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Sensitivity to UV Radiation of Small Nuclear RNA Synthesis in Mammalian Cells

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It was demonstrated previously that the synthesis of small nuclear RNA (snRNA) species U1 and U2 in human cells is very sensitive to UV radiation. In the present work, the UV sensitivity of U3, U4, and U5 snRNA synthesis is shown to be also high. The synthesis of U1, U2, U3, U4, and U5 snRNAs progressively decreased during the first 2 h after UV irradiation (this was not observed in polyadenylated RNA) and had not returned to normal rates 6 h after UV exposure. In contrast, the restoration of 5.8S rRNA synthesis began immediately after UV irradiation and was essentially complete 6 h later. A small fraction of U1 and U5 (and possibly U2 and U3) snRNA synthesis remained unaffected by high UV doses, when cell radiolabeling began 10 min after UV irradiation. The present data suggest that a factor other than the level of pyrimidine dimers in DNA (possibly, steps in the post-irradiation DNA repair process) plays an important role in the mechanism of UV-induced inhibition of U1–U5 snRNA synthesis.

UV radiation causes random formation of pyrimidine dimers in DNA, which result in premature termination of transcription (23). This effect has been used to determine the length of transcription units, since the probability of causing a UV lesion within a transcription unit is directly proportional to its length. Human U1 and U2 small nuclear RNA (snRNA) synthesis is very sensitive to UV radiation (4), suggesting that the UV target sizes of U1 and U2 snRNAs are much larger than those of the known precursors to these snRNAs (5). In contrast, the synthesis of small RNAs whose primary transcripts are not much longer than those of the mature species, like 4S and 5S RNA, is not affected by the same dose range of UV radiation (4, 8). Mammalian snRNA species U1, U2, U3, U4, and U5 share several common properties, including having N^2,N^2,7-trimethylguanosine-capped structures at their 5′ ends (3) and possibly being synthesized by RNA polymerase II (5, 7, 9, 12, 21, 22; S. C. Chandrasekharappa, J. H. Smith, and G. L. Eliceiri, J. Cell. Physiol., in press). The original goal of the present work was to determine whether U3, U4, and U5 snRNA synthesis is also sensitive to UV radiation; our results show that it is. U1 through U5 snRNA synthesis decreases during the first 2 h after UV irradiation and is not restored to normal levels for at least 6 h after UV exposure. These results suggest that a factor other than the pyrimidine dimer content of DNA is important in the UV inhibition of U1–U5 snRNA synthesis.

MATERIALS AND METHODS

KB or HeLa (human) cells, grown in spinner culture, were exposed to UV light as described earlier (at 15 cm from a GE G2578 lamp) (4). After specified times in spinner culture at 37°C, the cells were labeled with [^{3}H]uridine (40 μCi/ml) for 40 min, except for the experiment in Fig. 1 and 2 where labeling was for 80 min. The radioactive medium was removed, and the cells were incubated for 2 h in fresh medium supplemented with 20 mM uridine and 15 mM cytidine. Whole-cell RNA was extracted with phenol-chloroform in the presence of 7 M urea and sodium dodecyl sulfate (13). The RNA was precipitated with ethanol, incubated with chromatographically purified DNase I (DPFF; Worthington Diagnostics) (~1 mg/10 mg of nucleic acids) on ice for 2 h, extracted with phenol-chloroform again, and precipitated with ethanol. Small RNAs were analyzed by electrophoresis in 10% polyacrylamide gels in the presence of 7 M urea (18), followed by fluorography (1). The RNA bands falling within the linear response range in prefogged X-ray film strips were scanned with a densitometer (15), and the areas under the peaks were measured with an electronic planimeter. The areas under the peaks for every RNA band for each UV exposure were normalized by the value of the labeled 5S RNA of the same sample, since 5S RNA synthesis is known to be unaffected by UV radiation at this dose range (4, 8). The identification of U1 through U6 snRNAs and 5.8S and 5S rRNAs was based on fingerprinting and the characteristic and reproducible relative migration of these RNA species in 7 M urea–Tris-borate (pH 8.3)–

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EDTA–10% polyacrylamide gel electrophoresis (6, 16; data not shown).

The radiolabeling of polyadenylated [poly(A)$^+$] RNA was tested by passing RNA samples through nitrocellulose filters in the presence of 0.5 M KCl (2). Triplicate aliquots of whole-cell RNA preparations from various time points were passed slowly three times through nitrocellulose disks in the presence of a solution containing 0.5 M KCl, 10 mM Tris-hydrochloride (pH 7.6), and 1 mM $MgCl_2$ (2). Each filter was washed then with 10 ml of the same solution, dried, and counted by liquid scintillation. Quantitation of the radioactivity retained by the filters was adequate, both in terms of the number of counts (at least 12,000 cpm above background per filter) and variation among aliquots of the same sample (within ~10%).

RESULTS

We have reexamined the UV sensitivity of small RNA synthesis in cultured human cells, taking advantage of the much higher sensitivity of fluorography over that of liquid scintillation counting. Figure 1 shows that the UV sensitivity of KB cell U3, U4, and U5 RNA synthesis is as high as or higher than that of U1 and U2 RNA synthesis. The much greater sensitivity of fluorography made it possible to detect an unexpected feature of the synthesis of U1 and U5 RNAs, and possibly of U2 and U3 RNA synthesis as well. After a fast inhibition of the synthesis of most of a given RNA species, there was some remaining synthesis of that species which was resistant to increasing doses of UV radiation (Fig. 1). This does not appear to be due to an artifact of UV radiation, since the inhibition curve of 5.8S rRNA synthesis did not show this second phase in the same experiments (Fig. 1a). Figure 2 shows the RNA gel patterns of some of the time points summarized in Fig. 1. The remaining synthesis of U1, U2, U3, U4, and U5 RNAs at high UV doses was clearly seen (Fig. 2, lanes 2 and 3). In contrast, no 5.8S rRNA synthesis could be detected after 80 s of UV irradiation, even when the X-ray film was greatly overexposed (Fig. 2, lane 3). We are not aware of the synthesis of any RNA species whose UV inactivation curve exhibits both a UV-sensitive and a UV-resistant component.

The general mechanism of UV-induced inhibition of RNA synthesis is known to be premature termination of transcription at pyrimidine dimer sites (23). If this were the main cause of suppression of U1–U5 snRNA synthesis, their synthesis might be expected to be restored within 2 to 4 h after UV irradiation in human cells, since pyrimidine dimers are removed within this time in these cells (17). Surprisingly, the synthesis of U1, U2, U3, U4, and U5 snRNA decreased dramatically when cell radiolabeling began 130 min instead of 10 min after 5 or 10 s of UV irradiation (Fig. 3a). There was a sharp decrease in the labeling of these snRNAs after only 5 to 10 s of UV irradiation (Fig. 3a, lanes 2 and 4). Cells withdrawn from the same batch of irradiated cells, but whose labeling started 10 min after exposure to UV light, showed a considerably lower UV sensitivity of U1, U2, U3, U4, and U5 RNA synthesis (Fig. 3a, lanes 6 and 8), as shown also in Fig. 1. This effect appears to be specific, since 5.8S rRNA synthesis did not exhibit a marked reduction between 10 and 130 min after UV irradiation (Fig. 3a, lanes 2 and 6). To our knowledge, this is the first example of a specific RNA synthesis whose sensitivity to UV radiation increases with time after irradiation.

The radiolabeling of poly(A)$^+$ RNA was examined as a control of the synthesis of the major

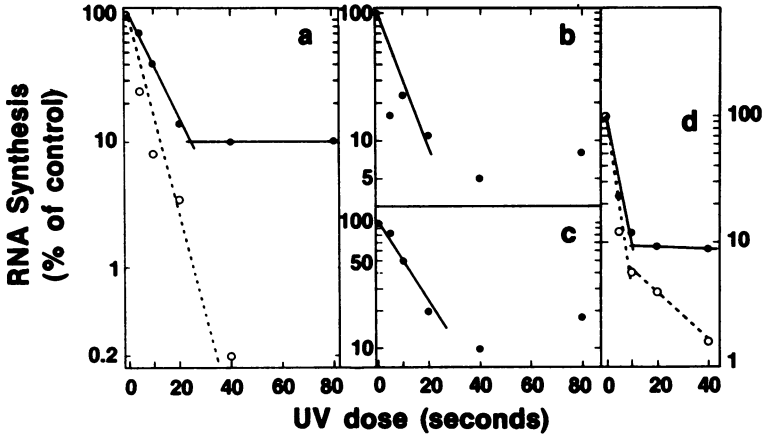

FIG. 1. Decay curves (a through d) of small RNA synthesis, when labeling began 10 min after UV irradiation. KB cells were used. All values have been normalized by those of labeled 5S RNA of the corresponding UV exposure. The decay curves shown are those of (a) U1 RNA (●) and 5.8S rRNA (○); (b) U3 RNA; (c) U2 RNA; and (d) U5 RNA (●) and U4 RNA (○).

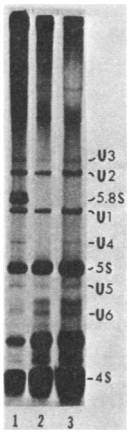

FIG. 2. Small RNA gel electrophoresis patterns of some of the time points from Fig. 1. Whole-cell labeled RNAs were extracted from non-irradiated control cells (lane 1), cells irradiated for 40 s (lane 2), and cells irradiated for 80 s (lane 3). After gel electrophoresis, the X-ray film exposures at −85°C were for 26 h (lanes 1 and 2) and 266 h (lane 3).

product of RNA polymerase II. Poly(A)$^+$ RNA is preferentially adsorbed on nitrocellulose filters in the presence of 0.5 M KCl (2). Radioisotope incorporation into poly(A)$^+$ RNA was tested in triplicate aliquots of whole-cell RNA from some time points of our experiments, as indicated above. Radiolabeling of 5S RNA was determined by densitometry of the autoradiography bands from duplicate gel lanes. For example, the ratio of labeled U1 RNA to 5S RNA fell over 10-fold between 10 and 130 min after 5 s of UV irradiation in the experiment shown in Fig. 3a (lanes 2 and 6), whereas the radioactive poly(A)$^+$ RNA/5S RNA ratio did not decrease. Therefore, enhanced inhibition of RNA synthesis during post-irradiation incubation is not a general characteristic of RNA polymerase II products.

There is no detectable excision of UV-induced pyrimidine dimers in mouse and hamster cells, and presumably rodent cells in general (14, 24, 25). If the decrease in U1–U5 snRNA synthesis observed in human cells within 2 h after UV irradiation were somehow related to the removal of pyrimidine dimers, one might not expect to see it in mouse cells. Mouse L cell U1, U2, U4, and U5 snRNA synthesis clearly diminished within 2 h after 5 s of UV exposure (Fig. 3b, lanes 10 and 11).

The differences in the synthesis of U1 through U5 snRNAs compared with that of 5.8S rRNA within 6 to 24 h of UV irradiation (5 s) of human cells are shown in Fig. 4. U1, U2, U3, U4, and U5 snRNA synthesis decreased within 10 to 130 min after UV exposure and remained clearly inhibited 6 h after irradiation (HeLa cells, Fig. 4b). Even if some recovery might occur after 6 h in KB cells, no further recovery was detected after 24 h (Fig. 4a). In contrast, the restoration of 5.8S rRNA synthesis began right after irradiation and was essentially complete within 6 h (Fig. 4). The synthesis of U6 snRNA was virtually unaffected (Fig. 4b).

DISCUSSION

In our previous work, we used cells prelabeled with [^{14}C]uridine before UV irradiation to normalize ^{3}H incorporation into various small RNAs after irradiation (4). In the present experiments, to simplify detection by fluorography, the simultaneous ^{3}H labeling of 5S RNA was used to normalize ^{3}H incorporation into the other snRNA species for each particular time point. This approach is justified on two accounts. First, 5S and 4S RNA synthesis has been shown to be unaffected at this UV dose range (4, 8). Second, comparable effects were observed with both methods (4; figures and data not shown). We chose to pulse-label and chase the label in these experiments to make it possible to detect small RNA species which are either not very abundant in the cell, like U4 snRNA, or are excised from larger RNAs late after synthesis, like 5.8S rRNA. Therefore, only metabolically stable transcripts would have been detected. The RNA species that we measured are

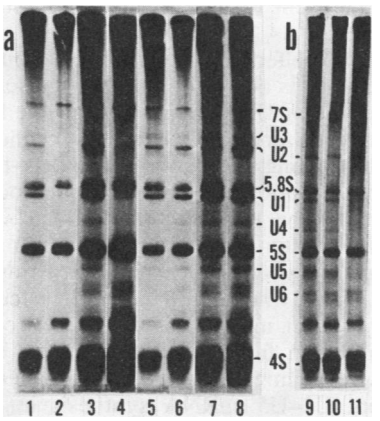

FIG. 3. Comparison of newly synthesized human (a) and mouse (b) small RNAs, when labeling began 130 or 10 min after UV irradiation. HeLa (a) and L (b) cells were used; aliquots of cells were labeled beginning 130 min after UV irradiation (lanes 1 through 4 and 11), whereas [^{3}H]uridine was added to other cell aliquots 10 min after exposure to UV light (lanes 5 through 8 and 10). The labeled RNAs shown were extracted from non-irradiated cells (lanes 1, 3, 5, 7, and 9), cells irradiated for 5 s (lanes 2, 6, 10, and 11), and cells irradiated for 10 s (lanes 4 and 8). After gel electrophoresis, the X-ray film exposures at −85°C were for 70 h (lanes 1, 2, 5, and 6) and 208 h (lanes 3, 4, 7, and 8).

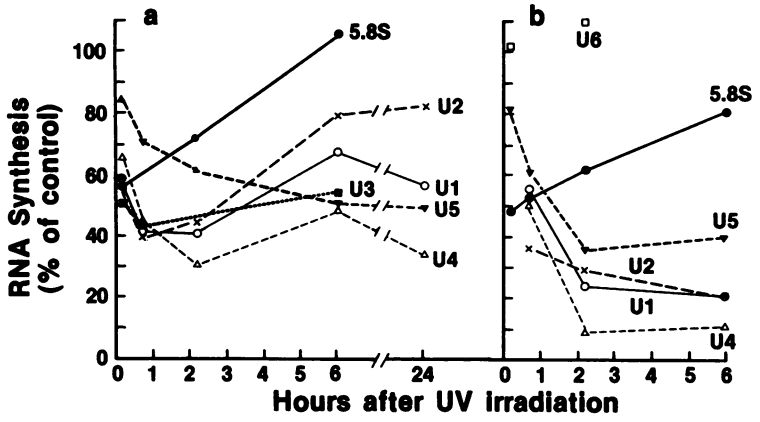

FIG. 4. Effect of post-irradiation incubation on the synthesis of various small RNA species. KB (a) and HeLa (b) cells were used. All values have been normalized by those of labeled 5S RNA of the corresponding time point. UV irradiation was for 5 s. The hours refer to the time between UV exposure and beginning of radiolabeling. The synthesis patterns shown are those of 5.8S (●); U1 (○); U2 (×); U3 (■); U4 (△); U5 (▼); and U6 RNA (□).

known to be metabolically stable (3, 26). The UV effects observed most likely represent inhibition of RNA synthesis, instead of enhanced degradation of specific RNA species, since comparable results were obtained when the label was not chased (4; data not shown). The apparent UV target size of U1 and U2 snRNA synthesis had been estimated previously, based on cell radiolabeling starting 10 min after UV irradiation (4). The present results render those estimates inadequate.

These UV effects raise questions about both the mechanisms of U1–U5 snRNA transcription and the type of UV damage responsible for the inhibitions observed. Our results cannot discriminate among some of the models for U1 through U5 snRNA synthesis that could be postulated. For example: the U1–U5 snRNA transcription units themselves might be long (although this is not supported by the transcription of cloned human U1 genes in a cell-free system or after injection into *Xenopus* oocytes [21]); a region far upstream from the U1–U5 snRNA genes might be necessary for their transcription; U1–U5 snRNA synthesis might require a rate-limiting transcription initiation factor whose synthesis is very sensitive to UV radiation.

Two observations suggest that the mechanism of UV inhibition of synthesis of U1–U5 snRNAs differs from that of 5.8S rRNA. First, U1–U5 snRNA synthesis decreased within the first 2 h after UV irradiation, whereas 5.8S rRNA synthesis was progressively recovering (Fig. 4). Second, 5.8S rRNA synthesis returned to normal within 6 h after UV exposure, whereas U1–U5 snRNA synthesis did not (Fig. 4). Premature termination of transcription at UV-induced pyrimidine dimers is believed to be the cause of UV inhibition of high-molecular-weight rRNA (and therefore, also 5.8S rRNA) synthesis (23). Our data on 5.8S rRNA synthesis are compatible with this model. Namely, there is an approximate correlation between the pyrimidine dimer DNA content and the inhibition of 5.8S rRNA synthesis; it is highest immediately after UV irradiation, decreasing gradually and completely within a few hours in human cells (16). Some factor other than the level of pyrimidine dimers in DNA appears to be important in the UV-induced inhibition of U1–U5 snRNA synthesis. This proposal is supported by the pattern of U1–U5 snRNA synthesis inhibition during the first 6 h after UV irradiation of human cells (Fig. 4), and the fact that similar results are obtained with cells that do not excise pyrimidine dimers (mouse L cells, Fig. 3b). Instead, post-irradiation events seem to have a major role in the UV inhibition of U1–U5 snRNA synthesis, since this inhibition becomes more pronounced with time after UV irradiation. The post-irradiation DNA repair reactions are the only known relevant processes. Of those reactions (incision, excision of pyrimidine dimers, polymerization, and ligation), the incision step appears to be the most likely candidate. There is no reason to suspect that any of the other steps could result in decreased transcription. In contrast, transcription from relaxed DNA is much lower than that from supercoiled DNA (e.g., references 10 and 11). If the RNA synthesis inhibition observed were related to changes in the torsional stress of U1–U5 snRNA transcriptional domains, it would be interesting to learn why they do not appear to affect the synthesis of high-molecular-weight rRNA. It is important to note that the increased

inhibition of U1–U5 snRNA synthesis during the first 2 h of post-irradiation incubation is not a general phenomenon of all RNA polymerase II products, since we did not detect it in poly(A)$^+$ RNA.

The biosynthesis of mammalian U1, U2, U3, U4, and U5 snRNAs exhibits several unique features. U1 snRNA and apparently also U2, U3, U4, and U5 RNA are the only known small RNAs synthesized by RNA polymerase II (5, 7, 9, 12, 21, 22; S. C. Chandrasekharappa, J. H. Smith, and G. L. Eliceiri, in press). U1 through U5 RNAs are the only known RNA polymerase II products whose 5'-end caps contain N^2,N^2,7-trimethylguanosine instead of 7-monomethylguanosine (3), and they are the only known small RNAs whose synthesis is very sensitive to UV radiation (4; the present work). There is a remarkable conservation of the sequence of at least the first few thousand nucleotides located upstream from the several human U1 genes that have been analyzed (19, 20). The present study demonstrates some additional unusual characteristics of the biosynthesis of U1 through U5 snRNAs. They are the only known RNAs whose synthesis progressively decreases during the first 2 h after UV irradiation and does not return to normal levels for at least 6 h after UV exposure. In addition, they are the only known RNA species whose synthesis exhibits a biphasic UV inactivation curve, with a UV-sensitive majority and a UV-resistant minority. The increased inhibition of U1–U5 snRNA synthesis within 2 h after UV irradiation observed in both human and mouse cells implies that the underlying mechanism behind this phenomenon may have been conserved through evolution since at least the time of the mammalian radiation. All these observations suggest that the mechanism of expression of the genes for U1 through U5 snRNAs may exhibit unique features.

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