Mammalian mitochondrial endonuclease activities specific for ultraviolet-irradiated DNA

Alan E. Tomkinson†, R. Thomas Bonk§, Joon Kim, Neil Bartfeld§ and Stuart Linn*  
Department of Biochemistry, University of California, Berkeley, CA 94720, USA

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ABSTRACT

Mitochondrial forms of uracil DNA glycosylase and UV endonuclease have been purified and characterized from the mouse plasmacytoma cell line, MPC-11. As in other cell types, the mitochondrial uracil DNA glycosylase has properties very similar to those of the nuclear enzyme, although in this case the mitochondrial activity was also distinguishable by extreme sensitivity to dilution. Three mitochondrial UV endonuclease activities are also similar to nuclear enzymes; however, the relative amounts of these enzyme activities in the mitochondria is significantly different from that in the nucleus. In particular, mitochondria contain a much higher proportion of an activity analogous to UV endonuclease III. Nuclear UV endonuclease III activity is absent from XP group D fibroblasts and XP group D lymphoblasts have reduced, but detectable levels of the mitochondrial form of this enzyme. This residual activity differs in its properties from the normal mitochondrial form of UV endonuclease III, however. The presence of these enzyme activities which function in base excision repair suggests that such DNA repair occurs in mitochondria. Alternatively, these enzymes might act to mark damaged mitochondrial genomes for subsequent degradation.

INTRODUCTION

Genetic information within mammalian mitochondria is contained in its multiple copy circular DNA chromosome (1). The nucleotide sequences of several mammalian mitochondrial genomes are known, and all of the proteins encoded can be assigned either to structural or respiratory functions. Therefore proteins involved in the replication and maintenance of the mitochondrial genome are encoded by nuclear genes.

Mitochondrial DNA has an increased susceptibility to DNA damaging agents (2—4), possibly because it is not organized into a chromatin structure. However, studies with yeast (5) and mouse or human cells (6) have not detected the removal of pyrimidine dimers from mitochondrial DNA and have found no evidence for a significant amount of recombinational DNA repair in mitochondrial DNA. It has been suggested that the absence of these types of DNA repair may be responsible for the high rate of mutation of mitochondrial DNA (7). Whereas it appears that nucleotide excision- and recombinational DNA repair do not occur in mitochondria, base excision repair might occur, since mitochondrial forms of uracil DNA glycosylase (8—10) and AP endonucleases (11) have been described.

Since mitochondria are the site of electron transport and oxidative phosphorylation, these organelles would be expected to contain a high concentration of active oxygen species (12—13). Indeed, it has been demonstrated that there is significantly more oxidative damage to mitochondrial DNA than to nuclear DNA (14). Endonuclease activities that recognize DNA lesions introduced by oxidizing agents such as osmium tetroxide, hydrogen peroxide, or ionizing- and UV irradiation, have been detected in bacteria (15, 16), yeast (17), and mammalian cells (18—20). These enzymes, which recognize damaged pyrimidines (21), remove the damaged bases and then cleave the DNA at the resultant AP site by a class I, β-elimination mechanism (22) to generate a 5′ phosphate and a 3′ unsaturated baseless sugar. In addition, a DNA glycosylase activity that excises hydroxymethyl uracil (HMU), a DNA lesion caused by ionizing radiation (23), has been purified from mammalian cells (24, 25) but has not been detected in bacteria or yeast cells (26).

In this paper we report that the mitochondria of mouse plasmacytoma cells contain uracil DNA glycosylase activity and endonuclease activities that recognize lesions introduced by high UV doses (UV endonucleases). Like bacteria, however, they do not contain detectable HMU DNA glycosylase activity. These enzymes are similar, but distinguishable from their extramitochondrial counterparts (27). The presence of these enzymes suggests that base excision repair may function in mitochondria to correct oxidative DNA damage.

Extracts from diploid cultured human fibroblast cells contain three UV endonuclease activities, one of which, UV endonuclease III, is either absent or present in substantially reduced amounts in extracts of xeroderma pigmentosum (XP) group D cells but present at normal levels in extracts from XP group A and group C cells (Choi, S.Y., Tomkinson, A.E. and Linn, S., unpublished observations). This paper also reports that the mitochondrial form of UV endonuclease III has altered properties and is present at

[Present addresses]  
† Imperial Cancer Research Fund, Blanche Lane, South Mimms, Potters Bar, Herts EN6 3LD, UK, §California College of Medicine, University of California, Irvine, CA 92717 and ¤University of Arizona, Department of Biochemistry, Tucson, AZ 85721, USA
significantly reduced levels in the mitochondria of XP group D lymphoblasts, suggesting a genetic relation between the mitochondrial and extramitochondrial forms of the enzyme.

MATERIALS AND METHODS

Materials

PM2 [3H-thymidine]DNA (5,000 cpm/nmol nucleotide) (28), PBS-2 [3H-uracil]DNA (150 cpm/pmol uracil) (29) and 3H-labelled SPO1 DNA (24) were prepared as described except that [5,6-3H]uridine was used for the SPO1 DNA. Sephacryl S-200 was purchased from Pharmacia; DEAE-cellulose (type 52) and P11 phosphocellulose were from Whatman; BA85S597 0.45μm nitrocellulose membrane filters were from Schleicher and Schuell.

Methods

Preparation of Whole Cell Extracts from MPC-11 Cells

Cells (8 liters at approximately 10^6 cells/ml) were grown and harvested as described by Nes (18) and the cell extract was prepared as described by Hollstein et al., (24). Briefly, the cells were lysed by sonication in 0.7M KCl to disrupt nuclear structures, diluted to 0.3M KCl and viscosity reduced by further sonication. After centrifugation to remove cellular debris, the supernatant was concentrated in an Amicon Diaflow apparatus.

Preparation of Mitochondria from MPC-11 Cells

Mitochondria were prepared basically as described by Tomkinson and Linn (30) from 16 liters of culture. In some preparations, 5mM CaCl_2 was added to the normal lysis buffer of 10 mM Tris-HCl (pH 7.5), 1.0 mM EDTA, 0.25 M sucrose (TE sucrose). Under these conditions nuclei remained intact and, after separation of nuclei, a nuclear extract was prepared as described by Anderson and Friedberg (8). After purification by differential centrifugation and sedimentation through a sucrose step gradient, mitochondria were either resuspended in TE sucrose for further purification through a linear sucrose gradient or lysed by sonication prior to estimation of mitochondrial enzyme activities and enzyme purification.

Preparation of Mitochondria from Human Normal and Xeroderma Pigmentosum Group D Lymphoblasts

5.6 Liters (6.7×10^8 cells) of GM 3714 normal lymphoblast cells or 5.6 liters (3.7×10^8 cells) of GM 2485A XP-D lymphoblast cells were grown in RPMI 1640 medium using roller bottles in the dark. Cells were harvested, washed and subcellular fractionation carried out as described by Tomkinson and Linn (30).

Preparation of Damaged DNA

Partial depurination of PM2 DNA was carried out at 70°C in 10 mM sodium citrate (pH 4.5) and 100 mM NaCl for 15 min so as to generate approximately 1.5 AP sites per DNA genome. PM2 DNA was irradiated in 10 mM Tris-HCl (pH 7.5) and 0.02% glycerol at 2.5J/m^2/sec with a Westinghouse germicidal lamp (G15T8). This total dose of 525 Joules produces 1-2 sites per genome (31).

Uracil DNA Glycosylase Assay

Reaction mixtures (50μl) which contained 40 mM Tris-HCl (pH 8.0), 2 mM EDTA and 4μM PBS-2 [3H-uracil] DNA were incubated for 30 min at 37°C. Reactions were chilled and then 10 μl of heat-denatured calf thymus DNA (10 mg/ml) and 60 μl of 0.8 M perchloric acid were added. After 10 min on ice, acid-insoluble material was removed by centrifugation for 15 min at 15,000 x g and radioactivity in the supernatant was determined by liquid scintillation counting. In some cases, an aliquot of the reaction mixture was removed prior to acid precipitation and filtered through Amicon YMT filters. Note that some of the uracil DNA glycosylase activity synthesized UV or AP DNA per min at 37°C.

Hydroxymethyluracil DNA Glycosylase Assay

Reaction mixtures (100 μl) contained 40 mM Tris-HCl (pH 8.0), 2 mM EDTA and 28 μM SPO1 [3H]DNA. After incubation for 60 min at 37°C, unlabelled markers were added and the reactions were immediately filtered through Amicon YMT filters. The filtrate was separated by HPLC as described by Hollstein et al. (24). One unit of this glycosylase activity releases 1 pmol of HMU per min at 37°C.

Endonuclease Assays

Reactions were incubated for 15 min at 37°C under the conditions described and then stopped by the addition of 200 μl of 0.3 M K_2HPO_4 (pH 12.4), which denatures the PM2 DNA. After 3 min, the samples were neutralized with 100 μl of 1 M KH_2PO_4 (pH 4.0) and 200 μl of 5 M NaCl was added. This treatment renatures Form I, but not Form II PM2 DNA. Each sample was finally diluted to 5 ml with 40 mM Tris-HCl (pH 8.0), 1 M NaCl and filtered through a nitrocellulose filter, which selectively retains denatured DNA. Filters were washed with 5 ml of 0.3 M NaCl and 0.03 M sodium citrate, dried and counted by liquid scintillation. These were compared to a sample which had been prepared by treating an aliquot of the damaged DNA with 200 μl of 0.3 M K_2HPO_4 (pH 13.2) for 5 min (in order to completely denature the DNA) followed by neutralization and further treatment as above. The number of nicks introduced per PM2 genome was calculated assuming a Poisson distribution of UV lesions or AP sites as described (28). To estimate the amount of activity specific for UV lesions or AP sites, assays were carried out on both damaged and untreated DNA. One unit of endonuclease activity introduces 1 fmol of nicks specifically into UV or AP DNA per min at 37°C. These assays were linear over at least 30 min and responses were linear with enzyme added between roughly 50% above the blank (no enzyme) value and roughly 1.5 nicks introduced per duplex circle.

DNA Synthesis Reactions

Reactions for incision (100 μl) contained 47 μM PM2 DNA (contributing 10 mM NaCl and 1 mM sodium citrate) and 50 mM Tris-HCl (pH 7.5), 10 mM MgCl_2 for the AP endonuclease or 50 mM Tris-HCl (pH 8.0), 3 mM EDTA, 40 mM KC1 for the UV endonuclease. Incubation was at 37°C and the reactions were terminated by heating for 5 min at 70°C. Aliquots of 10 μl were removed to determine the extent of endonucleolytic incision. Reactions for synthesis (200 μl) contained 70 mM potassium phosphate (pH 7.5), 9 mM 2-mercaptoethanol, 7 mM MgCl_2, 90 μM each dATP, dGTP, and dCTP, 85 μCi of [32P]dTTP (1000 cpm/pmol), 3 nmol of the incised PM2 [3H]DNA and 0.05 unit of E. coli DNA polymerase I. Incubation was at 37°C and aliquots of 50 μl were removed at regular intervals, chilled on ice and then mixed with
TABLE 1 Subcellular Distribution of Uracil DNA Glycosylase Activity

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Vol (ml)</th>
<th>Protein (mg)</th>
<th>Units of Uracil DNA Glycosylase</th>
<th>Units of HMU DNA Glycosylase</th>
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</thead>
<tbody>
<tr>
<td>Cytosol/soluble</td>
<td>95</td>
<td>330</td>
<td>540</td>
<td>14</td>
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<td>Nuclear</td>
<td>48</td>
<td>240</td>
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<td>62</td>
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<tr>
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<td>12</td>
<td>18</td>
<td>110</td>
<td>&lt;0.5</td>
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Subcellular fractionation of mouse plasmacytoma cells was performed as described in Methods, in the presence of 5 mM CaCl₂. Protein was quantitated according to Lowry et al. (32) and enzyme assays were performed as described in Methods.

TABLE 2 Purification of Mitochondrial UV Endonuclease Activities

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Activity (units/10⁶ ml)</th>
<th>Protein (mg)</th>
<th>Specific Activity (U/mg)</th>
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</thead>
<tbody>
<tr>
<td>Cytosol</td>
<td>161</td>
<td>4.88 x 10⁵</td>
<td>3147</td>
<td>155</td>
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<tr>
<td>Sonicated</td>
<td>7</td>
<td>35,500</td>
<td>150</td>
<td>225</td>
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<td>Mitochondria</td>
<td>158</td>
<td>2930</td>
<td>195</td>
<td>365</td>
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<tr>
<td>DEAE-cellulose</td>
<td>18</td>
<td>1040</td>
<td>108</td>
<td>420</td>
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<tr>
<td>Dialyzed P-cell I</td>
<td>18</td>
<td>454</td>
<td>0.8</td>
<td>420</td>
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<tr>
<td>DEAE-cellulose</td>
<td>3.5</td>
<td>735</td>
<td>0.39</td>
<td>1880</td>
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<td>0.5</td>
<td>129</td>
<td>0.045</td>
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<tr>
<td>Phosphocellulose II</td>
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<td>2.2</td>
<td>365</td>
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<td>(peak fraction)</td>
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<tr>
<td>(peak fraction)</td>
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<td>0.068</td>
<td>465</td>
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MPC-11 cells were fractionated and UV endonuclease activity in the cytoplasmic and mitochondrial fractions measured as described in Methods. The mitochondrial UV endonuclease activities were further purified by procedures similar to those described by Tomkinson et al. (11) for the purification of mitochondrial AP endonuclease. Briefly, the mitochondria were lysed by sonication in 50 mM Tris-HCl (pH 7.5), 0.4 M NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol and 0.05% Triton X-100 (Buffer A). After centrifugation at 27,000 x g for 30 min, the supernatant was applied to a 3-ml DEAE-cellulose column equilibrated with Buffer A and proteins eluted with Buffer A. Fractions containing significant absorbance at 280 nm were pooled, dialysed against 40 mM Tris-HCl (pH 8.0), 10 mM KC1, 1 mM EDTA, 1 mM EDTA, 10 mM 2-mercaptoethanol and 0.05% Triton X-100 (Buffer B) containing 1 µg/ml leupeptin, 1 µg/ml pepstatin and applied to a phosphocellulose column equilibrated with Buffer B. Proteins were eluted with equivalent gradient from Buffer B to Buffer C containing 1.2 M KCl. Three activities, designated I, II and III, eluted at 0.75 M, 0.5 M and 10 mM KCl, respectively. Each peak was dialysed against 5 mM potassium phosphate (pH 8.0), 1 mM EDTA, 0.5 mM DTT and 0.01% Triton X-100 (Buffer C) and then applied to a DEAE-cellulose column equilibrated with Buffer C. Proteins were eluted with a linear gradient from Buffer C to Buffer C containing 395 mM potassium phosphate. The UV endonuclease activities I, II and III eluted at 70 mM, 35 mM and 100 mM potassium phosphate, respectively.

RESULTS

Association of Uracil DNA Glycosylase and UV Specific Endonuclease Activities with Murine Cell Mitochondria

When mitochondria are purified by differential centrifugation and sedimentation through a sucrose step gradient, they are essentially free from cytoplasmic contamination (34) and lysosomal enzymes (11). Under conditions which favor nuclear integrity, 5% of the total uracil DNA glycosylase activity was associated with the mitochondria (Table 1). This value is comparable to those reported for other cell types (8, 9).

Subcellular fractions were also assayed for HMU DNA glycosylase activity (Table 1). Such activity was detected only in the nuclear and cytoplasmic fractions—mitochondria appeared to lack this activity. Of course, an activity might be present in mitochondria that is labile or has unusual reaction requirements.

The subcellular fractionation method employed to prepare mitochondria to be used as the starting material for enzyme purifications causes disruption of nuclei with the consequent presence of nuclear enzymes in the cytoplasmic fraction. Under these conditions, approximately 7% of the total cellular UV specific endonuclease activity (Table 2) and a similar percentage of the total uracil DNA glycosylase activity (data not shown) was associated with the mitochondrial fraction.

When intact mitochondria were further purified by sedimentation through a linear sucrose gradient, UV endonuclease activity and uracil DNA glycosylase activity co-sedimented with the mitochondrial marker enzyme, cytochrome oxidase. Disruption of mitochondria by exposure to hypotonic solution increases the amount of uracil DNA glycosylase selectively in fractions which sediment with mitochondria, but not in fractions which sediment as free protein. In general, a 2 to 3.5-fold increase of uracil DNA glycosylase and a 3 to 4-fold increase in UV specific endonuclease activity was observed when comparing assays of sonicated or osmotically shocked mitochondria to those of intact organelles. Together these results suggest that the uracil DNA glycosylase and UV specific endonuclease activities detected in mitochondrial fractions are likely to be naturally associated with the mitochondria.

Comparison of Mitochondrial and Extramitochondrial Forms of Uracil DNA Glycosylase

Uracil DNA glycosylase activities were purified from both a whole cell extract and a mitochondrial fraction prepared as described in Methods. Intact mitochondria were resuspended in 20 mM Tris-HCl (pH 8.0), 1 mM EDTA, 0.3 M KCl, 10 mM 2-mercaptoethanol before lysis by sonication. Proteins in the whole cell extract and the mitochondrial fraction were separated by Sephacryl S-200 chromatography and assayed for uracil DNA glycosylase activity. The mitochondrial form eluted at a position corresponding to an apparent molecular weight of 50- to 60 kDa, whereas the extramitochondrial enzyme eluted at a position corresponding to an apparent molecular weight of 30- to 40 kDa. (These reproducible differences are surprising as both forms have the same sedimentation coefficients.) Active fractions were pooled, dialyzed, and chromatographed on phosphocellulose P11.
endonuclease I has a broad pH optima between 7.0 and 8.5, between 7.0 and 8.5. All three enzymes are most active in 50 mM Tris-HCl at pH 8.0 and each demonstrates an increase in activity required the presence of 0.05% Triton X-100 in the dilution buffer to retain activity upon dilution. The mitochondrial enzyme was further purified by chromatography on Sephadex G-75 and hydroxylapatite. Unfortunately, when the most active fraction was subjected to SDS-PAGE, no protein could be detected upon silver staining. Catalytic properties of this preparation were similar to those described for other mitochondrial forms of the enzyme (9, 36).

### Purification of Mitochondrial UV Endonuclease Activities

To test whether distinguishable UV endonuclease activity was associated with mitochondria, the activity was purified. In initial experiments, the mitochondrial extract was prepared and fractionated by Sepacryl S-200 chromatography as described for whole cell extracts. Three peaks of UV endonuclease activity were resolved (data not shown) and these had elution positions similar to the activities separated from whole cell extracts (Tomkinson, A.E. and Linn, S. unpublished observation; 27), although the relative amounts of the activities were significantly different.

Using a different fractionation procedure, which is described in Table 2, three peaks of UV endonuclease activity, designated I, II and III, were resolved by phosphocellulose chromatography (Figure 1).

Each of these had an inseparable AP endonuclease activity suggesting that they each belong in the class of bifunctional enzymes, which are similar to *E. coli* endonuclease III in that they possess both DNA glycosylase and AP endonuclease activity. The sedimentation co-efficients of enzymes I, II and III were 3.5, 2.75 and 3.0, respectively. These S values correspond to molecular weights of 50 kD, 35 kD and 40 kD, respectively (assuming globular conformations). A polypeptide of 36 kD in molecular weights of 50 kD, 35 kD and 40 kD, respectively.

### Co-factor Requirements

None of the UV endonucleases requires a divalent cation for activity and each is active in the presence of EDTA on both UV-irradiated and depurinated DNA. The enzymes are stimulated roughly two-fold by 5 mM MgCl₂, however. Mitochondrial UV endonuclease I has a broad pH optima between 7.0 and 8.5, endonuclease II between 6.5 and 8.5 and the endonuclease III between 7.0 and 8.5. All three enzymes are most active in 50 mM Tris-HCl at pH 8.0 and each demonstrates an increase in non-specific activity as the pH drops below 6.0. The KCl optima for enzymes I, II and III are 75 mM, 100 mM and 50 mM, respectively, with no less than 50% maximal activity in the range of 0—100 mM KCl. Whereas enzymes I and III are most active in 0.005% Triton X-100, enzyme II is equally active between 0.005% and 0.02%.

### Substrate Specificities

The mitochondrial endonuclease activities strongly preferred DNA containing lesions due to UV-irradiation or acid-depurination. Little endonucleolytic activity was detected on undamaged DNA. The enzymes were each 2- to 3-times more active on UV-irradiated supercoiled PM2 DNA than on the same substrate which had been relaxed by the action of topoisomerase (Table 3).

### Type of Cleavage at AP Sites

AP endonucleases have been distinguished by whether they generate an efficient primer for DNA synthesis after cleavage of the AP site. All three of the mitochondrial enzymes appear to cleave in a manner analogous to *E. coli* endonuclease III,
leaving a sugar residue at the 3' terminus that does not efficiently prime DNA synthesis (Fig. 2). The mitochondrial AP endonuclease which generates a 3'-OH terminal nucleotide (11) was included as a positive control.

If a 'UV endonuclease' acts as a DNA glycosylase, and also
as an AP endonuclease as do the extra-mitochondrial UV
endonuclease I and II (27), and, if the glycosylase action
significantly precedes the AP endonuclease reaction, addition of
exogenous AP endonuclease might stimulate the observed rate
of nicking of UV-irradiated DNA. This was not the case with
mitochondrial UV endonuclease II, however (data not shown).
Unfortunately, the low levels of each of the mitochondrial UV
endonucleases precluded reproducibly assaying directly for DNA
glycosylase activity.

Human Mitochondrial UV Endonucleases from Normal and
Xeroderma Pigmentosum, Complementation Group D
Lymphoblasts
The three UV endonucleases characterized in mouse cells have
analogous in human cells (27; Choi, S.-Y., Tomkinson, A.E., and
Linn, S., unpublished observation). UV endonuclease III activity
is largely or totally absent specifically from whole cell extracts
from xeroderma pigmentosum group D cells but present at normal
levels in extracts from XP group A and group C cells (Choi,
Therefore we examined mitochondrial fractions from XP group
D lymphoblasts for the mitochondrial UV endonuclease III
activity.

Mitochondria were prepared from human lymphoblasts as
described in Methods and UV endonuclease activities purified
by essentially the same procedures used for the mouse mitochondrial UV endonucleases. The profile of UV endonuclease activities from normal and XP group D cells after phosphocellulose chromatography is shown in Figure 3 and the levels are given in Table 4 where a preparation from mouse
MPC-11 cells is also included for comparison. In these experiments, UV endonucleases I and II were eluted together
by a high salt step wash, while UV endonuclease III passed
through the column. The XP group D cell mitochondria contained
one-third of the pass-through activity compared with normal
mitochondria, whereas the XP group D cytoplasm had almost
none. When the residual activity in the XP group D mitochondria
phosphocellulose pass-through fraction was chromatographed
upon DEAE-cellulose, it eluted at 200 mM potassium phosphate,
whereas UV endonuclease III from normal mitochondria elutes
at 100 mM potassium phosphate (see Table 2). Moreover, this
activity was 1.8-fold more active on relaxed, then supercoiled
irradiated DNA (rather than preferring supercoiled DNA). Thus
the activity from XP group D mitochondria differs both
qualitatively and quantitatively from that of normal mitochondria.
Possibly the mitochondrial and cytoplasmic forms of UV
endonuclease III may be related genetically, but not similarly
regulated or processed.
constitutes 20—30% of the total mitochondrial UV endonuclease activity, whereas the mitochondrial form of UV endonuclease III constitutes 7% of the total extramitochondrial UV endonuclease activity, and it appears that the two forms appear to be present in all mammalian cell types examined. By analogy with the extramitochondrial forms of these enzymes, it is assumed that the mitochondrial form of UV endonuclease I and II, which can be resolved into three peaks that have similar enzymatic activities, uracil DNA glycosylase and UV specific endonucleases I and II, initially described by Nes (18), have such similar properties that they maybe derived from the same gene.

A DNA glycosylase activity that excises hydroxymethyl uracil (HMU) has been characterized from mouse MPC-11 cells (24). This activity was present in the cytoplasmic or nuclear fractions but could not be detected in the mitochondrial fraction. HMU can be formed in DNA by attack on thymine by free radicals formed by the action of ionizing radiation (23), or it can form by deamination and oxidation of 5-methylcytosine (25). 5-Methylcytosine (5-meC) is involved in the regulation of gene expression in mammalian cells, so the main role of HMU DNA glycosylase might be to maintain the levels of 5-methylcytosine in the mammalian nuclear genome. However, transcription of the mammalian mitochondrial genome is not regulated by 5-meC (41) and consequently these organelles would not require HMU DNA glycosylase. Parenthetically, bacterial or yeast cells, which likewise do not utilize 5-meC in DNA to regulate gene expression, also lack the enzyme (26).

The question of whether the mammalian nuclear genome has separate genes encoding similar nuclear and mitochondrial AP endonucleases, UV endonucleases and uracil DNA glycosylases will be answered by gene cloning experiments. Nonetheless, the mitochondrial location of these enzymes, in addition to mitochondrial DNA polymerase (42) and DNA ligase (43) provide the enzyme machinery for DNA base excision repair in these organelles. It is also noteworthy that Satoh, et al. (44) reported the removal of alkylated bases from the mammalian mitochondrial genome, presumably by base excision repair. It is equally possible, however, that these enzymes do not function in DNA repair, but act instead to initiate the degradation of damaged mitochondrial DNA, thus assuring the survival only of the population of undamaged mitochondrial genomes. Along such lines, bulky adducts such as pyrimidine dimers, which do not appear to be excised from mitochondrial DNA (5,6), might block replication directly and hence need no further processing.

**DISCUSSION**

Mouse cell mitochondria contain the base excision repair activities, uracil DNA glycosylase and UV specific endonuclease with associated AP endonuclease activity as judged by the criteria that in preparations of intact mitochondria these enzymes co-sediment with mitochondrial marker enzymes and that there is a significant increase in activity following disruption of mitochondria. The association of 5—10% of total cellular uracil DNA glycosylase activity with mitochondria has been reported previously (8, 9) and the rat liver nuclear and mitochondrial forms of this enzyme have been purified to apparent homogeneity. When compared, these forms were distinguishable by small but significant differences in physical and catalytic properties (10, 36). In yeast, there appears to be distinct nuclear genes coding for the nuclear and mitochondrial forms of uracil DNA glycosylase (39) (as well as DNA polymerase (40)). In these studies of the mitochondrial and extramitochondrial forms of uracil DNA glycosylase from mouse cells, similar small differences were detected, in particular the sensitivity of the extramitochondrial form to dilution. This requirement for Triton X-100 in the dilution buffer to retain activity was also observed for the extramitochondrial form of UV endonuclease I (27).

Mouse mitochondria also contain UV endonuclease activity which can be resolved into three peaks that have similar enzymatic and physical properties to the enzymes purified and characterized from whole cell extracts of mouse MPC-11 cells (27). UV endonucleases I and II, initially described by Nes (18), have similar properties that they maybe derived from the same gene and differ by proteolysis or covalent modification. Surprisingly, however, the two forms appear to be present in all mammalian cell types examined. By analogy with the extramitochondrial forms of these enzymes, it is assumed that the mitochondrial analogue removes oxidative lesions such as thymine glycol from the mitochondrial genome. These lesions are generally found after the relatively large doses of UV radiation used here.

Extramitochondrial UV endonuclease III activity constitutes 7% of the total extramitochondrial UV endonuclease activity, whereas the mitochondrial form of UV endonuclease III constitutes 20—30% of the total mitochondrial UV endonuclease activity. Extracts from human fibroblasts also contain three UV endonuclease activities. UV endonuclease III activity is either absent or present at very reduced levels in XP group D fibroblasts and it seems probable that UV endonuclease III and AP endonuclease I, which is also deficient in XP group D cells (28), are associated with the same polypeptide. XP group D lymphoblasts contain little or none of the extramitochondrial UV endonuclease III and reduced levels of mitochondrial endonuclease III or a modified endonuclease, suggesting a relation between the two enzymes.

**REFERENCES**


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<th>Cell Type</th>
<th>Cytoplasmic</th>
<th>Mitochondrial</th>
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<tr>
<td></td>
<td>UV endo I &amp; II</td>
<td>UV endo I &amp; II</td>
</tr>
<tr>
<td>MPC-11</td>
<td>2300 (7.3%)</td>
<td>98 (22.8%)</td>
</tr>
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<td>35 (8.0%)</td>
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<td>activity, units</td>
<td>89 (0.7%)</td>
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<td>protein, mg</td>
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<td>35 (8.0%)</td>
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<td>XP-D Lymphoblasts (GM 2485A)</td>
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Data for human lymphoblasts is from the experiment described in Fig. 3, that for mouse MPC-11 cells is similarly derived as described in Methods.