Inhibition of Protein Synthesis Does Not Antagonize Induction of Uv-Induced Sister-Chromatid Exchange in Xeroderma Pigmentosum Cells

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ABSTRACT. Cycloheximide strongly antagonizes the induction of sister-chromatid exchanges by ethyl methanesulfonate or mitomycin C in human skin fibroblast and xeroderma pigmentosum cells (group A). Analogous behavior has been observed in several other species including Chinese hamster and plant cells. This report documents an exception to that pattern: cycloheximide fails to antagonize UV-induced sister chromatid exchange in xeroderma pigmentosum cells, whereas it does in normal human skin fibroblast cells. A genetic defect in these cells is postulated to alter the UV-mediated DNA recombination process.

We reported previously that in Chinese hamster cells inhibition of protein synthesis antagonizes mutagen-induced sisterchromatid exchange (SCE). We proposed the existence of a newly synthesized protein class which functions in SCE formation at DNA replication forks (5, 6, 7). Similar experiments have suggested the existence of such a protein class in human lymphocytes (3) and in plant cells (8), implying that these proteins may be universally present in eukaryotes.

Since these results suggest that SCE formation is dependent upon protein synthesis, inhibitors of protein synthesis provide a useful tool to investigate the function of this class, its relationship to UV-induced DNA repair (1, 2), and to DNA replication (9). In this report we describe similar experiments performed with skin fibroblasts from a xeroderma pigmentosum patient. In these cells we have observed an exception to our earlier observation in that cycloheximide fails to inhibit UV-induced SCE. These results suggest the existence of a unique relationship between UV-sensitivity and somatic DNA recombination in these cells.

MATERIALS AND METHODS

Human primary fibroblast strains from a xeroderma pigmentosum (XP) patient (XP3OS, complementation group A) and normal human skin (NHSF) were used throughout this study. Both cells were gifts from Dr. H. Takebe of Kyoto University in Japan.

The cells were routinely cultured in Dulbecco's modified MEM medium (Nissui Pharm. Co., Japan) supplemented with 20% fetal calf serum (Grand Island Biological Co., U.S.A.), 100 µg/ml penicillin and 100 µg/ml streptomycin. After 24 h in medium containing

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2×10^{-6} M 5-bromodeoxyuridine (BUdR medium), cells were treated with the indicated concentrations of ethyl methanesulfonate (EMS) or mitomycin C (MMC), either alone or in combination with cycloheximide (CH), for a further 42 h in BUdR medium. Immediately after exposure to the indicated doses of UV light, emitted from a low-pressure germicidal lamp, cells were cultured for 82 h in BUdR medium. In the case of UV exposure, CH was present throughout the period.

To minimize the influence of sampling time on SCE frequencies, cells were analyzed every 6 h during the last 24 h of the culture period (1). Cytological preparation was according to the standard air-drying method. The fluorescence plus Giemsa (FPG) method (4) was used to visualize SCE. Each data point is based on the examination of more than 50 metaphase cells.

RESULTS AND DISCUSSION

As described previously (5), the influence of protein synthesis inhibitors on SCE formation is very similar after treatment with a variety of mutagens. We have therefore chosen two chemical mutagens (EMS and MMC) and a physical mutagen (UV) for induction of SCE. Each of these agents produces a different spectrum of DNA lesions.

First we examined the effects of these mutagens on the frequency of SCE (Fig. 1). In all cases a significant increase was observed in both XP3OS and NHSF cells. The frequency of chemically induced SCE increased dose-dependently, showing little or no difference between XP3OS and NHSF cells (Fig. 1A and 1B). In contrast, SCE induction by UV occurs at a higher rate in XP3OS than in NHSF cells (Fig. 1C). At 1.0 J/m^2 of UV, the SCE frequency in XP3OS cells is about 2-fold greater than in NHSF cells, and almost the same as in NHSF cells at 2.0 J/m^2 . The increases are all linear functions of the mutagen dose within the ranges tested. Except for UV-

Fig. 1. Induction of SCE by EMS, MMC, and UV in XP3OS and NHSF cells. Vertical bars indicate standard deviation of the mean.

* Statistically significant difference from the curve for NHSF cells ($p \leq 0.05$, covariance analysis) (4).

(○—○) XP3OS, (△----△) NHSF.

irradiated XP3OS cells, the frequencies of induced SCE and the dose-dependencies all correspond to the values reported previously in a Chinese hamster cell line (5).

To investigate the effect of inhibition of protein synthesis on SCE levels, we selected mutagen doses which are adequate to induce SCE at a frequency of approximately 35 SCE/cell: 2×10^{-3} M for EMS 10^{-7} M for MMC, and 0.5 or 1.0 J/m² of UV for XP3OS and 1.0 or 2.0 J/m² for NHSF. In the presence of CH, a significant dose-dependent decrease was observed in the frequency of chemically induced SCE in both NHSF and XP3OS cells (Fig. 2A). At 10^{-6} M CH, the frequency of induced SCE fell to one third or less for both EMS- and MMC-induced SCE. Since responses to these mutagens are very similar, the CH-effect occurs regardless of how DNA damage is induced. It should be emphasized that this effect is observed even in XP3OS cells when they are exposed to the chemical mutagens. In each case, induced

Fig. 2. Influence of CH on SCE induced by EMS, MMC, and UV. Cells were treated with 2×10^{-3} M EMS or 10^{-7} M MMC, alone or in combination with the indicated concentrations of CH, using the protocol described in MATERIALS AND METHODS. UV-irradiation was carried out at doses of 0.5 (○) or 1.0 (●) J/m² for XP3OS cells, and 1.0 (△) or 2.0 (▲) J/m² for NHSF cells. Immediately after irradiation, the cells were cultured in BUdR medium with or without CH. Sampling times for cells treated with CH were prolonged up to 60 h (EMS and MMC) and to 82 h (UV) to correct for mitotic delay. Antagonization ratio (%) = $[(B - A)/(C - A)] \times 100$, where A, B, and C are background SCE frequency, antagonized SCE frequency with CH, and induced SCE frequency without CH, respectively. Vertical bars indicate standard deviations of the mean. The values in round and square brackets represent the mitotic index and chromosome aberration frequencies, respectively. (○—○) XP3OS, (△—△) NHSF.

* Statistically significant increase from the curves for NHSF cells ($p \leq 0.05$, covariance analysis) (4).

chromosome aberrations were not antagonized by CH at the concentrations tested (Fig. 2). The reduction in SCE is not due to selective mitotic delay, because the mitotic indices did not vary significantly between XP3OS and NHSF cells when tested at one concentration of these agents (Fig. 2), and because the multiple sampling method was employed (1).

In an earlier report of this series we proposed the existence of proteins which function to increase the recombination frequency between sister DNA strands at replicational forks (5). Our previous reports (5, 8) implied that such proteins are probably universally present in species in which SCE occurs. As shown in Fig. 2A, both XP3OS and NHSF cells exhibit a similar CH-dependent decrease in induced SCE after exposure to either EMS or MMC. This result suggests that the proposed process, which involves recombination proteins, must be present and functional in XP3OS cells as well as NHSF cells.

This process also appears to be operative following UV-induced DNA damage in NHSF cells (Fig. 2C), in which CH exhibits an effect similar to that observed after MMC and EMS treatment (Fig. 2A and 2B). A surprising exception was, however, observed in XP3OS cells, where no strong inhibition by CH was found in UV-irradiated XP3OS cells. This result has been observed three times at two UV doses. In each case the difference is statistically significant. We therefore conclude that protein synthesis is not essential for the induction in UV-irradiated XP3OS cells.

Cytologically observed SCE reflects a recombinational process between sister DNA molecules. The inhibition of protein synthesis must decrease the pool size of proteins related to the recombinational process.

A simple explanation for the observation may be that CH acts by inhibiting DNA chain elongation, thereby allowing more time for repair of SCE-inducing lesions. According to this hypothesis this inhibition of DNA synthesis by CH in UV-irradiated XP3OS cells is ineffective because the lesions are not removed during the delay in DNA synthesis.

Our previous studies suggest, however, that this explanation is inadequate. The effect of CH on SCE probably results from the lack of a protein which functions in the recombination process rather than from slowing of chain elongation (5), and occurs even if DNA lesions for SCE are not removed over long periods (6, 7). We have found, furthermore, that a Chinese hamster mutant resistant to methyl methanesulfonate is much less susceptible to induction of SCE than the parental line, but the CH-effect for SCE does not occur in this mutant (Sono and Sakaguchi, in preparation).

What we could say here, therefore, is that there may be several different ways that SCE occurs, and that one of the ways may be supplementally or selectively chosen by difference in the type of DNA damage.

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