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Failure of RNA Synthesis to Recover after UV Irradiation: An Early Defect in Cells from Individuals with Cockayne's Syndrome and Xeroderma Pigmentosum[1]

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ABSTRACT

Previous work has shown that in cells from the ultraviolet-sensitive genetic disorder, Cockayne's syndrome, DNA synthesis fails to recover after ultraviolet irradiation, despite the fact that these cells have no detectable defect in either excision or daughter-strand repair pathways. We now show that Cockayne cells, as well as cells from a number of patients with xeroderma pigmentosum, are sensitive to the lethal effects of UV irradiation in stationary phase under conditions in which no DNA is synthesized after irradiation. Furthermore, in normal and defective human fibroblasts, RNA synthesis is depressed after UV irradiation. In normal (dividing) cells, RNA synthesis recovers very rapidly, but this recovery does not occur in Cockayne cells, and it is reduced or absent in xeroderma pigmentosum cells from different complementation groups. Qualitatively, similar results are obtained with cells in stationary phase. The recovery of RNA synthesis in the various defective cell strains is not correlated with the overall extent of excision repair, but there is some correlation between recovery of RNA synthesis and cell survival after ultraviolet irradiation. These results implicate recovery of RNA synthesis as an important early response to ultraviolet irradiation.

INTRODUCTION

CS[3] is an autosomal recessive disorder characterized by growth retardation, skeletal and retinal abnormalities, neurological defects, mental retardation, and sun sensitivity (6, 10). Although the sun sensitivity is manifest at the cellular level as hypersensitivity to the lethal effects of UV irradiation (2, 24, 32), there is at present no biochemical evidence for a defect in excision or daughter strand (postreplication) repair (1, 2, 20, 24, 32). This contrasts with the sun-sensitive syndrome XP, which is known to involve a deficiency in either excision or daughter strand repair (30). However, the decreased survival (2, 24, 32), increased UV-induced sister chromatid exchanges (24), and increased UV mutability (3) of CS cells imply that there is a defect in DNA repair in this disorder. This is supported by evidence that CS cells have a reduced ability to carry out host cell reactivation of adenovirus 5 using whole virus (Ref. 8, but see Ref. 12), and of SV40 with a DNA transfection assay.[4]

In a previous paper (20), we demonstrated that although Cockayne cells appeared normal in excision repair, as measured by conventional procedures, they were unable to recover from the inhibition of DNA synthesis resulting from exposure to UV irradiation. In normal cells, DNA synthesis recovered in 5 to 8 hr after a fluence of 4 J/sq m (20).

In this paper, we describe the effects of UV irradiation on RNA synthesis in normal, Cockayne, and XP cells and another sun-sensitive syndrome, 11961 (4, 20). A preliminary account of some of this work has appeared elsewhere (21).

MATERIALS AND METHODS

Cell Strains. The normal, Cockayne, and xeroderma pigmentosum cell strains are listed in Table 1. Cells were maintained in Eagle's minimum essential medium supplemented with 15% fetal calf serum.

DNA and RNA Synthesis. Cells were seeded in 5-cm dishes at 10^5/dish in 3 ml medium. Two days after seeding, the medium was removed. Cells were either unirradiated or UV irradiated at 254 nm at a fluence rate of 0.5 J/sq m/sec and 2 ml of medium were added to each plate. At various times after UV irradiation, the medium was removed from duplicate plates and replaced with 2 ml of medium containing 5- or 10-μCi/ml of either [^{3}H]uridine (30 Ci/mmol) for measurement of RNA synthesis or [^{3}H]thymidine (20 Ci/mmol) for measurement of DNA synthesis. Labeling times were 15, 30, or 60 min for RNA and 60 min for DNA. At the end of the pulse, the medium was removed, the cell monolayer was washed with Dulbecco's A buffered saline, and 0.3 ml 2% sodium dodecyl sulfate was added to the dishes. The acid-insoluble radioactivity was determined by using the procedure described in an earlier work (20).

Measurement of Survival in Stationary Phase. The procedure of Kantor *et al.* (16) was used. Cells (1.3×10^5) were seeded onto 5-cm dishes. The following day, the medium was replaced with fresh medium containing 0.5% fetal calf serum. Five to 7 days later, the medium was removed, the cells were exposed to UV irradiation at a fluence rate of 0.5 or 1.2 J/sq m/sec, and fresh medium containing 0.5% serum was added. After incubation for a further 5 to 7 days, the medium containing dead (nonadhering cells) was removed and discarded, and the adhering cells were detached by trypsinization and counted with a hemocytometer.

RESULTS

DNA Synthesis after UV in XP and CS. We have shown previously that a number of Cockayne cell strains and the UV-sensitive 11961 cells fail to recover normal rates of DNA synthesis after UV irradiation (20). Work by other groups (27, 29) has demonstrated that, following UV irradiation, DNA synthesis is initially depressed in both normal and XP cell strains and that it recovers in 5 to 8 hr in normal cells but not in excision-defective XP cells from Complementation Group A. Subsequent work has shown that, in XP variants, DNA synthesis recovers to normal levels but at a slightly slower rate than in cells from normal donors (7).

The results in Chart 1 show that in a further 2 CS strains

[1] Work supported in part by Euratom Grant 166-76-1-BIO-UK.
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[3] The abbreviations used are: CS, Cockayne syndrome; XP, xeroderma pigmentosum; UDS, unscheduled DNA synthesis; ER, excision repair.
[4] P. Abrahams, personal communication.
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DNA synthesis failed to recover; whereas, in cells from both parents of one of these CS patients, DNA synthesis recovered in the same way as in normal cells. Also shown in Chart 1 are the responses of a number of XP cell strains of different complementation groups. Whereas the DNA synthesis in strains from Groups A (no UDS), G (2% UDS), and D (30% UDS) failed to recover, in an XP variant (normal UDS) and an XP from Group C (25% UDS) it did recover appreciably.

Survival after UV Irradiation of Cells in Stationary Phase. To ascertain whether the CS gene product was involved solely in the recovery of DNA synthesis and whether the failure to bring about this recovery was the primary defect in CS cells or a secondary manifestation of some other primary defect, the effects of UV irradiation on nongrowing cells were analyzed. Human cells cease dividing when the serum concentration is reduced to 0.5% (16). After 7 days in serum-depleted medium, cells were UV irradiated. Five to 7 days later, when dead cells had detached from the plates, the adhering cells were counted. Autoradiographic experiments (results not shown) demonstrated that during the whole postirradiation period, a maximum of 15% of the cells went through an S phase. Thus, the lethal effects of UV could be measured in cells which essentially never synthesized DNA after the irradiation.

Chart 2*a* shows data obtained previously in this laboratory for the survival of several CS strains (24) and measured by colony-forming ability but plotted on a linear scale against UV fluence. Chart 2*b* shows the combined results of many experiments in which the survival of cells in stationary phase was

Table 1

Cell strains used in experiments

Genotype	Strain designation	Excision repair	% survival at 4 J/sq m[a]	Recovery of RNA synthesis[b]
Normal	1BR	N[c] (22)	60 (24)	++
	2BI[d]	N (22)	60 (24)	
	54BR[e]	N[f]	60	++
	GM730[g]	N[f]	60	
Cockayne	CS698CTO[h]	N (13)	17 (24)	–
	CS697CTO[h]	N[f]	11 (24)	–
	CS1AN[g]	N (2, 20)	0.4 (24)	–
	CS1BI[d]	N[f]	8.5	–
	CS1LO[i]	N[f]	12	–
Cockayne Heterozygote	CSH1BI[d]	NT	50	++
	CSH2BI[d]	NT	64	
Sun-sensitive	11961[j]	N (4)	2.6 (4)	–
XP-A	XP4LO[j]	0 (22)	0 (3, 4, 24)	–
XP-C	XP106LO[j]	25 (28)	2[k]	+
XP-D	XP1BR[l]	30 (22)	0 (3)	–
XP-G	XP2BI[d]	2 (17)	0 (17)	–
XP-variant	XP30RO[m]	N (19)	48 (3, 4)	+

[a] All survival data are from this laboratory using the same UV source and dosimetry as in our experiments. A value of 0 indicates < 0.5%. Uncited data are from C. F. Arlett and S. A. Harcourt, personal communications.

[b] Details in Chart 3: ++, normal recovery; +, slow or reduced recovery; –, very little recovery.

[c] N, normal range; NT, not tested. Measurements are of UDS or excision of endonuclease-sensitive sites.

[d] Supplied by A. M. R. Taylor, Birmingham, England.

[e] Supplied by C. F. Tredgold, Brighton, England.

[f] Unpublished observations.

[g] Supplied by Human Genetic Mutant Cell Repository, Camden, N. J.

[h] Supplied by M. Buchwald, Toronto, Ontario, Canada.

[i] Supplied by F. Giannelli, London, England.

[j] Supplied by P. Hall-Smith, Brighton, England.

[k] Very poor plating efficiency. Value is only approximate.

[l] Supplied by D. A. Burns, London, England.

[m] Supplied by D. Bootsma, Rotterdam, the Netherlands.

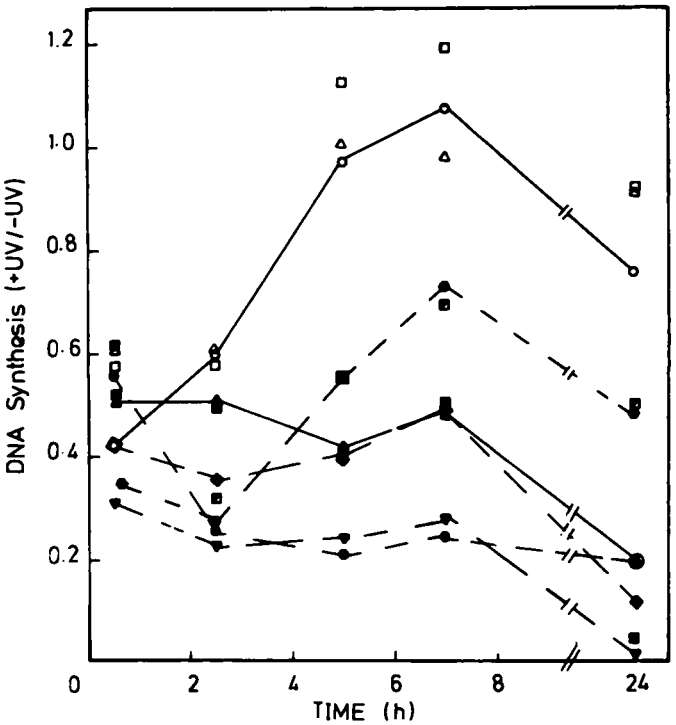

Chart 1. DNA synthesis after UV irradiation of CS and XP cells. Cells not irradiated or irradiated with 4 J/sq m were pulse labeled for 1 hr at different times after irradiation, and the amount of acid-insoluble radioactivity was determined. Normal cell: ○, 1BR (4). CS heterozygotes: □, CSH1BI (2); △, CSH2BI (2). CS homozygotes: ■, CS1LO (2); ▲, CS1BI (2). – – – –, XP cells: ●, XP4LO (Group A-1); ◩, XP106LO (Group C-2); ◆, XP1BR (Group D-2); ▼, XP2BI (Group G-1); ●, XP30RO (variant-3). Numbers in parentheses, number of experiments; *points*, means.

measured as described above. Both CS and XP cells were sensitive to the killing effect of UV irradiation in stationary phase. One of the normal cell strains was appreciably more sensitive than were the other 3 strains, but it was still significantly more resistant than the most resistant CS strain at UV fluences above 10 J/sq m. Although each strain was some 5 times more resistant to UV when measured in this assay than when measured by colony-forming ability of exponentially growing cells (*cf. abscissae* of Chart 2*b* and Chart 2*a*), as also found and discussed by Kantor *et al.* (16), the relative sensitivities of the strains remained quite similar. Since the stationary-phase cells never synthesized DNA after irradiation, these results show that the hypersensitivity of CS cells does not require DNA replication in order to be expressed. We therefore decided to measure the effect of UV on RNA synthesis, since RNA is synthesized in both growing and stationary cells.

RNA Synthesis after UV Irradiation in Growing Cells. RNA synthesis was measured as the rate of incorporation of [^{3}H]-uridine during a 15-, 30-, or 60-min pulse. Since the ratios of incorporation in irradiated to unirradiated cells were very similar for the different pulse times, the data have been combined. Chart 3 shows the kinetics of RNA synthesis in exponentially growing cells after irradiation with 4 J/sq m. The results are plotted as the ratio of [^{3}H]uridine incorporation in irradiated cells to that in unirradiated cells. Cells of all strains remained attached to the dishes for at least 24 hr after irradiation.

RNA synthesis was depressed in normal cells immediately after UV irradiation to a level 60 to 75% of that in unirradiated cells (Chart 3*a*). Over the next 3 hr, RNA synthesis recovered completely. By 7 hr, synthesis was slightly higher in irradiated

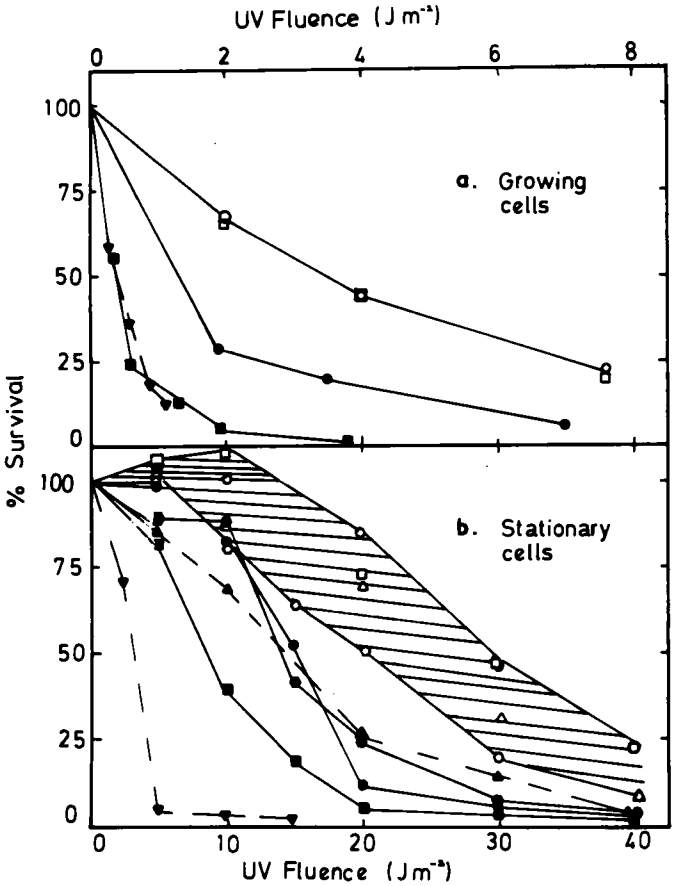

Chart 2. Cell killing in stationary phase. *a*, colony-forming ability. Data taken from Ref. 24. *b*, stationary phase. Survival of adhering cells after UV irradiation of cells cultivated in medium containing 0.5% serum. Normal cells: ○, 1BR (16); □, 2BI (2); △, 54BR (2); ◯, GM730 (7). CS cells: ●, CS698CT0 (10); ■, CS1AN (4); ⬤, CS697CT0 (5). – – – –, XP cells: ▼, XP2BI (Group G-2); ▲, XP106LO (Group C-5). *Points*, means of several experiments; numbers in parentheses, number of experiments.

cells. In contrast, in all 5 CS strains and the UV-sensitive 11961 cells, although RNA synthesis showed the same initial depression, it did not recover significantly. This effect was very reproducible. (For clarity, lines are drawn through only 4 sets of *points* in Fig. 3*a*.) The Cockayne heterozygote was indistinguishable from the normal cells. Qualitatively similar effects were seen after a UV fluence of 8 J/sq m.

Chart 3*b* shows the kinetics of RNA synthesis in a number of XPs from different complementation groups and with different excision capacities. The levels of ER (measured by UDS) are listed in Table 1. RNA synthesis recovered almost normally in the XP variant which is not deficient in excision repair. In XP106LO (25% of normal ER), it recovered to normal levels but at a reduced rate. This was found in 4 separate experiments. In XP1BR, XP4LO, and XP2BI, RNA synthesis did not recover, and their levels of RNA synthesis were similar to those of Cockayne cells.

No significant differences were seen in the response of protein synthesis to UV in a number of normal, CS, and XP strains used in this study.[5]

RNA Synthesis in Stationary Cells. Cells were maintained in 0.5% serum as described above and were UV irradiated with 4 J/sq m. Chart 4 shows the kinetics of RNA synthesis. The recovery of RNA synthesis in normal stationary-phase cells did not differ significantly from that in growing cells. In CS cells, RNA synthesis recovered very little in the first 6 hr, but it did recover slowly over a longer time, as might be anticipated from the survival data (Chart 2*b*). In the XP2BI cells, it did not recover. After higher UV fluences, normal cells recovered control RNA synthesis rates after 24 hr, whereas CS cells did not (data not shown).

DISCUSSION

In an earlier paper, we showed that the CS gene product was required for the recovery of DNA synthesis after UV (14, 20). In the present work (Chart 2), we have shown that CS cells were hypersensitive to the lethal effects of UV even when they were in a nondividing state under conditions in which the cells never replicated their DNA after irradiation (16).[5] This procedure is to be contrasted with the measurement of liquid-holding recovery, in which cells are irradiated and incubated in stationary phase but then subcultured to assay for colony-forming ability (*e.g.*, see Refs. 23 and 31).

These results with stationary-phase cells show that the failure of DNA synthesis to recover in proliferating CS cells (20), although likely to be an important factor leading to the reduced colony-forming ability of CS cells after UV irradiation, cannot be the primary defect in these cells. It is rather a secondary manifestation of some other primary defect.

Further evidence in support of this suggestion comes from our observations on the recovery of RNA synthesis. This preceded the recovery of DNA synthesis in normal cells, and it was complete by 2.5 hr, at which time DNA synthesis was still maximally depressed (Chart 1; Ref. 20). RNA synthesis also failed to recover in CS cells in both the dividing and the nondividing states (Charts 3 and 4). Thus, a difference in rates of RNA synthesis between normal and CS cells could already be seen 1 hr after irradiation, even though the initial depression was similar in all cells studied. As in previous cellular studies (4, 20), the sun-sensitive 11961 cells behaved in a manner very similar to that of CS cells.

The inhibition and recovery of RNA synthesis after UV irradiation have been studied in a number of reports using different cell systems. The work of Hackett and Sauerbier (11) showed that irradiation of mouse cells produced lesions which terminated transcription of 45S ribosomal precursor RNA, leading to the production of truncated preribosomal RNA chains. Initiation of RNA synthesis was unaffected by UV; therefore, the number of RNA chains produced was the same in irradiated and unirradiated cells. Similar results were obtained with heterogeneous nRNA (9), but in this case the truncated RNA chains appeared to be rapidly degraded. Both of these studies were consistent with transcription-terminating lesions being produced at a frequency of about 1/1000 base-pairs/100 J/sq m. If these findings also pertain to human fibroblasts, this frequency of transcription-terminating lesions compares well with the value of 1 pyrimidine dimer/1100 base-pairs/100 J/sq m (calculated from the data of Fig. 2*a* of Ref. 18). This is consistent, as Hackett and Sauerbier (11) suggested, with pyrimidine dimers causing the termination of growing RNA chains. Hackett and Sauerbier (11) also found that UV irradiation did not affect the rates of uptake or phosphorylation of uridine; therefore, incorporation of uridine was a true reflection

[5] Unpublished observations.

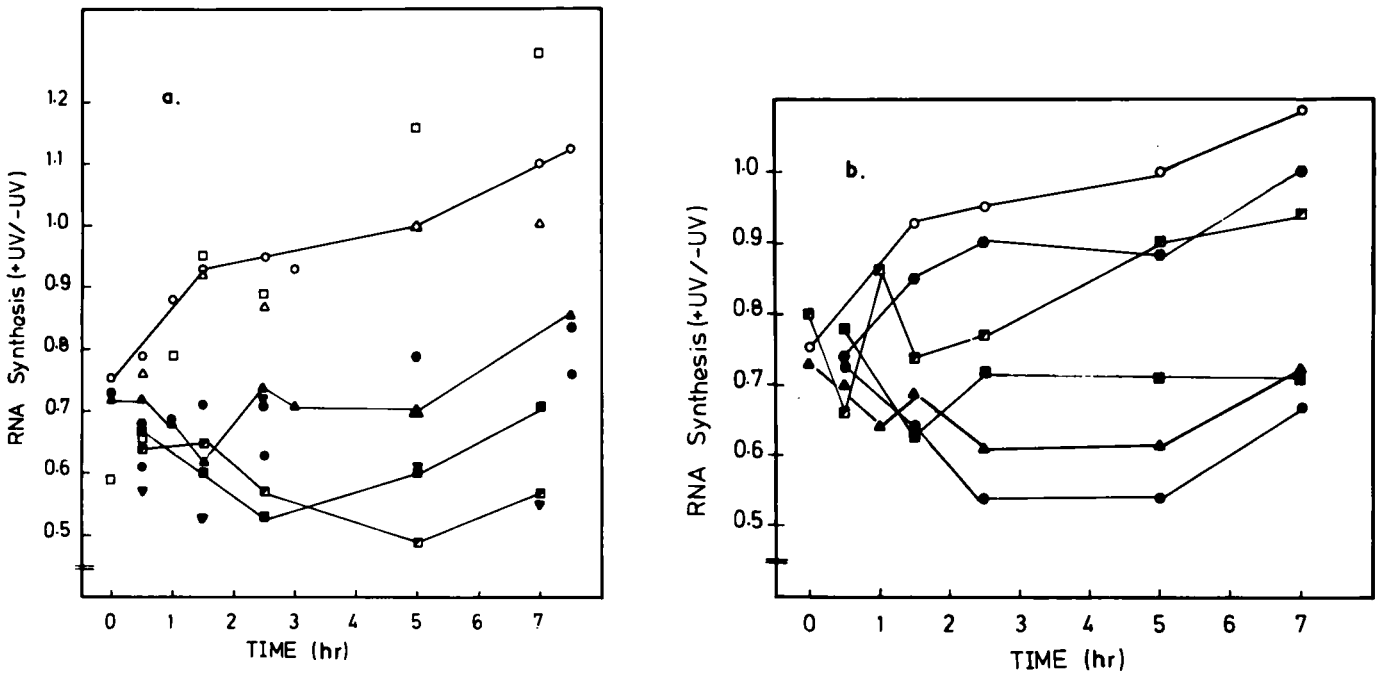

Chart 3. Recovery of RNA synthesis in growing cells. Cells unirradiated or UV-irradiated with 4 J/sq m were pulse-labeled with [^{3}H]uridine at different times after irradiation, and the acid-insoluble radioactivity was determined. *a*, normal cells; ○, 1BR (19); □, 54BR (3). Cockayne heterozygote: △, CSH1BI (3). Cockayne homozygotes: ●, CS698CT0 (6); ⬣, CS697CT0 (2); ▲, CS1AN (6); ▼, CS1BI (3); ◩, CS1L0 (2). Sun sensitive: ■, 11961 (3). *b*, normal cell: ○, 1BR (19). XP cells: ●, XP4L0 (Group A-2); ◩, XP106L0 (Group C-4); ■, XP1BR (Group D-2); ▲, XP2BI (Group G-6); ⬣, XP30R0 (variant-2). Numbers in parentheses, number of experiments; *points*, means.

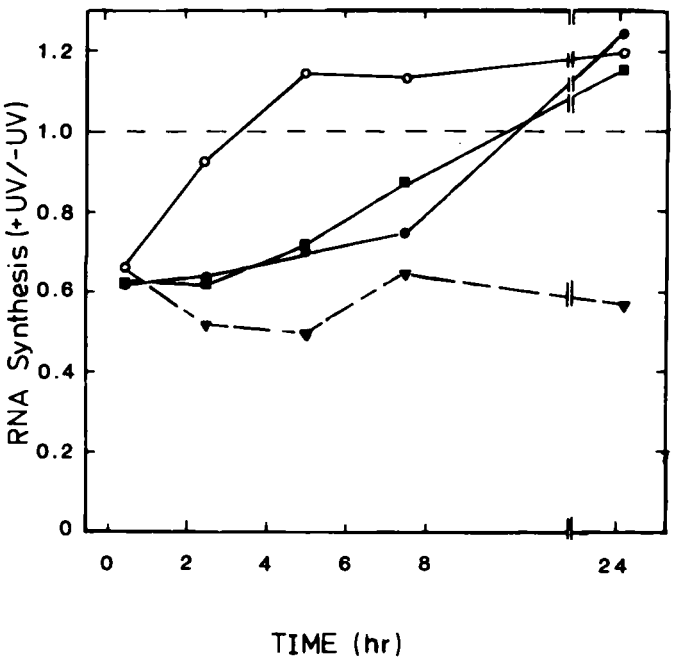

Chart 4. Recovery of RNA synthesis in stationary cells. Cells incubated in medium containing 0.5% serum were unirradiated or UV irradiated with 4 J/sq m, incubated in medium containing 0.5% serum and pulse-labeled at different times with [^{3}H]uridine. ○, 1BR (4); ●, CS698CT0 (2); ■, CS1AN (2); ▼, XP2BI (2). Numbers in parentheses, number of experiments; *points*, means.

of RNA synthesis. We may infer, therefore, that the inhibition of RNA synthesis seen in our cells also results from termination of transcription of all classes of RNA by pyrimidine dimers.

Recovery of RNA Synthesis and Excision Repair. Our results also allow us to comment on the relationship of the recovery of RNA synthesis to the extent of excision repair in different cells. Nocentini (26), using monkey CV-1 cells, and Kantor and Hull (15), using stationary human fibroblasts, found an inhibition of RNA synthesis by UV that was followed by a recovery. They used UV fluences which were, however, considerably greater than those used in our study, and the recovery was much slower. Both of these authors suggested that the recovery of RNA synthesis resulted from ER of pyrimidine dimers.

It is therefore instructive to compare the recovery of RNA and DNA synthesis in our experiments with the overall rates of ER in the different cell strains under study (Table 1, Columns 3 and 5). The XP cells from Groups A and G (XP4L0 and XP2BI) have essentially no ER (Table 1), and RNA and DNA synthesis did not recover at all. In the XP from Group C (XP106L0, 25% ER) DNA and RNA synthesis recovered partially (Charts 1 and 3*b*) with kinetics similar to that of the variant XP30R0 (normal ER). On the other hand, in XP1BR (Group D), which has a capacity for ER (30%) very similar to that of XP106L0, and in CS and 11961 cells (normal ER), nucleic acid synthesis did not recover.

The recoveries of DNA synthesis in XP-A, C, and variants in our experiments are very similar to results reported by others (7, 25, 27, 29). In particular, as in our experiments, Moustacchi *et al.* (25) found that DNA synthesis recovered with similar kinetics in an XP Group C and an XP variant cell strain, but the significance of this observation was not discussed. Thus, recoveries of RNA and DNA synthesis do not, as others have suggested (15, 26), correlate well with the overall rate of ER.

The work of Ben-Hur *et al.* (5) is consistent with our findings. They showed that, after exposure of hamster V79 cells to 5 J/sq m, the transcriptionally controlled induction of ornithine decarboxylase by serum enrichment was reduced by about

50%. If, however, 2 to 4 hr elapsed between UV irradiation and commencement of induction, the induction had almost returned to normal levels. These results could be interpreted as showing that RNA synthesis was able to recover rapidly after UV irradiation, even though the cells used in this study had very little excision repair.

Schemes for Recovery of RNA Synthesis. There are at least 2 possible explanations for the rapid recovery of RNA synthesis in normal cells.

First the transcription machinery could be altered in such a way that pyrimidine dimers would no longer cause termination of growing RNA chains, but instead transcription is able to proceed past the lesions. On this model, the alteration of the transcriptional machinery would require the products of the CS gene and some of the XP genes.

Alternatively, the transcription-terminating lesions may be removed. As discussed above, extensive excision repair is neither necessary nor sufficient for RNA synthesis to recover. It is conceivable, however, that transcription-terminating lesions, *i.e.*, those lesions in the transcribing regions of DNA, are potentially more lethal than the bulk of the lesions, and that these, comprising only a small fraction of the total damage (<10%), may be excised rapidly in normal cells by some special ER mechanism. On this model, the CS gene product would control this special ER pathway, but it would not be involved in overall ER. These ideas are purely speculative at the moment, and there is at present no evidence to support the idea of rapid excision repair in transcribing regions of DNA. We are testing this hypothesis. The above discussion does provide a framework for future experimentation on CS cells. Other workers have proposed that CS cells are defective in the rejoining step of ER (13, 14), but results to be presented elsewhere do not support this contention.

Recovery of Nucleic Acid Synthesis and Cell Survival. Within the group of XPs studied, there is some correlation between the recoveries of RNA and DNA synthesis and cell survival; the order for all 3 parameters being XP variant > XP-C > XP-D ≃ XP-G ≃ XP-A. Also, as a group, the sensitivity of CS cells to killing by UV correlates with the inability of RNA and DNA synthesis to recover, but within the group of CS cell strains this correlation no longer pertains. Although strains CS697CT0 and CS698CT0 are considerably more resistant to the lethal effects of UV than are the other CS cell strains (Ref. 24; see also Chart 2*a*), the kinetics of RNA and DNA synthesis were indistinguishable for all the CS strains studied. In addition, although substantial recovery of nucleic acid synthesis occurs in XP106L0, its survival is in the same range as that of the CS cells (Table 1). Thus, both recovery of nucleic acid synthesis and extensive excision repair are essential if the cells are to survive.

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