SHORT COMMUNICATION

Presence of an Endonuclease Specific for Ultraviolet Light-irradiated Deoxyribonucleic Acid in a Ultraviolet Light-sensitive Mouse Cell Mutant Q31

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It has been shown that rodent cell lines permanently established in cell culture are unable to excise pyrimidine dimers into acid-soluble form but that they have an activity to nick ultraviolet light (UV)-irradiated DNA in nuclear crude extracts. From mouse cell line L5178Y mutagenized with N-methyl-N'-nitro-N-nitrosoguanidine, Sato and Hieda (1979) isolated a clone, designated Q31, which showed a high sensitivity to 4-nitroquinoline-1-oxide and UV but not to X-rays. Sato and Setlow (1981) measured the disappearance of UV-specific endonuclease-sensitive sites from the DNA of UV-irradiated mutant Q31 and parental L5178Y cells and found that Q31 cells were deficient in excision repair of pyrimidine dimers as compared to parental cells. These results indicate that the first incision step of excision repair might be impaired in Q31 cells. Hence we have examined the possibility if the Q31 cells have a defect in an endonuclease specific for UV-irradiated DNA in alkaline sucrose gradients after treatment with nuclear extracts.

The preparation of crude nuclear extracts of mouse leukemia L5178Y cells and the UV-sensitive Q31 cells was essentially the same as described elsewhere. The activity of an endonuclease specific for UV-irradiated DNA was measured by sedimenting irradiated and unirradiated bacteriophage φX174 RF (replicative form) I DNA after treatment with the extracts for 15 min at 37°C in the presence of ethylenediaminetetraacetic acid (EDTA) (Fig. 1). When unirradiated φX174 RF I DNA was treated with crude nuclear extracts from L5178Y and Q31 cells and sedimented in alkaline sucrose gradients, the same amount of linear DNA molecules appeared around fraction 17, indicating that both L5178Y and Q31 nuclear extracts had approximately similar endonuclease activities toward un-irradiated DNA (Fig. 1a). This was also the case for the UV-irradiated DNA (Fig. 1b).
Fig. 1. Sedimentation of UV-irradiated and unirradiated \( ^{14}\text{C}\)-\( \phi \text{X174} \) RF I DNA in alkaline sucrose gradients after incubation for 15 min at 37°C with nuclear crude extracts from mouse cells. Unirradiated DNA (a) and DNA irradiated with 300 J/m\(^2\) (b) were incubated without extracts (\( \Delta \)), with L5178Y extract (\( \odot \)), and with Q31 extract (\( \bullet \)). The reaction mixture (100 \( \mu \text{l} \)) contained 10 mM Tris-HCl (pH 8.0), 100 mM KCl, 3 mM EDTA, 10 mM mercaptoethanol, 2.5 \( \mu \text{g} \) \( ^{14}\text{C}\)-\( \phi \text{X174} \) RF I DNA (ca. 10,000 cpm) and 312 \( \mu \text{g} \) protein of nuclear crude extract from L5178Y cells or 334 \( \mu \text{g} \) protein of extract from Q31 cells. After incubation, DNA was sedimented in alkaline sucrose gradients (0.9 M Nacl/0.1 M NaOH/1 mM EDTA, pH 12) for 2.5 hours at 35,000 rpm in an RPS40T-2 rotor of Hitachi ultracentrifuge at 20°C. After fractionation onto filter paper disks, acid-insoluble radioactivity was determined in a liquid scintillation counter. Preparation of \( \phi \text{X174} \) RF I DNA labeled with (2-\(^{14}\text{C}\)) thymidine was basically the same as described by Tanaka and Sekiguchi (1975). DNA was irradiated with a low pressure mercury germicidal lamp (Toshiba, 15 W) at a dose rate of 2 W/m\(^2\) for 150 seconds (total dose, 300 J/m\(^2\)). Peaks 4, 8, and 17 correspond to covalently closed circular (ccc), open circular (oc), and linear molecules, respectively. Since even a single nick in ccc or oc molecules increases the amount of linear molecules, the assay using ccc molecules as substrate is one of the most sensitive methods to measure an endonuclease activity.
Larger amount of nicked DNA was found in irradiated DNA than in unirradiated DNA. There was, however, little difference between the amount of nicked DNA treated with L5178Y extract and that with Q31's.

From these results, it can be said that in the nuclear extract of mouse cells there is an endonuclease which can specifically incise UV-irradiated DNA in the presence of EDTA. These results may exclude the possibility that a higher sensitivity of Q31 cells to UV was due to lack of this endonuclease. Our results, however, do not necessarily indicate that mutant Q31 cells have as many endonucleases specific for UV-irradiated DNA as wild type L5178Y cells, since we have not examined the possible existence of the other endonucleases specific for UV-irradiated DNA which require ATP and Mg\(^{2+}\) ion as shown in *Escherichia coli*\(^{6,6}\). Our finding that UV-sensitive Q31 mouse cell clone had as efficient nicking activity toward UV-irradiated DNA as wild type L5178Y cells may not be inconsistent with the notion that an endonuclease which specifically incises UV-irradiated DNA in the presence of EDTA is not a true UV endonuclease which actually plays a role *in vivo*. Which of those endonucleases, the one incising in the presence of EDTA or the other incising in the presence of ATP and Mg\(^{2+}\), is a genuine one, remains unsolved.

Another possibility is that the endonuclease specific for UV-irradiated DNA found in Q31 cells is a genuine endonuclease but can not work *in vivo* because as described below. Cleaver (1968) discovered that xeroderma pigmentosum (XP) cells, which show a high UV sensitivity and low host cell reactivation capacity for UV-irradiated virus, had a defect in excision repair of pyrimidine dimers\(^{7}\). Furthermore, pyrimidine dimers present in UV-irradiated DNA were excised by extracts of XP cells, whereas dimers were not removed from the chromatin of A group XP cells irradiated with UV, an indication that the dimer excision from chromatin requires other factors than those from DNA\(^{9}\). The UV-sensitive Q31 mouse cells might have a defect in one of factors to release dimers from chromatin.

REFERENCES