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MASTER

# EXCISION REPAIR OF BULKY LESIONS IN THE DNA OF MAMMALIAN CELLS

SECOND TYPE OF TITLE

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## INTRODUCTION

Although the excision of pyrimidine dimers from UV-irradiated human cells has been known since 1968 (1) and excision has been the subject of a number of symposia and recent reviews (2-4), the details of the process still elude us. We assume that the excision of pyrimidine dimers is of the nucleotide excision type, (a) by analogy with bacteria, (b) because it is of the large patch type, (c) because although few single strand breaks accumulate during excision in normal human cells, the numbers that accumulate in excision defective cells are much less, (d) because the introduction of an exogenous UV endonuclease enhances repair and survival in excision defective cells and (e) because inhibitors of the polymerization steps such as hydroxyurea and cytosine arabinoside result in the accumulation of single strand breaks (5, 6). (However, the number of breaks observed is much less than the number of dimers removed in uninhibited cells, indicating that the various steps in excision repair act as if they are linked to one another (7).) The various measures of excision repair of pyrimidine dimers - the best studied lesion to date because they are easy to identify and measure in a number of ways - give general agreeement (8)but different investigators obtain conflicting results in details such as the dependence on time, dose and the method of measurement (9-11). Nevertheless, the various techniques indicate that excision repair of dimers varies widely among cell lines and strains. For example, rodent cells are low excisers compared to normal human cells (12), and among humans the cells of most of the individuals with xeroderma pigmentosum (XP) are defective in excision repair (13).

Table 1: Ways in Which Some Chemical Damages Mimic UV Damage in Human Cells

1. UV-sensitive cells (XP) are more sensitive to the chemical than normal cells.
2. Chemically treated viruses show a higher survival on normal cells than on XP cells.
3. XP cells deficient in repair of UV damage are also deficient in excision of chemical damage.
4. Excision repair of UV and of chemical damage involves long patches (approx. 100 nucleotides).
5. XP complementation groups observed for repair of chemical damage are the same as those for UV damage.

The repair of a number of bulky chemical adducts such as those derived from N-acetoxy-acetylaminofluorene, benzo(a)pyrene and dimethylbenzanthracene also seems to be by nucleotide exicision because their repair mimics in a number of ways the repair of UV (254nm) damage (14). (See Table I) Thus, we infer that the many genes, identified as XP complementation groups, controlling UV excision repair also control the repair of bulky adducts. The existence of seven complementation groups implies the existence of rather complicated control mechanisms or enzymic sequences in the excision repair of bulky adducts. The repair of UV damage and certain chemical damages must have a number of steps in common and since for UV it seems as if the rate limiting step is the endonucleolytic one, it is reasonable to suppose that it is also rate limiting for the repair of chemical damages.

SATURATION OF EXCISION REPAIR

The kinetics of excision repair is complicated if for no other reason than that chromatin is not a uniform substance but is composed of both linker and core regions and repair is initially more rapid in the linker regions for both UV-irradiated and AAAF treated human cells (15, 16). Nevertheless, the repair systems seem to saturate at high doses of UV or chemical treatment (17) (although some investigators do not observe such saturation (11, 18)) implying either that UV affects the repair system directly or indirectly as a result of the accumulation of photoproducts that inhibit repair or, as seems much more reasonable, that repair saturates because of the excess of substrate compared to a rate limiting enzymic step. For example, at a UV dose that exhibits saturation of repair in human cells, $20 J/m^2$ of 254nm, the effects on know enzymic systems are negligible. If the same rate limiting step exists for the repair of UV and bulky chemical adducts one would expect treatment of repair proficient cells with a combination of agents at high

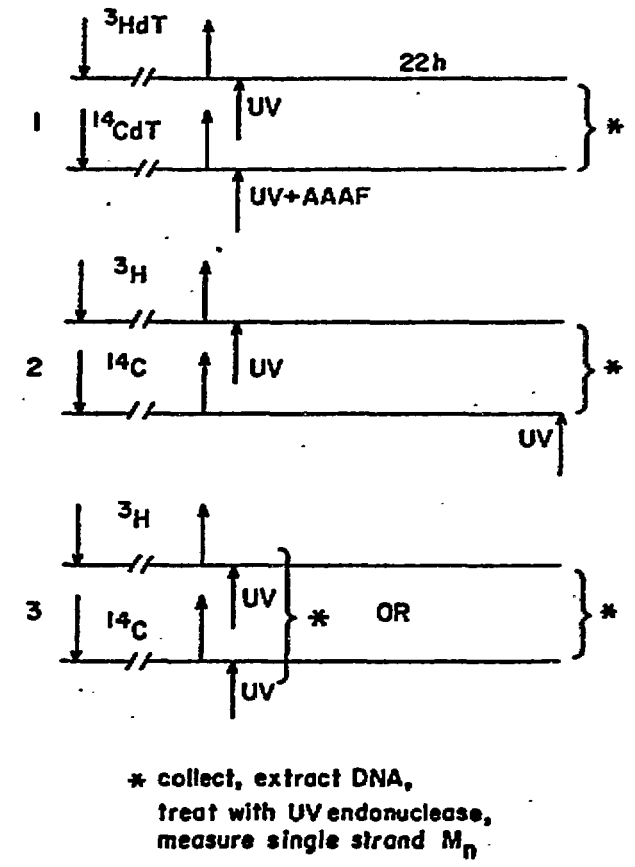


Fig. 1. The experimental procedures used to measure (1) the effect of AAAF on dimer excision (2) excision, and (3) the precision of the assay. UV: 20 J/m$^2$ of 254nm; AAAF: 20 µM for 20 min; labeling time before UV: 36h with $^3$H-thymidine (0.3 µCi/ml) or $^{14}$C-thymidine (0.04 µCi/ml).

doses would result in no more unscheduled DNA synthesis than from individual treatments and that the chemical would inhibit the excision of dimers. Such expectations are not fulfilled. The results of treating normal human excising strains with combinations of agents resulted in additivity of repair and no inhibition of dimer excision, whereas treatment of repair deficient cells indicated that the combined treatment gave less repair than either agent separately and the chemical agent inhibited excision of dimers by greater than 50% (8, 19).We speculated that although XP heterozygoes fall within the normal range of excision repair when treated with UV alone, they might respond in an intermediate fashion and that by use of the sensitive endonuclease site technique we might detect an intermediate level of dimer excision between the low inhibition

observed with normal cells and the very large inhibition observed with XP cells. The experiments described below illustrate the precision attainable with the endonuclease assay, the fact that the amount of observed excision depends upon the isotope used to label cells, and that XP heterozygotes are, on the average, between normals and XPs.

## EXCISION IN XP HETEROZYGOTES

The procedures followed for these experiments are shown in Fig. 1. The experiment of interest is Part 1, which measures directly the difference in excision between cells irradiated and cells irradiatd and treated with AAAF. Part 2 is to estimate the normal amount of repair observed in 22 hours and Part 3 represents the necessary control experiments. Fig. 2 shows typical alkaline sedimentation data for the experiments outlined in Parts 1 and 2 of Fig. 1. It is clear that as a result of the combined treatment (Fig. 2A) there is less repair than after UV alone and one can estimate from the data in Fig. 2 that there is an approximate 35% inhibition by the combined treatment of the normal excision shown in Fig. 2B. Life would be simple except that the control experiments (Fig. 3) indicate that the situation is more complicated because even though the different radioactive labels do not affect the initial numbers of pyrimidine dimers, they do affect the amount of observed excision in normal human cells. Hence, the experiments shown in Part 1 of Fig. 1 were all done with the labels reversed. Typical data are shown in Fig. 4. The reversal of the labels changes the sedimentation patterns but the isotope effect is not sufficient to explain the inhibition in Fig. 2A. We averaged the inhibitions observed in experiments such as in Fig. 4 and obtained the results shown in

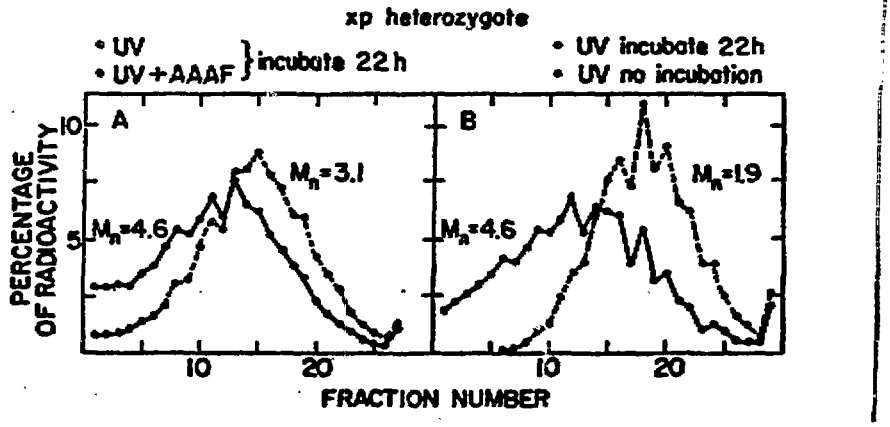


Fig. 2. Sedimentation profiles, in alkali, of the DNAs from Experiments (1) and (2) of Fig. 1.

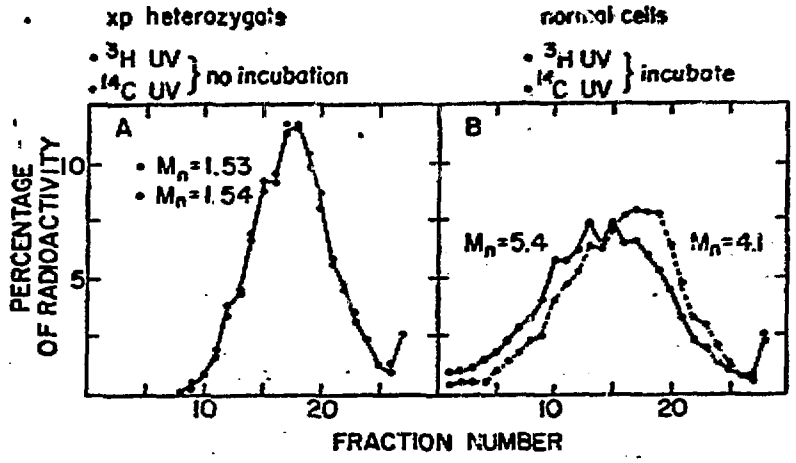


Fig. 3. Sedimentation profiles in alkali, of the DNA from Experiment (3) of Fig. 1.

Table 2. They illustrate that, on the average, XP heterozygotes show more inhibition of dimer excision by AAAF than do normal cells although there is a heterogeneity in the heterozygote population and the heterogeneity follows no clear genetic rules. Thus, these data indicate that the enzyme systems in XP heterozogotes act as if they are mixtures of normals and homozygotes.

TABLE 2. Inhibition of Dimer Excision ($20J/m^2$, 22h) in Presumptive XP Heterozygote Fibroblasts by 20 μM AAAF

| Strain | Percentage Inhibition | Remarks |
|---|---|---|
| Normals | 7 | see refs. |
| CeAr(CRL*1165) | 4 | mother XPC |
| ReKo(CRL1202) | 3 | mother XPD |
| BeTim(CRL1254) | (7) | mother XPA |
| BeAr(CRL1167) | 18 | father XPC |
| EmAr(CRL1168) | 21 | brother XPC |
| DaKam (CRL1278) | 21 | father XP |
| LoWen(CRL1159) | 30 | mother XPD |
| GM**1631 | 27 | father XP |
| GM1632 | 33 | mother XP |
| GM0241 | 39 | mother XP |
| GM2034 | 21 | mother XP |

* American Type Culture Collection
** Human Genetic Mutant Cell Repository.

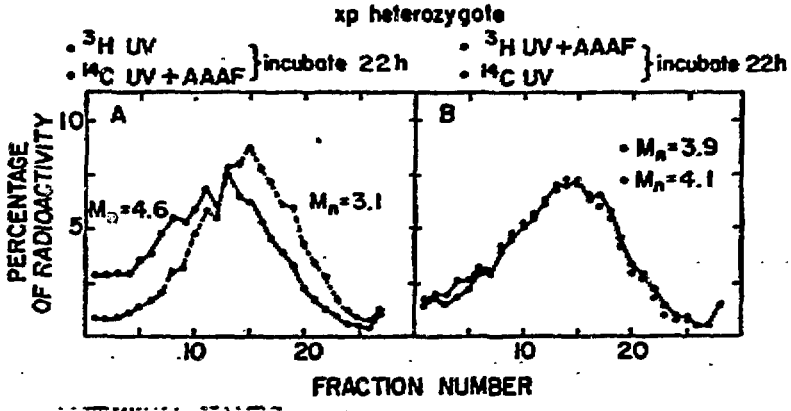


Fig. 4. Sedimentaton profiles, in alkali of the DNAs from Experiment (1) of Fig. 1 with the radioactive labels reversed.

Other investigators (20) have not obtained the same results as we have for normal human fibroblasts and at present we are not able to explain the discrepancy between the two sets of outwardly convincing experiments. The difference in excision repair between cells labeled with $^{3}$H-dThd and $^{14}$C-dThd depends on the time at which repair is measured. For example, 6 to 12 h after UV the difference between the two labels is almost 50%, but as repair begins to approach completion, the difference drops and after 20 h only amounts to approximately 10%. We suspect that the isotope effect results from the difference in radiation damage from the more numerous and less energetic $^{3}$H decays as compared to $^{14}$C and the result of such damage on the progression of cells through the cell cycle. The faster repair shown by $^{3}$H labeled cells can be mimicked in $^{14}$C labeled cells by irradiating the latter 24 hours before UV with 500 rad of x-rays (500 rads is approximately the dose received by $^{3}$H labeled cells during the 24 hours before UV irradiation) but no effect is observed if the irradiation takes place immediately before UV.

## ACKNOWLEDGMENT

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## REFERENCES

1. J. D. Regan, J. E. Trosko, and W. L. Carrver, Evidence for excision of ultraviolet induced pyrimidine dimers from the DNA of human cells in vitro, Biophys. J. 8:319 (1968).

2. P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, editors, "DNA Repair Mechanisms," Academic Press, N. Y. (1978).
3. L. Grossman, A. Braun, R. Feldberg, and I. Mahler, Enzymatic repair of DNA, Ann. Rev. Biochem. 44:19 (1975).
4. P. C. Hanawalt, P. K. Cooper, A. K. Ganesan, and C. A. Smith, DNA repair in bacteria and mammalian cells, Ann. Rev. Biochem. 48:783 (1979).
5. A. R. S. Collins, S. L. Schor, and R. T. Johnson, The inhibition of repair in UV irradiated human cells, Mutat. Res. 42:413 (1977).
6. W. C. Dunn and J. D. Regan, Inhibition of DNA excision repair in human cells by arabinofuranosyl cytosine: effect on normal and xeroderma pigmentosum cells, Molec. Pharmacol. 15:367 (1979).
7. R. B. Setlow and E. Grist, unpublished observation.
8. F. E. Ahmed and R. B. Setlow, DNA repair in xeroderma pigmentosum cells treated with combinations of ultraviolet radiation and N-acetoxy-2-acetylaminofluorene, Cancer Res. 39:471 (1979).
9. J. I. Williams and J. E. Cleaver, Excision repair of ultraviolet damage in mammalian cells. Evidence for two steps in the excision of pyrimidine dimers, Biophys. J. 22:265 (1978).
10. U. K. Ehmann, K. H. Cook, and E. C. Friedberg, The kinetics of thymine dimer excision in ultraviolet irradiated human cells, Biophys. J. 22:249 (1978).
11. F. E. Ahmed and R. B. Setlow, Kinetics of DNA repair in ultraviolet irradiated and N-acetoxy-2-acetylaminofluorene-treated mammalian cells, Biophys J. 24:665 (1978).
12. R. W. Hart and R. B. Setlow, Correlation between deoxyribonucleic acid excision-repair and life-span in a number of mammalian species, Proc. Natl. Acad. Sci. USA 71:2169 (1974).
13. E. C. Friedberg, U. K. Ehmann, and J. I. Williams, Human diseases associated with defective DNA repair, Adv. Radiat. Biol. 8:85 (1979).
14. R. B. Setlow and F. E. Ahmed, DNA repair in human cells exposed to combinations of carcinogenic agents, in: 13th Jerusalem Symposium on Carcinogenesis: fundamental mechanisms and environmental effects, D. Reidel, Dordrecht (1980).
15. J. E. Cleaver, Nucleosome structure controls rates of excision repair in DNA of human cells, Nature 270:451 (1977).

16. M. J. Smerdon, M. B. Kastan, and M. W. Lieberman, Distribution of repair incorporated nucleotides and nucleosome rearrangement in the chromatin of normal and xeroderma pigmentosum fibroblasts, Biochemistry 18:3732 (1979).

17. F. E. Ahmed and R. B. Setlow, Saturation of DNA repair in mammalian cells, Photochem. Photobiol. 29:983 (1979).

18. D. A. Scudiero, Decreased DNA repair synthesis and defective colony-forming ability of ataxia telangiectasia fibroblast cell strains treated with N-methyl-N'-nitro-N-guanidine, Cancer Res. 40:984 (1980).

19. F. E. Ahmed and R. B. Setlow, Excision repair in ataxia telangiectasia, Fanconi's anemia, Cockayne syndrome, and Bloom's syndrome after treatment with ultraviolet radiation and N-acetoxy-2-acetylaminofluorene, Biochim. Biophys. Acta. 521:805 (1978).

20. A. J. Brown, T. H. Fickel, J. E. Cleaver, P. H. M. Lohman, M. H. Wade, and R. Waters, Overlapping pathways for repair of damage from ultraviolet light and chemical carcinogens in human fibroblasts, Cancer Res. 39:2522 (1979).