DNA REPAIR IN HUMAN/EMBRYONIC CHICK HETEROKARYONS

ABILITY OF EACH SPECIES TO AID THE OTHER IN THE REMOVAL OF ULTRAVIOLET-INDUCED DAMAGE

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ABSTRACT Cultured human and embryonic chick fibroblasts possess different enzyme-mediated processes to repair cyclobutyl pyrimidine dimers induced in their deoxyribonucleic acid (DNA) by ultraviolet (UV) radiation. While dimers are corrected in human cells by excision repair, a photoenzymatic repair process exists in embryonic chick cells for the removal of these potentially deleterious UV photoproducts. We have utilized a sensitive enzymatic assay to monitor the disappearance, i.e. repair, of dimer-containing sites in fused populations of human and chick cells primarily consisting of multinucleate human/chick heterokaryons. Fused cultures were constructed such that UV photoproducts were present only in chick DNA when evaluating excision repair and only in human DNA when evaluating photoenzymatic repair. Based on the kinetics of site removal observed in these cultures we are led to conclude the following: Within heterokaryons per se the photoreactivating enzyme derived from chick nuclei and at least one excision-repair enzyme (presumably a UV endonuclease) derived from human nuclei act on UV-damaged DNA in foreign nuclei with an efficiency equal to that displayed toward their own nuclear DNA. Hence, after cell fusion these chick and human repair enzymes are apparently able to diffuse into foreign nuclei and once therein competently attack UV-irradiated DNA independently of its origin. In harmony with the situation in nonfused parental cultures, in heterokaryons the chick photoenzymatic repair process rapidly removed all dimer-containing sites from human DNA including the residual fraction normally acted upon slowly by the human excision-repair process.

INTRODUCTION

Enzymatic mechanisms which repair damage induced in DNA by ultraviolet (UV) radiation (e.g., cyclobutyl dimers between adjacent intrastrand pyrimidines) have been identified in numerous cell strains established in culture from a broad range of animals (Cleaver, 1974; Cook, 1970). One mechanism, termed excision repair, proceeds by a series of enzyme-mediated reactions leading to the release of the defective nucleotide sequence from the DNA followed by repair synthesis and ligation to close the resulting

single-strand gap. A second extensively studied mechanism, known as photoenzymatic repair, accomplishes the same objective *in situ* by simply cleaving the cyclobutane ring, thereby directly converting the pyrimidine dimer into two normal monomers. While the excision-repair enzymes rely on metabolic energy, the photoreactivating enzyme displays an absolute requirement for 340–500 nm light as an energy source.

Although a repair enzyme is expected to act on its own DNA in vivo, the ability to operate also on foreign DNA is a property of special interest which cannot be predicted from first principles and must therefore be measured empirically. While the activities of several repair enzymes toward foreign DNA in phage-infected cells of the bacterium *Escherichia coli* are well-understood (Setlow and Carrier, 1968; Muraoka and Kondo, 1969; Boyle and Setlow, 1970; Taketo et al., 1972), relatively little information of a similar nature is available for animal cell systems. To combine within the same mammalian cell, repair enzymes and DNA from diverse sources (e.g., human and rodent), analogous to phage-infected *Escherichia coli,* multinucleate heterokaryons have been constructed from cells of two different species by the technique of somatic cell hybridization (Harris and Watkins, 1965). In hybrid cells obtained in this manner, enzymes peculiar to one species have been observed to promote unscheduled synthesis in UV-damaged DNA derived from the other species (Darzynkiewicz and Chelmicka-Szorc, 1972; Darzynkiewicz et al., 1972; Goldstein and Lin, 1972). This nonconservative form of DNA synthesis presumably reflects the gap-filling step in excision repair (Painter and Cleaver, 1969), indicating that certain excision-repair enzymes of human and rodent origin can migrate into foreign nuclei and then act on UV photoproducts in the DNA within the chromatin. These studies, however, do not provide quantitative data on the kinetics of repair. Unfortunately, this leaves unanswered probably the most important question pertaining to this phenomenon, namely: What is the relative efficiency displayed by eukaryotic repair enzymes when operating on damage to DNA in foreign versus native chromatin?

This situation motivated us to undertake a series of experiments described in brief elsewhere (Paterson and Lohman, 1974) and in detail here. Our novel investigative system exploits: (*a*) the complementary dimer-repair properties exhibited by human fibroblasts (excision-proficient but photoenzymatic-deficient [Regan et al., 1968; Cleaver, 1966]) and embryonic chick fibroblasts (photoenzymatic-proficient but excision-deficient [Paterson et al., 1974 *b*]); and (*b*) a sensitive enzymatic assay (Paterson et al., 1973) characterized by its ability to monitor DNA with high resolution for the in vivo disappearance of one specific class of UV-damaged sites (i.e. those containing pyrimidine dimers). Such sites are detected in this new assay by their susceptibility to attack by a UV endonuclease purified from *Micrococcus luteus.* Human and chick cells were fused to form giant heterokaryons containing many intact nuclei and a mixture of cytoplasm derived from both strains. The experimental design was such that UV photoproducts were induced only in chick DNA when assessing excision repair involving human enzymes and only in human DNA when assessing photoenzymatic repair by chick enzymes. This protocol permitted the examination of both types of repair within the same biological background. The most reasonable interpre-

tation of our data is that in these human/chick heterokaryons the photoreactivating enzyme from chick nuclei and one or more excision-repair enzymes from human nuclei readily operate on the UV-damaged DNA in the other nuclei with an efficiency comparable to that directed toward their own nuclear DNA.

MATERIALS AND METHODS

Cell Strains

Three (two human, one chick) primary fibroblast strains were used: AH, derived from a healthy human volunteer (Kleijer and Bootsma, 1971); XP4RO, established from a patient having the classical form of xeroderma pigmentosum (XP) (Bootsma et al., 1970); and embryonic chick cells (designated as CEF), freshly isolated from a sterile biopsy of a 10-day chick (White Leghorn) embryo (Paterson et al., 1974 *b*).

Cell Cultivation

Monolayer cultures of each strain were routinely grown at 37° in Roux flasks containing F12 medium (Ham, 1965) supplemented with 15% (vol/vol) fetal calf serum, 100 IU/ml penicillin and 100 μg/ml streptomycin.

DNA Labeling

When required, radioactive labeling of the DNA was accomplished by seeding cells in Falcon petri dishes (diam, 15 cm; each receiving ~ 10^6 cells) containing the F12 growth medium supplemented with either 0.5 μCi/ml (2 Ci/mmol) [methyl-^{3}H]thymidine (dThd) or 1.0 μCi/ml (53 Ci/mol) [2-^{14}C]dThd (Amersham/Searle Corp., Arlington Heights, Ill.) and then incubating the cultures for 48 h in a CO_2 incubator (i.e. a 37° water-saturated atmosphere of 5% CO_2 – 95% air).

UV Irradiation

Only the ^{3}H-labeled cultures were exposed to UV radiation. These monolayer cultures were washed twice in Hanks' balanced salt solution (Hanks and Wallace, 1949). After completely removing the washing solution, the preparations were exposed to an incident UV fluence of 10 J/m^2 emitted from a TUV low pressure mercury tube (15 watt) (predominantly 254-nm light; exposure rate, 0.8 J/m^2 per s; Philips Electronic Instruments, Mount Vernon, N.Y.). Immediately thereafter both these UV-irradiated cultures as well as the remaining unirradiated (^{14}C-labeled and unlabeled) cultures were trypsinized and the cells were collected and held at 4° until cell fusion. Due to the rapid rate of photoenzymatic repair in embryonic chick cells (Paterson et al., 1974 *b*) these and all subsequent manipulations of the cultures were performed, unless specified otherwise, under yellow light (i.e. "gold" fluorescent lamps).

Cell Fusion

In all cases the fused cultures were constructed from one radioactive strain (either a ^{3}H-labeled, UV-damaged one or a ^{14}C-labeled, undamaged one) in combination with either one or, in one set of experiments, two nonradioactive (undamaged) strains. Cell fusion was achieved by a standard regimen (Harris and Watkins, 1965). In a typical fusion ~ 10^7 cells of each selected strain were mixed with heavily UV-irradiated (incident fluence, 550 J/m^2) Sendai virus (hemagglutination titer, 500 U) in 2 ml of Hanks' solution (Hanks and Wallace, 1949). The fusion suspension was maintained at 4° for 4 min, briefly shaken manually to test cell aggregation and hence fusion, and then incubated at 37° for 25 min. Regardless of the fusion pair under study,

no removal of pyrimidine dimer-containing sites was detected during this short incubation period (unpublished data). Hence, the fusion procedure did not interfere with monitoring (*a*) the initial number of these damaged sites induced by the radiation treatment and (*b*) the time course of disappearance of such sites during subsequent incubation of the fused cultures. Finally the fused cell preparations were gently (to minimize break up of the cell clumps) seeded in Falcon petri dishes (diam, 9 cm; each dish receiving the equivalent of $\sim 2 \times 10^6$ parental cells) containing prewarmed F12 growth medium.

Incubation of Fused Cultures

The petri dishes were immediately placed in 37° incubators and the fused cell samples were then incubated for varied times either in total darkness or under fluorescent black light (320–420 nm; flux ~ 8 J/m^2 per s) to permit photoenzymatic repair (Paterson et al., 1974 *b*). In one experiment, some fused cultures were first placed in the dark for 24 h before exposure to black light for the remainder of the incubation period. After the completion of incubation, the cells were collected and stored at −70° (Paterson et al., 1973).

In Vitro Enzymatic Assay

The protocol used to measure the amount of UV-induced damage remaining in the irradiated DNA of fused cultures after incubation included: (*a*) coextraction of DNA from the two parallel fused cultures containing ^{3}H-labeled, irradiated DNA and ^{14}C-labeled, unirradiated DNA; (*b*) incubation of the isolated DNA with and (as a control) without a UV endonuclease (purified from the bacterium *Micrococcus luteus*) selectively active toward sites altered by pyrimidine dimers in UV-irradiated DNA (Paterson et al., 1973); (*c*) sedimentation of the DNA samples in alkaline 5–20% (vol/vol) sucrose gradients; and (*d*) computer analysis of the ensuing radioactivity profiles to determine the average molecular weight of the DNAs and hence the number of single-strand scissions specifically introduced in the UV-damaged DNA by the enzyme treatment. (The number of strand breaks per unit mass varies inversely with enzyme-induced changes in the number-average molecular weight as given by a simple expression [Paterson, 1974]. For reasons advanced by others [Regan et al., 1971], the number-average molecular weight was determined by computing the weight-average molecular weight and then, assuming the distribution to be random, halving this value.) This series of treatments has been described in detail elsewhere (Paterson et al., 1973).

RESULTS

Measurement of UV-damaged Sites in Cellular DNA

The radioactivity profiles of UV-irradiated and unirradiated chick DNA, depicted in Fig. 1, demonstrate the potential of the in vitro enzymatic assay to quantify lesions (observed as nuclease-susceptible sites) in the UV-damaged DNA of fused cell cultures derived from human and embryonic chick cells. When the DNA extracted from CEF/AH hybrids was incubated with the purified UV endonuclease and then sedimented, a marked difference was observed in alkaline sucrose gradients between the molecular weights of the UV-damaged and undamaged chick DNAs. This difference corresponded to 1.6 single-strand breaks per 10^7 daltons (see Paterson, 1974, for details concerning this computation). Incubation of the extracted DNA sample without the endonuclease resulted in a molecular weight change in the irradiated compared to the unirradiated macromolecules of 0.1 breaks per 10^7 daltons. Hence the embryonic chick DNA exposed in vivo to a UV fluence of 10 J/m^2 contained, on the average, 1.5 sites per 10^7 daltons which were susceptible to endonucleolytic attack by the *Micrococcus* enzyme. (The measurement is accurate to within ± 0.1 sites per 10^7 daltons.) This number of nuclease-

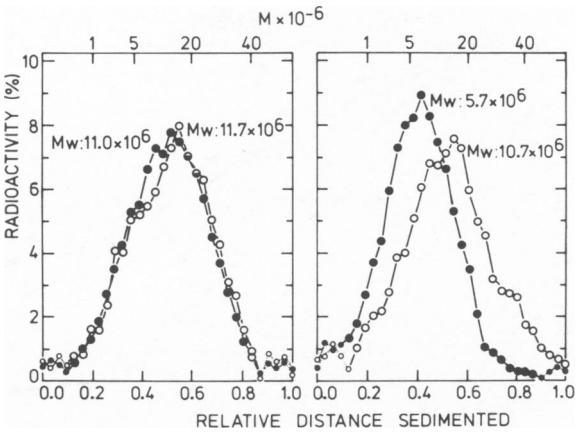

FIGURE 1 Alkaline sucrose gradient profiles of radioactive embryonic chick DNA extracted from CEF/AH hybrid cultures after incubation in the absence (left panel) or presence (right panel) of a UV endonuclease from *M. luteus*. The hybrid cultures were obtained by fusing in one case UV-irradiated (10 J/m^2), ^{3}H-labeled chick cells (•) and in the other case unirradiated, ^{14}C-labeled chick cells (○) with unirradiated, unlabeled human cells. Immediately after fusion the two CEF/AH cultures were collected and mixed thoroughly. The DNA was then extracted *in toto*, incubated with and without the *M. luteus* enzyme and finally analyzed by velocity sedimentation (40,000 rpm at 20° for 150 min in a Beckman SW 50.1 rotor [Beckman Instruments Inc., Palo Alto, Calif.]) as described (Paterson et al., 1973). The portions of each profile drawn with a dotted line correspond to gradient fractions clearly divorced from the main body of radioactivity and therefore excluded in the computation of weight-average molecular weight (Mw). M, molecular weight (daltons).

susceptible sites equals within experimental error the measured number of pyrimidine dimers (~1.7 per 10^7 daltons[1]) in the same UV-damaged chick DNA as determined by radiochromatography (Setlow et al., 1969) and is also in very close agreement with that found in similar studies on nonfused, parental cells (Paterson et al., 1974 *b*). Thus neither the cell fusion procedure nor the presence of the nonradioactive, undamaged human DNA affected the excellent sensitivity of the assay, i.e. the stoichiometric conversion of intact dimer-containing sites to UV endonuclease-nicked ones in the radiation-damaged chick DNA.

In Vivo Removal of Sites from CEF and XP DNA by Excision Repair

To assess the ability of human excision-repair enzymes to act on nuclease-susceptible sites in UV-damaged DNA within foreign (i.e. embryonic chick) nuclei, CEF fibroblasts, labeled with [^{3}H]dThd, were exposed to 254-nm light, and fused with unlabeled, undamaged AH cells. The resulting CEF/AH cell samples were then incubated in total darkness (conditions precluding photoenzymatic repair) for various times and finally assayed to determine the numbers of nuclease-susceptible sites in the chick DNA. The results are shown in Fig. 2. About 50% of the sites induced initially were eliminated from the chick DNA in 24 h with little additional removal occurring during a subsequent 6 h period. Substitution of normal, repair-proficient AH

[1] Calculation based on: (*a*) percent of the total thymine (Thy) content in dimers: 0.03 (unpublished data); (*b*) ratio of dimers in (chick) DNA containing ~26 mol % Thy: Thy-Thy:Thy-Cyt:Cyt-Cyt = 5:4:1 (Setlow and Carrier, 1966).

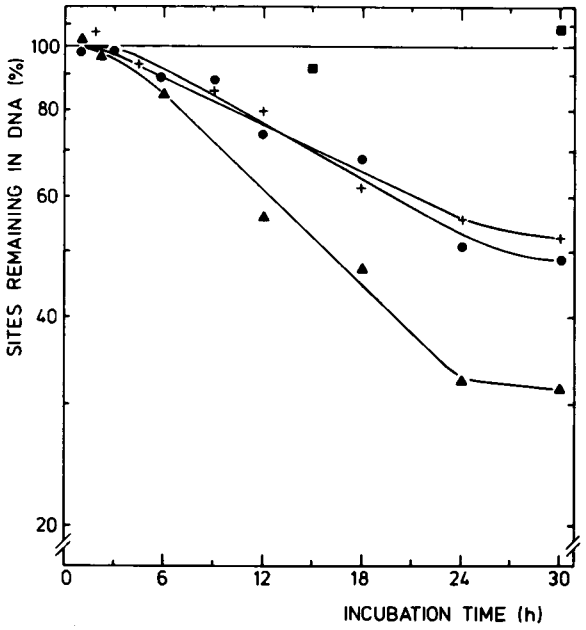

FIGURE 2 Time course of disappearance of nuclease-susceptible sites from UV-damaged (10 J/m^2), ^{3}H-labeled chick DNA in fused cultures of embryonic chick and human cells during incubation in the dark. The experimental protocol was as outlined in the legend of Fig. 1 except that after hybridization the cultures were incubated for indicated times before performing the in vitro enzymatic assay. The number of sites remaining in the DNA of the incubated samples is presented as a percentage of that detected in the parallel unincubated sample (arbitrarily set at 100%). Each point represents the average of independent triplicate determinations (standard deviation $\leq 13\%$). Combination of fused strains (only the first member in each fusion was prelabeled with [^{3}H]dThd and preexposed to 254-nm radiation): AH/AH, ▲; CEF/XP4RO, ■; CEF/AH, ●; XP4RO/AH, +.

cells by XP4RO ones severely defective in site removal (Paterson et al., 1973), however, resulted in chick/human hybrids unable to act on nuclease-susceptible sites in chick DNA. These data clearly demonstrate the involvement of at least one human excision-repair enzyme in the removal of sites from chick DNA in the CEF/AH hybrids. When the same analysis was performed on XP4RO/AH fused cultures, nuclease-susceptible sites disappeared from the UV-irradiated XP4RO DNA with kinetics closely resembling those observed for the irradiated CEF DNA in CEF/AH fused cultures. It seems reasonable to conclude from these parallel studies on chick/human and human/human hybrids that after cell fusion at least one excision-repair enzyme of human origin freely migrates to, and then eliminates nuclease-susceptible sites from foreign DNA in chick nuclei with an efficiency equal to that exhibited toward native DNA in human nuclei. The human enzyme involved, which is nonfunctional in XP4RO cells, is probably a UV endonuclease mediating the introduction of a single-strand incision adjacent to the pyrimidine dimer (Paterson et al., 1973; 1974 *a*).

Both curves in Fig. 2 illustrating the kinetics of site removal in CEF/AH and XP4RO/AH fused cells are characterized by three distinct components: (*a*) an initial shoulder lasting up to 3 h; (*b*) a steep exponential decline during the next 21 h; and (*c*) a second, less abrupt exponential decline extending beyond 48 h (data not shown after 30 h). Although its precise bio-

chemical basis is ambiguous, the shoulder is observed only in UV-irradiated hybrids assayed immediately after cell fusion (see Discussion). The two exponential phases probably correspond to the fast and slow components of site removal observed earlier in similar studies on nonfused (Paterson et al., 1973; Wilkins and Hart, 1974) and fused (Paterson et al., 1974 *a*) human cells.

We have also followed the removal of nuclease-susceptible sites in AH/AH hybrids—fused cultures expected to exhibit a maximal ability to execute excision repair. The resulting site-removal curve (Fig. 2) is similar to those obtained for CEF/AH and XP4RO/AH hybrids. However, in comparison to the other two, the AH/AH preparations eliminated sites more rapidly and to a greater extent (i.e. ~70% vs. ~50% in 24 h). This enhancement in both the rate and extent of site removal may be explained on the basis of heterogeneity among the cells comprising the fused cultures. Within the total cell population there existed not only the desired multinucleate heterokaryons arising from both fusion members but also nonfused, mononucleate cells and, to a lesser extent, multinucleate cells both of which originated from only one member. In fact, within the CEF/AH and XP4RO/AH hybrids 20–25% of the total nuclei resided in mononucleate cells (unpublished data). While both the heterokaryons and the remaining cells of single ancestry would be expected to perform site removal in AH/AH cultures, only the heterokaryons should be functional in CEF/AH and XP4RO/AH cultures. In keeping with this expectation ~30% (100% × [70% − 50%]/70%) fewer sites were eliminated from the UV-damaged DNA in the latter two fused pairs than in the former one. And since the AH/AH cultures required the same incubation period to complete the initial exponential decline as the CEF/AH and XP4RO/AH cultures (i.e. ~24 h), each curve for these latter two hybrids is presumably a composite of two curves representing two subpopulations of cells. One curve represents cells derived from only one parent (containing ~30% of the total UV-damaged DNA) and would exhibit no site removal; the other represents heterokaryons and would be similar in shape to that seen here for the AH/AH fused cultures.

In Vivo Removal of Sites from XP DNA by Photoenzymatic Repair

An experiment reciprocal to the one described above was performed to test whether the chick photoreactivating enzyme, like the human excision-repair enzyme(s), could eliminate nuclease-susceptible sites from foreign DNA. Fused samples prepared from ^{3}H-labeled, UV-irradiated XP4RO fibroblasts and untreated CEF fibroblasts were incubated for various times under black light and then assayed for the frequency of nuclease-susceptible sites remaining in the extracted XP4RO DNA. The kinetics of site removal for these human/chick hybrids, shown in Fig. 3, indicate that after a lag of 6 h ~80% of the initial sites disappeared in a monophasic exponential fashion during the next 10 h with no additional elimination thereafter. The site-removal mechanism displayed an absolute requirement for light because all sites persisted in the damaged DNA within both CEF/CEF and XP4RO/CEF preparations when these fused cultures were incubated in the dark. In agreement with an earlier study in which human cells were reported to lack photoenzymatic repair (Cleaver, 1966) XP4RO/XP4RO cultures, when held in the light, failed to eliminate nuclease-susceptible sites. Hence the process active on UV-damaged human DNA in XP4RO/CEF hybrids was photoenzymatic repair contributed by the chick member.

Data in Fig. 3 also demonstrate that CEF/CEF hybrids (i.e. fused cultures expected to perform photoenzymatic repair with maximal competence) removed all sites from their UV-injured DNA after 10 h under black light. As proposed earlier to account for the observed deficiency in site removal by excision repair in CEF/AH and XP4RO/AH hybrids, the fraction (20%) of uncorrected sites in human DNA within XP4RO/CEF samples probably resided in DNA located in cells (either mono- or multinucleate) exclusively human in origin and, consequently, inaccessible to the chick photoreactivating enzyme. The abrupt cessation in site removal observed after 16 h is consistent with this interpretation. It seems then that within the subpopulation of XP4RO/CEF heterokaryons virtually all sites were removed by the chick enzymes. A

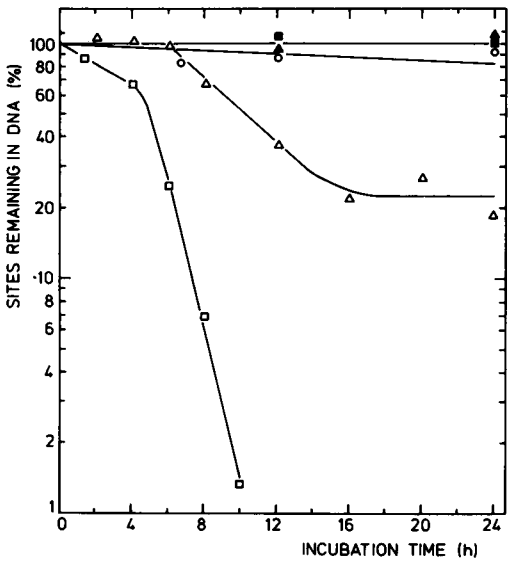

FIGURE 3

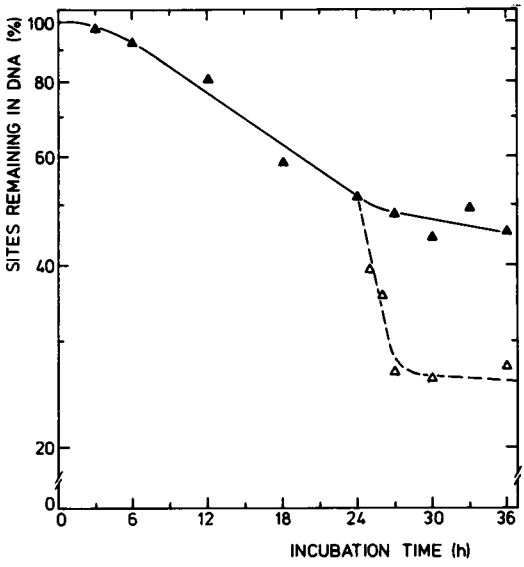

FIGURE 4

FIGURE 3 Time course of disappearance of nuclease-susceptible sites from UV-damaged (10 J/m^2), ^{3}H-labeled human DNA in hybrid cultures of human and embryonic chick cells during incubation in the dark (closed symbols) or under black light (open symbols). Data were obtained as in Fig. 2. Each point represents the average of independent triplicate determinations (standard deviation $\leq 11\%$). Combination of fused strains (only the first member was prelabeled and preexposed to 254-nm radiation): XP4RO/XP4RO, circles; XP4RO/CEF, triangles; CEF/CEF, squares.

FIGURE 4 Time course of in vivo disappearance of nuclease-susceptible sites from UV-damaged (10 J/m^2), ^{3}H-labeled DNA of XP4RO cells after fusion with both AH and CEF undamaged, unlabeled cells. Data were obtained as in Fig. 2. Samples of the hybridized culture derived from these three strains were incubated in the dark for up to 36 h and assayed at various intervals during this period (closed symbols). After 24 h of dark incubation some samples were illuminated with black light for various times and then assayed (open symbols). Each point represents the average of independent triplicate determinations (standard deviation $\leq 10\%$).

comparison of exponential slopes also indicates that sites disappeared more rapidly from CEF/CEF than from XP4RO/CEF cultures. Here again, the curve depicting site removal in XP4RO/CEF hybrids can be resolved into two components, one corresponding to multinucleate heterokaryons and the other cells of single ancestry. The exponential region in the curve for the heterokaryon fraction would be expected to approximate roughly that shown here for the CEF/CEF cultures except that it would be displayed toward higher incubation times due to the broader shoulder in the XP4RO/CEF composite curve (see Fig. 3).

Unfortunately, a CEF/CEF hybrid, analogous to the XP4RO/AH one used earlier, could not be constructed due to the nonavailability of a chick strain defective in photoenzymatic repair. On the basis of the above results, however, it seems reasonable to conclude that the chick photoreactivating enzyme in multinucleate human/chick cells can diffuse to, and then operate on foreign DNA within human nuclei with an efficiency consistent with that which would be displayed toward native DNA in chick nuclei.

Extent of Excision Repair Compared with Photoenzymatic Repair

A comparison of the relative extent of site removal by excision repair and photoenzymatic repair in human/chick hybrids (Figs. 2 and 3) indicates that under our experimental conditions the latter eliminated more nuclease-susceptible sites, not only in chick, but also in human DNA during a 24 h period. That is, regardless of the source of the DNA, the chick photorepair process evidently acted on essentially all sites while the excision-repair process, involving one or more human enzymes, attacked at most 70%. To substantiate this observation we followed the disappearance of nuclease-susceptible sites in a hybridized culture constructed from ^{3}H-labeled, UV-irradiated XP4RO cells in combination with both AH and CEF unlabeled, unirradiated cells. Fused samples derived from these three strains were then incubated in total darkness for various times up to 24 h, a period of sufficient duration to permit the fast component of excision repair to go to completion (see Fig. 2); the remaining fused samples were then further incubated in duplicate, one maintained in the dark while the other was exposed to black light. Data in Fig. 4 show that the excision-repair mechanism removed ~50% of the initial sites from the XP4RO DNA within 24 h and not more than an additional 5% during the the next 12 h. In contrast, a further 20% of the initial damage was eliminated within the short span of 3 hr after subsequent exposure to light, demonstrating that chick-mediated photoenzymatic repair acted on that fraction of nuclease-susceptible sites in UV-damaged human DNA which, otherwise, was insensitive to excision repair. This result could arise in fused cultures predominantly consisting of (*a*) trihybrid heterokaryons of XP4RO, AH, and CEF origin or (*b*) a mixture of bihybrid heterokaryons of XP4RO/AH and XP4RO/CEF origin. The second possibility seems most unlikely in that cultures incubated under black light immediately after fusion removed almost the same fraction (~70%) of sites (data not shown) but within 12 h, not 24 h. This implies that ~50% of the total sites removed were operated on by photoenzymatic repair as in 12 h only about 20% were eliminated in the dark (see Fig. 4). Taking into account that ~20% of the original nuclei remained in unfused cells, it follows that a large majority of the UV-damaged XP4RO nuclei resided in multinucleate cells capable of performing both types of repair and thus such cells were mainly derived from all three parental cell types. Consequently, it seems fairly certain that the chick photoenzymatic repair process can indeed quickly monomerize that fraction of sites in human DNA normally subject to removal by the slow component of excision repair (Paterson et al., 1973; 1974 *a*; Wilkins and Hart, 1974).

DISCUSSION

These experiments demonstrate that after artificial fusion of human and embryonic chick fibroblasts, DNA repair enzymes unique to each species are not only functionally conserved in the resulting multinucleate heterokaryons but are readily able to act on UV-damaged DNA within foreign nuclei derived from the fusion partner. Both the chick photoreactivating enzyme and presumably the human UV endonuclease specific for dimers appeared to operate on nuclease-susceptible sites independently of the origin of the irradiated DNA. Although the enzymatic assay monitored only the disappearance of these dimer-containing sites and not overall repair, there can be little doubt that the site removal observed here reflects bona fide repair actually gone to completion (Paterson et al., 1973; 1974 *a*; 1974 *b*).

In earlier studies on nonfused human (Paterson et al., 1973) and embryonic chick (Paterson et al., 1974 *b*) cells as well as fused human cells (Paterson et al., 1974 *a*), the

kinetics of disappearance of nuclease-susceptible sites from UV-damaged DNA in vivo exhibited an exponential decline. The equivalent curves for site removal presented in this communication, however, are characterized by a shoulder before the onset of an exponential descent (see Figs. 2–4). We attribute the appearance of the shoulder to a modification in experimental design. In the earlier investigation on fused human strains (Paterson et al., 1974 *a*) the cultures, after cell hybridization, were held overnight before exposure to UV radiation and subsequent incubation to measure repair. By contrast, in the present study the radiation treatment was administered before cell hybridization (due to the necessity of preferentially damaging either human or chick DNA) and the fused samples were then immediately subjected to repair incubation. Hence in the earlier, but not in the present, study the cells had ~14 h to adjust to a fused state before being damaged by UV irradiation. Although no attempt has been made to identify the precise molecular basis for the delay in site removal (ranging from 2 to 3 h for excision repair up to ~6 h for photoenzymatic repair) the following two possibilities come to mind: (*a*) Redistribution of the existing repair-enzyme molecules among the various nuclei within the newly formed heterokaryons (and possibly *de novo* synthesis and distribution of additional molecules) may require considerable time. (*b*) Exposure to UV-inactivated Sendai virus may be temporarily harmful to the general metabolism of cells and consequently inhibitory to the repair processes. It will be noted that the site-removal curve for XP4RO/CEF hybrids incubated in black light possesses a pronounced shoulder relative to that found in the corresponding curve for CEF/CEF hybrids (Fig. 3). In contrast, the shoulders of the comparable curves depicting site removal by excision repair in CEF/AH and AH/AH hybrids are not appreciably different (Fig. 2). Although at this point alternate explanations are equally tenable, it is tempting to speculate on the basis of these observations that the chick photoreactivating enzyme, but not the human excision-repair one(s), tends to be localized within its own nucleus. In support of this notion, indirect evidence would seem to indicate that the photoreactivating enzyme in cells of frog liver (Cook and McGrath, 1967) and *E. coli* (Muraoka and Kondo, 1969; Paterson and Roozen, 1972) is preferentially concentrated near DNA.

Recent data of Wilkins and Hart (1974) suggest that the residual fraction of nuclease-susceptible sites removed by the slow component of excision repair is confined to stretches of DNA normally enveloped with nucleoproteins and, consequently, physically inaccessible to the enzymes mediating this repair process. If this explanation is correct then the chick photoreactivating enzyme is specifically able to penetrate the suggested protein sheath masking the dimers. Until the molecular architecture of the eukaryotic chromosome is better understood it is perhaps premature to speculate further on the underlying principle which permits the chick photoenzymatic repair process, but not the human excision-repair process, to remove quickly all dimers from UV-irradiated DNA. At any rate it should be of interest to determine whether the rapid removal of this otherwise persistent fraction of dimers by the chick photorepair mechanism has any survival value to the human cell.

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