Journal of Photoscience (2002), Vol. 9(2), pp. 182-185

Multiple functions of human UV DNA repair endonuclease III

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There are 3 UV DNA repair endonuclease activities in mammalian cells that cleave UV-irradiated DNA. Interestingly, mammalian UV endonuclease III with MW of 26.7kD has a lyase activity on AP sites. It also cleaves the phosphodiester bond within a cyclobutane pyrimidine dimer. Genomic analysis of human repair endonuclease III gene revealed that this gene has 100% sequence identity with ribosomal protein S3 (rpS3). Therefore, rpS3 seems to function both in translation and in DNA repair. This gene of about 6.1 kb contains 6 introns and 7 exons, and the first and fifth introns of human rpS3 gene contain functional U15 small nucleolar (sno) RNAs which appear to be involved in ribosome assembly. It is to be noted that the column profile of the endonuclease activity of rpS3 appears to be altered in Xeroderma Pigmentosum (XP) group D cells compared to normal cells indicating that this protein is involved in XP disease as well. XP is a human disease characterized by high sensitivity of skin by UV- or sun-light irradiation and by high frequency of developing skin cancers. We also report here that rpS3 protein is involved in other cellular functions.

Key words : UV, endonuclease, rpS3, DNA repair, Xeroderma Pigmentosum

When cells were exposed to UV, DNA damages are induced such as pyrimidine dimers and modified bases. Accumulation of DNA damages within a cell is one of the causes of mutagenesis, carcinogenesis, and inhibition of DNA replication [1,2] and degenerative diseases. However, these damages are corrected by several repair systems such as excision repair to protect the organism.

What is an UV endonuclease?

UV endonucleases are DNA excision repair enzymes that repair damaged DNA by UV-irradiation. They are bi-functional enzymes that remove damaged base by DNA glycosylase activity to form AP (apurinic/apyrimidinic) site and excise phosphodiester bond at AP site by AP endonuclease activity, or have an excision endonuclease activity to recognize and excise bulky damaged sites [3]. AP endonuclease activity divides into two classes according to cleavage site at AP site (Figure 1), classifies class I AP endonuclease which incises on the 3'-side of an AP site via β-lyase mechanism to generate an inefficient primer for DNA polymerases, and class II AP endonuclease which incises on the 5'-side of an AP site to produce an efficient 3'-hydroxyl nucleotide primer for DNA polymerases, generally [4,5]. Therefore, class I AP endonuclease is called as AP lyase. Thus, the concerted actions of class I and class II AP endonucleases should release a β-elimination product to fill the damaged site by DNA polymerases (Figure 2). There are three endonucleases from mammalian

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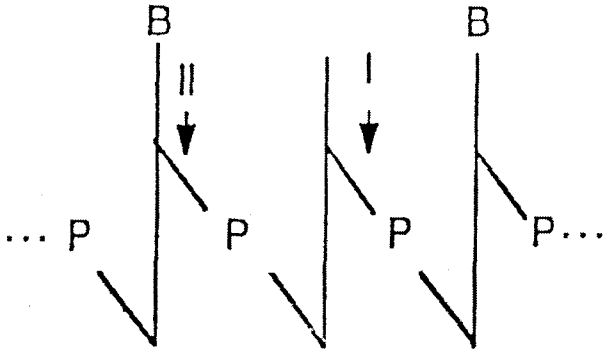

Figure 1. Classification of AP endonucleases

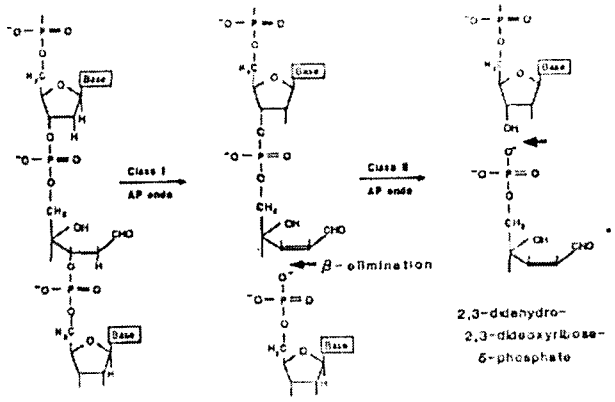

Figure 2. Conserted action of class I and class II AP endonucleases.

and human cells that specifically cleave damaged DNA which was heavily irradiated with UV light (UV endonucleases I,II and III). UV endonuclease I and II are class I AP endonuclease that cleave phosphodiester bond at UV-damaged or AP site by β-elimination mechanism [6,7]. Although acted on both supercoiled and relaxed closed circular UV irradiated DNA, UV endonuclease I preferred relaxed form and UV endonuclease II supercoiled form [6]. Another activity of UV endonuclease I is thymine glycol DNA glycosylase activity. UV endonuclease III, whose sequence has 100% identity with ribosomal protein S3 [5], also has class I AP lyase activity on AP DNA. This protein is peculiar in that it cleaves phosphodiester bond within a cyclobutane pyrimidine dimer (Figure 3).

Dual functions of UV endonuclease III

RpS3 forms part of the domain on the ribosome where the initiation of translation occurs; it can be cross-linked to eukaryotic initiation factors eIF-2 and eIF-3, and it appears to be directly involved in ribosome-mRNA-aminoacyl tRNA interactions during translation [8-10]. Other groups found that Drosophila rpS3 has DNA repair activities for 8-oxoguanine and abasic site, a DNA deoxyribophospho-diesterase antivity, and a DNA glycosylase activety [11-13].

Therefore, rpS3 seems to have a dual function, both in translation and in DNA repair.

XP-D and UV endonuclease III

Repair activity corresponding to UV-endonuclease III was missing from extracts of XP group D cells [14]. UV endonuclease III activity from XP-D cells was significantly lower or absent compared with that from the normal cells. UV endonuclease I and II activities, however, did not differ between the XP-D and normal cells [5]. Xeroderma Pigmentosum (XP) is a highly cancer-prone disorder that is associated with defects in nucleotide excision repair [15]. Unchanged rpS3 cDNA sequence in XP-D cells (GenBank Accesion Nos. U14990, U14991, U14992 (1994)) implys that protein-protein interaction or posttranslational modification of rpS3 are important in the DNA repair with respect to XP disease.

Genomic structure or rpS3 and U15b sonRNA

RpS3 gene encodes a polypeptide of 243 amino acids from the human chromosome 11q13. This region shows a high incidence of structural abnormality of genes, gene

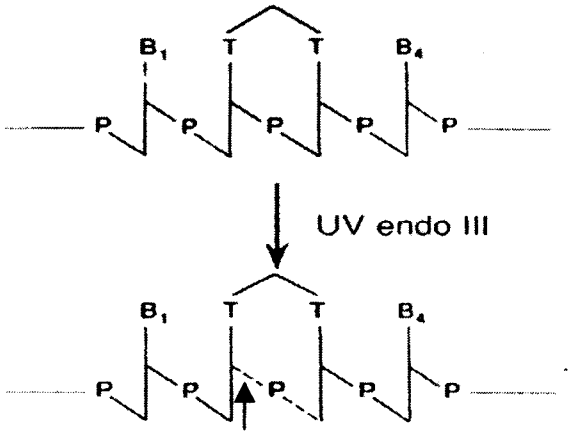

Figure 3. UV endonuclease III cleaves phospodiester bond between pyrimidine dimer.

amplification, multiple endocrine neoplasia type 1, breast carcinoma and B-cell neoplasm [16]. RpS3 gene also shows the high expression from colorectal carcinoma cells [17]. Human rpS3 gene, composed of six introns and seven exons, contains two functional U15 snoRNA genes, U15a and U15b in the first and fifth introns [18]. The relation between snoRNA and repair activity remains to be investigated.

DNA repair, cell survival and cell death

RpS3 is involved in translation as a ribosomal protein and in DNA repair as a UV endonuclease III. Cells with DNA damage either repair or pass the damage and continue to grow. However, accumulation of DNA damages within the cell is one of the causes of degenerative diseases such as cancers and aging. If the damages are too severe to repair, cells will decide to commit suicide or programmed cell death. These responses are modulated to maximize cellular survival while minimizing the chance of carcinogenesis. Therefore, it is possible that human endonuclease III/rpS3 is closely related in pathways of survival in cells. Our recent data show that rpS3 is also involved in cell survival and death. These functions seem to be regulated cellular mechanisms by post-translational modifications including phosphorylation, dephosphorylation and conjugation of a protein. The regulation mechanism of cell death by rpS3 through protein modification remains to be elucidated.

ACKNOWLEDGEMENT

This work was supported in part by KOSEF 99-1-20900-005-5 grant from the Ministry of Science and Technology of Korea.

REFERENCES

1. Brash, D.E. and Haseltine, W. A. (1982) UV-induced mutation hotspots occur at DNA damage hotspot. *Nature* 298, 189-192.

2. Berger A. and Edenberg M. J. (1986) Pyrimidine dimmers block simian virus 40 replication forks. *Mol. Cell. Biol.* 6, 3443-3450.

3. Demple, B. and Linn, S. (1980) DNA N-glycosylases and UV repair. *Nature* 287, 203-208.

4. Kim, J. and Linn, S. (1988) The mechanisms of action of E. coli endonuclease III and T4 UV endonuclease (endonuclease V) at AP sites. *Nucleic Acids Res.* 11, 1135-1141.

5. Kim, J., Chubatsu, L. S., Admon, A., Stahl, J., Fellous, R., and Linn, S. (1995) Implication of mammalian ribosomal protein S3 in the processing of DNA damage. *J. Biol. Chem.* 270, 13620-13629.

6. Kim, J. and Linn, S. (1989) Purification and characterization of UV endonucleases I and II from murine plasmacytoma cells. *J. Biol. Chem.* 264, 2739-2745.

7. Nes, I. F. (1980) Purification and properties of a mouse-cell DNA-repair endonuclease, which recognizes lesions in DNA induced by ultraviolet light, depurination, gamma-rays, and OsO4 treatment. *Eur. J. Biochem.* 112, 161-168.

8. Westerman, P., Heumann, W., Bommer, U.A., Bielka, H., Nygare, O., and Hultin, T. (1979) *FEBS Lett.* 97, 101-104.

9. Tolan, D.R., Hershey, J.W., and Traut, R.T. (1983) Crosslinking of eukaryotic initiation factor eIF3 to the 40S ribosomal subunit from rabbit reticulocytes. *Biochemie.* 65, 427-436.

10. Bommer,U.A., Lutsch, G., Stahl, J., and Bielka, H. (1991). Eukaryotic initiation factors eIF-2 and eIF-3: interactions, structure and localization in ribosomal initiation complexes. *Biochimie.* 73, 1007-1019.

11. Yacoub, A., Augeri, L., Kelley, M.R., Doetsch, P.W., and Doetsch, W.A. (1996). A drosophila ribosomal protein contains 8-oxoguanine and abasic site DNA repair activities. *EMBO J.* 15, 2306-2312.

12. Sandigursky, M., Yacoub, A., Kelley, M.R., Deutsch, W.A., and Franklin, W.A. (1997). The drosophila ribosomal protein S3 conains a DNA deoxyribophosphodiesterase (dRpase) activity. *J. Biol. Chem.* 272, 17480-17484.

13. Deutsch, W.A., Yacoub, A., Jaruga, P., Zastawny, T.H., and Dizdaroglu, M. (1997). Characterization and mechanism of action of drosophila ribosomal protein S3 DNA glycosylase activity for the removal of oxidative damaged DNA bases. *J. Biol. Chem.* 272, 32857-32860.

14. Kuhnlein, V., Lee, B., Penhoet, E.E., adn Linn, S. (1978). Xeroderma pigmentosum fibroblasts of the D group lack an apurinic DNA endonuclease species with a low apparent Km. *Nucleic Acids Res.* 5, 951-960.

15. Kraemer, K.H., Lee, M.M., and Scotto, J. (1984). DNA repair protects against cutaneous and internal neoplasia: evidence from xeroderma pigmentosum. *Carcinogenesis* 5, 511-514.

16. Polakiewicz, R. D., Munroe, D. J., Sait, S. N., Tycowski, K. T., Nowak, N. J., Shows, T. B., Housman, D. E., and Page, D. C. (1995) Mapping of ribosomal protein S3 and internally nested snoRNA U15A gene to human chromosome 11q13.3-q13.5. *Genomics* 20, 577-580.

17. Pogue-Geile, K., Geiser, J. R., Shu, M., Miller, C., Wool, I. G., Meisler, A. I., and Pipas, J. M. (1991) Ribosomal protein genes are overexpressed in colorectal cancer: isolation of a cDNA clone encoding the human S3 ribosomal protein. *Mol. Cell Biol.* 11, 3842-3849.

18. Lim, Y., Lee, S. M., Lee, J. Y., Moon, E. P., Lee, B. J., and Kim, J. (2002) Complete genomic structure of human rpS3: identification of functional U15b snoRNA in the fifth intron. *Gene* 286, 291-297.