RAD6* protein of *Saccharomyces cerevisiae* polyubiquitinates histones, and its acidic domain mediated the specificity. *Genes. Dev.* **2**, 1476-1485.
23. Schild, D., J. Johnston, C Chang, and R.K. Mortimer, 1984. Cloning and mapping of *Saccharomyces cervisiase* photoreactivation gene *PHR1*. *Mol. Cell. Biol.* **4**, 1864.
24. Maniatis, T., E.F. Fritsh, J. Sambrook, 1982. Molecular cloning. A laboratory mannual. *Cold Spring Harbor.*
25. Jang, Y.K., Y.H. Jin, E.M. Kim, F. Fabre, S.H. Hong, and S.D. park, 1993. Molecular cloning of rhp51^{+} gene in *Schizosccharomyces pombe*, whose amino acid sequence is highly conserved from prokarytic RecA to the mammalian Rad51 homolog. *Gene* : **169**, 125-130.
26. Perozzi, G., and S. Prakash, 1986. *RAD7* gene of *Saccharomyses cerevisiae* : transcript, nucleotide sequence analysis and functional relationship between the *RAD7* and *RAD23* gene products. *Mol. Cell. Biol.* **6**, 1497-1507.
27. Phipps, J., A. Nasim, and D.R. Miller, 1985. Recovery, repair, and mutagenesis in *Schizosaccharomyces pombe*. Adv. *Genetics*. **23**, 1-72.
28. Praekelt, U.M., and P.A. Macock, 1990. *HSP12*, a new small heat shock gene of *Saccharomyces cerevisiae* : analysis of structure, regulation and function. *Mol. Gen. Genet.* **233**, 97-106.
29. Madura, K., and S. Prakash, 1990. Transcript levels of the *Saccharomyces cerevisiae* DNA repair gene *RAD23* increase in response to UV light and in meiosis but remain constant in the mitotic cell cycle. *Necleic Acid Res.* **18**, 4737-4742.
30. Higgns, D.R., S. Prakash, P. Reynolds, R. Polakowska, S. Weber, and L. Prakash, 1983. Isolation and characterization of the *RAD3* gene of *saccharomyces cerevisiae* and inviability of rad3 deletion mitants. *Proc. Natl. Acad. Sce. USA* **80**, 5680-5684.