Requirement for ERCC-1 and ERCC-3 Gene Products in DNA Excision Repair in Vitro

COMPLEMENTATION USING RODENT AND HUMAN CELL EXTRACTS

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Numerous rodent cell lines exist that have defects in nucleotide excision repair of DNA caused by alterations in genes that fall into 10 different complementation groups. The precise roles in the repair of these genes are unknown. We report here that extracts from Chinese hamster ovary cells of excision repair-defective complementation groups 1 and 3 are defective in DNA excision repair in a cell-free system. In vitro complementation can be achieved by mixing extracts from the two groups with one another. In addition, extracts from a human cell line representing xeroderma pigmentosum complementation group B could complement rodent complementation group 1 extracts, but not group 3 extracts. This is consistent with an identity of the ERCC-3 and xeroderma pigmentosum group B genes. Cellular evidence points toward a defect in the incision of damaged DNA in group 1 and 3 mutants. Since the ERCC-1 and ERCC-3 proteins are required for the in vitro reaction, it appears that both gene products are directly involved in the enzymatic incision of damaged DNA, or in preincision reactions. The experiments reported here provide the biochemical basis of an approach to analyze the function of these nucleotide excision repair proteins.

Cells use the versatile process of nucleotide excision repair to remove damaged segments of DNA from one strand of the double helix and to resynthesize a new segment. Nucleotide excision repair acts on a wide diversity of DNA lesions, including pyrimidine dimer photoproducts produced by UV irradiation and adducts produced by many chemical agents. The mechanistic details are not well-understood in mammalian cells, but the outline of the process can be visualized in a general way. A group of proteins cooperates to incise DNA at a site of damage, creating a short gap that is filled in by a DNA polymerase and sealed by a DNA ligase.

Many components of the excision repair system have been revealed indirectly through the analysis of mutant cell lines with aberrant DNA repair. These mutants have been derived from two sources: human syndromes associated with repair defects and damage-sensitive rodent cell lines isolated in the laboratory. In particular, cells from individuals with the sun-sensitive disorders xeroderma pigmentosum (XP) and Cockayne’s syndrome exhibit reduced DNA excision repair.

These relatively rare inherited conditions exhibit considerable genetic diversity, with seven complementation groups and a variant form in XP (A to G, and V) and three or more complementation groups in Cockayne’s syndrome (1–3).

In addition to these human mutants, hundreds of excision repair mutants have been isolated from rodent cell lines, mainly from Chinese hamster cells. These mutant lines have been assigned to 10 different complementation groups by analysis of cell hybrids (2, 4–9). Some of the groups appear to correspond to human XP groups, and some do not. The human genes that correct repair defects in rodent cells have been designated ERCC (excision repair cross-complementing) genes.

One approach to the analysis of excision repair has been by molecular cloning of the genes involved. By transfection of repair mutants with DNA from normal cells, sequences can be selected that confer mutagen resistance. Genes cloned in this way include XPAC (the XP-A correcting gene), ERCC-1, ERCC-5, ERCC-6, and two ERCC genes that are apparently equivalent to XP genes, ERCC-3/XPBC, and ERCC-2/XPDC (2, 10, 11). The predicted protein sequences of these genes have features that suggest that their products interact with DNA. However, despite hints of activities deduced from the DNA sequences, few biochemical functions have been firmly established, and proteins have yet to be isolated in an active form in sufficient quantities for study.

What are the possible functions of the different XP and ERCC gene products? Some of them must be enzymes involved in recognition of DNA damage and in endonucleolytic incision at damaged sites, analogous to the UvrABC proteins of Escherichia coli (12, 13). Others may play a role in the disassembly of chromatin to allow access for repair enzymes. Some proteins may be accessory factors that link transcription to DNA repair, for example by displacing transcriptional complexes so that repair can proceed or, conversely, by directing repair proteins to actively transcribed regions of DNA (14–16). It is also possible that some ERCC or XP genes could encode regulatory products that control the expression or activity of other repair genes.

In this paper, we report initial studies of the biochemical functions of two ERCC gene products. The experimental system is based on cell-free extracts that can mediate DNA excision repair in a strategy analogous to that previously used by other investigators to study DNA replication or transcription. DNA repair carried out by enzymes in soluble extracts from mammalian cell lines is assessed by monitoring the introduction of short patches of nucleotides into circular plasmids containing DNA adducts (17). Using this approach, it has been shown that human cell extracts can carry out repair synthesis in DNA damaged by UV light, psoralens, and platinating agents (17–24) and that this repair synthesis is...
localized to sites of DNA damage (25), where it leads to removal of DNA lesions (26). Extracts from cells belonging to the classical complementation groups of XP are deficient in repair replication in damaged circular DNA (17). Pursuing this observation, we have recently used biochemical complementation of XP-A cell extracts as an assay for purification of the XP-A repair protein (27).

Although a similar strategy should lead to the isolation of other XP proteins, the use of XP mutants alone will not yield insights into all the components of DNA repair. It seems essential to establish whether extracts from the many excision repair-deficient rodent cell lines and complementation groups can be used to aid in the isolation and study of functionally active DNA excision repair proteins. For the experiments reported here, we have chosen extracts from rodent lines representing two complementation groups with quite different characteristics, groups 1 and 3.

Group 1 mutants are very sensitive to UV light, to chemical agents that yield damage subject to excision repair, and to agents that produce DNA interstrand cross-links such as mitomycin C. The genes that can correct group 1 mutants, ERCC-1, have a predicted protein sequence that displays a conserved region, which suggests that the ERCC-1 protein may bind to DNA (28). The ERCC-1 gene does not appear to correspond to any of the XP genes (29).

Group 3 mutants are sensitive to UV light and many chemical agents, but are much less sensitive to mitomycin C than are group 1 mutants (30-32). ERCC-3 displays sequence similarity to characteristic domains in a superfamily of ATP-dependent DNA and RNA helicases (33). The ERCC-3 gene was recently reported to correspond to that defective in the rare XP group B (33).

We report here that extracts from cells of both groups 1 and 3 are defective in repair synthesis in soluble extracts; and thus, both the ERCC-1 and ERCC-3 proteins are required for the in vitro repair reaction. Restoration of repair synthesis by biochemical complementation can be achieved by mixing extracts from the two groups with one another. Furthermore, we demonstrate interspecies complementation by mixing extracts from human and rodent cells. The results provide a basis for the purification and assay of ERCC proteins in active form either from mammalian cell extracts or from sources where the cloned gene products are overexpressed.

**MATERIALS AND METHODS**

**Cell Lines**—The Chinese hamster ovary cell line AAS and the UV-sensitive mutant UV20 were obtained from Larry Thompson (Lawrence Livermore National Laboratory, Livermore, CA). Mutants UV4 and UV24 were obtained from David Busch (Armed Forces Institute of Pathology, Washington, D. C.). CHO-9 and the UV-induced mutants CHO 43-3B and CHO 27-1 were isolated by Wood and Burki (5). The human XP-B lymphoblastoid cell line GM2252A was obtained from the National Institute of General Medical Sciences Human Mutant Cell Repository (Coriell Institute, Camden, NJ).

All cell lines were tested and found to be free of Mycoplasma. In most cases, cells were grown in 900-cm² plastic roller bottles in E medium supplemented with 10% fetal calf serum. The attached cells were removed with trypsin/EDTA solution and washed twice in ice-cold phosphate-buffered saline without Ca²⁺ or Mg²⁺ before preparation of extracts. XP-B cultures were grown in悬浮 medium for 3 days, the plates were examined microscopically and stained with 1% methylene blue in 1% acetic acid. After exposure to these UV fluences, resistant cells (CHO-9 and AAS) remained attached to the plates, but virtually all cells from UV-sensitive lines were killed by 4 (or 8) J/m² and had detached from the growth surface.

**Cell-free Extracts**—Two-liter suspensions of cells in late exponential growth phase at 6-8×10⁶ cells/ml in 40-400-cm² roller bottles (3-4×10⁴ cells) were used as appropriate for each preparation. Extracts were prepared as previously described (17, 34). A Teflon pestle and glass homogenizer were used for disrupting human lymphoid cells. CHO cells were more difficult to lyse, even in hypotonic buffer, and required the use of a closer fitting ground glass homogenizer.

**Plasmid DNA**—The 2988-base pair plasmid pBluescript KS⁺ (Stratagene) and the related 3740-base pair plasmid pHM14 (35) were grown in E. coli host strain JM109. Purified pBluescript KS⁺ DNA was irradiated with 450 J/m² UV light (peak wavelength = 254 nm). Both plasmids were treated with Nt protein (purified by C. J. Jones in this laboratory), and closed-circular forms were extensively purified by sucrose gradient sedimentation as described (17, 36). pBluescript KS⁺ was modified with N-acetoxy-2-(acetylamino)fluorene (in parallel with mock-treated pHM14) according to published procedures (37) by Robert P. P. Fuchs (Institute of Molecular and Cellular Biology, Strasbourg, France).

**DNA Repair Synthesis**—Reaction mixtures (50 μl) contained (final concentrations) 250 ng of nonirradiated closed-circular pHM14and 250 ng of irradiated plasmid pBluescript KS⁺, 45 μM HEPES/KOH (pH 7.8), 70 mM KCl, 7.4 mM MgCl₂, 0.9 mM dithiothreitol, 0.1 mM EDTA, 20 μM each dGTP, dCTP, and TTP, 8 μM dATP, 74 kBq of [α-³²P]dATP (110 TBq/mmol), 2 μM dATP, 40 mM phosphocreatine, 3.4% bovine serum albumin, and 50-400 μg of extract protein. In each 50-μl reaction mixture, 20 μl consisted of extract dialysis buffer, and the composition of this buffer was taken into account in calculating the above concentrations of components. Reaction mixtures were incubated for 5 h at 30 °C. Under these conditions maximal synthesis in all reactions was reached by 2 h, after which no further repair events were initiated. We have found that the overall quality and reproducibility of results can be kept high by using a fresh stock of [α-³²P]dATP (<1 week old).

**Quantification of Repair**—Plasmid DNA was purified from the reaction mixtures as described (17), except that proteinase K digestion was carried out at 56 °C for 40 min after reactions with CHO extracts and before phenol extraction and ethanol precipitation of the DNA. The plasmids were linearized with BamHI and electrophoresed overnight on a 1% agarose gel containing 0.5 μg/ml ethidium bromide. Incorporation of [³²P]dAMP into the DNA was quantified by autoradiography and phosphorimaging scanning of the excised DNA bands and standardized for variation in DNA recovery by densitometry of a negative of a photograph of the ethidium bromide-stained gel. DNA standardization was sometimes aided by including [³²P]-labeled (4500 cpm) closed-circular pHM14 in each reaction. The pHM14 band was excised from the dried gel and solubilized (0.5 M HCl, 90 °C) before scintillation counting.

**RESULTS**

**DNA Repair Synthesis by Extracts from CHO Cell Lines**—DNA repair synthesis carried out by cell extracts is assessed by monitoring the introduction of short patches of nucleotides into damaged closed-circular plasmid DNA (17). A radiolabeled deoxyribonucleotide is included in the reaction mixture so that synthesis can be detected by autoradiography after agarose gel electrophoresis of the reaction products and quantified by measuring incorporation of radioactive material into damaged plasmid DNA. A slightly larger undamaged plasmid is present in each reaction as an internal control. Little or no incorporation into this plasmid occurs, provided the closed-circular DNA is carefully purified (36).

The first goal was to establish whether extracts from rodent complementation group 1 and 3 mutants were defective in repair synthesis in vitro. The cell lines utilized are summarized in Table I. Whole cell extracts were prepared from growing cells and examined for the ability to catalyze damage-dependent DNA repair synthesis in vitro. Extracts to be directly compared were prepared simultaneously to reduce possible experimental variations, and two to four separate sets of each parent/mutant pair were made on different occasions to con-
**TABLE I**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Group</th>
<th>Origin</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO-9</td>
<td>Group</td>
<td>CHO-KK wild type</td>
<td>5</td>
</tr>
<tr>
<td>CHO 43-3B</td>
<td>Group</td>
<td>Derived from CHO-9</td>
<td>5</td>
</tr>
<tr>
<td>CHO 83-95-1A</td>
<td>Group</td>
<td>Transfectant of CHO 43-3B with ERCC-1</td>
<td>39</td>
</tr>
<tr>
<td>CHO 27-1</td>
<td>Group</td>
<td>Derived from CHO-9</td>
<td>5</td>
</tr>
<tr>
<td>AA8</td>
<td>Group</td>
<td>CHO-K1 wild type</td>
<td>58</td>
</tr>
<tr>
<td>UV4</td>
<td>Group</td>
<td>Derived from AA8</td>
<td>4</td>
</tr>
<tr>
<td>UV20</td>
<td>Group</td>
<td>Derived from AA8</td>
<td>58</td>
</tr>
<tr>
<td>UV24</td>
<td>Group</td>
<td>Derived from AA8</td>
<td>4</td>
</tr>
<tr>
<td>GM2252</td>
<td>Group</td>
<td>Human lymphoblastoid</td>
<td>59</td>
</tr>
</tbody>
</table>

**Fig. 1.** DNA repair synthesis by extracts from CHO-9 cells and UV-sensitive mutant cells of repair complementation group 1. A, each reaction included 250 ng each of UV-irradiated pBluescript KS plasmid and undamaged pHM14. Experiments were prepared in parallel from CHO-9 or CHO 43-3B cells cultured in roller bottles, and extract protein was used as indicated. Upper, autoradiograph of dried agarose gel; lower, photograph of the ethidium bromide-stained gel showing the linearized plasmid DNA. B, quantification of the data from A. The graph shows femtomoles of dAMP incorporated into UV-damaged plasmid after normalization to account for minor variations in DNA recovery between samples. Extracts from CHO-9 or CHO 43-3B are shown. C, DNA repair synthesis carried out by extracts from group 1 mutant expressing human ERCC-1 gene. Reactions contained extract protein from CHO-9, CHO 43-3B, or CHO 83-95-1A cells. All three extracts were prepared from cells grown in suspension; CHO 43-3B and CHO 83-95-1A extracts were prepared in parallel.

Fig. 2. DNA repair synthesis by extracts from CHO-9 cells and mutant cells from complementation group 3. Reactions included extract protein from CHO 27-1 and CHO-9 cells as indicated. These extracts were prepared in parallel from cells cultured in suspension. Upper, autoradiograph of dried agarose gel; lower, photograph of the ethidium bromide-stained gel showing the linearized plasmid DNA.
by mixing extracts from cells of complementation groups 1 and 3. Each reaction included 250 ng each of UV-irradiated pBluescript KS plasmid and undamaged pHM14. The DNA content was approximately equal in all lanes; only the autoradiographs of the gels are shown for simplicity (see Table II for quantification of the data). In each lane, the total extract protein was kept constant at 200 µg. Upper, CHO 43-3B extract (left-most lane) mixed with CHO 27-1 extract (right-most lane); center, XP-B extract (left-most lane) mixed with CHO 43-3B extract (right-most lane); lower, CHO 27-1 extract (left-most lane) mixed with XP-B extract (right-most lane).

**TABLE II**

Quantification of data from Fig. 3

The numbers given are femtomoles of dAMP incorporated per reaction after normalization for the amount of DNA recovered per sample.

<table>
<thead>
<tr>
<th>Extract mixture</th>
<th>Extracts A + B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>260 + 125 + 100 + 75 + 0 +</td>
</tr>
<tr>
<td></td>
<td>0 µg 75 µg 100 µg 125 µg 200 µg</td>
</tr>
<tr>
<td></td>
<td>protein  protein  protein  protein  protein</td>
</tr>
<tr>
<td>A:CHO 43-3B + B:CHO 27-1 (B)</td>
<td>80 310 490 440 230</td>
</tr>
<tr>
<td>A:XP-B + B:CHO 43-3B</td>
<td>80 380 320 240 70</td>
</tr>
<tr>
<td>A:CHO 27-1 + B:XP-B</td>
<td>200 130 120 90 80</td>
</tr>
</tbody>
</table>

mixed with extracts from a human lymphoblastoid XP-B cell line that carries an alteration in the *ERCC-3* gene (Fig. 3, center). This demonstrates that interspecies complementation of excision repair between group 1 and 3 mutants is possible. However, no complementation was observed when extract from the *ERCC-3* mutant CHO 27-1 was mixed with human XP-B extract (Fig. 3, lower). Mixing of XP-B and CHO 27-1 extracts led only to the dilution of the residual synthesis in the CHO 27-1 extract, and no combination gave an activity greater than that achieved by 200 µg of CHO 27-1 extract protein alone. These *in vitro* results are thus consistent with genetic complementation experiments with intact fibroblast cells that have demonstrated that the *ERCC-3* gene is equivalent to the *XP-B* gene (33). In other experiments (not shown), complementation could be achieved by mixing extract from XP-A cells with extract from either CHO 43-3B or CHO 27-1 cells.

Quantification of the data in Fig. 3 gave the results summarized in Table II. The presence of ~200 µg of total extract protein in each reaction mixture was needed for reliable complementation with mixed extracts. Reactions with 100 µg or less of total extract protein showed little or no detectable complementation of damage-dependent repair synthesis (data not shown).

Mutants CHO 43-3B and CHO 27-1 were derived from the parental cell line CHO-9. Most other existing UV-sensitive mutants were derived from the AA8 cell line, and so extracts of such cells were tested for a deficiency in repair synthesis. Group 1 mutants UV4 and UV20 and Group 3 mutant UV24 all showed significantly less synthesis than the parental line AA8. Data from a representative experiment are included in Fig. 4A. We note that a background of UV-dependent synthesis occurs to some extent in reactions with all extracts from group 1 and 3 cell lines. Some of this background may reflect residual DNA excision repair, but the precise origin of this synthesis is not clear and is discussed further below. It is minimized at a concentration of 70 mM KCl in the reaction mixture. Reproducibly, more residual damage-dependent synthesis was observed with extracts of mutant cells derived from the AA8 cell line (UV4, UV20, and UV24) than with extracts of mutant cells derived from the CHO-9 cell line (CHO 43-3B and CHO 27-1).

Further mixing experiments to verify and extend the complementation results were carried out with extracts of mutant cell lines derived from the CHO AA8 cell line. Extremes from two independently derived ERCC-1 mutants could not complement one another, but could complement ERCC-3 mutant extracts (Fig. 4). Repair synthesis with extracts from mutants UV4, UV20, and UV24 ranged from 15 to 40% of repair synthesis with an equivalent amount of AA8 extract protein. In repeated parallel experiments, mixtures of group 1 and 3 cell extracts always gave 65–70% of the synthesis carried out by the same amount of AA8 extract protein in the same experiment. Additionally, repair-deficient extracts derived from mutants of either the CHO-9 or AA8 cell line gave consistent results when mixed with one another. A mixture of equal amounts of CHO 43-3B (group 1) extract with UV4 (group 1) extract showed no complementation of repair synthesis, but mixing 100 µg of UV24 (group 3) extract with 100 µg of CHO 43-3B extract gave repair synthesis above that achieved by 200 µg of either extract alone (Fig. 4B).

The repair synthesis activity exhibited by the extracts from UV-sensitive mutants of CHO cells therefore seems to reflect, in a general way, the repair capacity of the cells. The complementation experiments suggest that each extract is deficient in a human lymphoblastoid XP-B cell line that carries an alteration in the *ERCC-3* gene (Fig. 3, center). This demonstrates that interspecies complementation of excision repair between group 1 and 3 mutants is possible. However, no complementation was observed when extract from the *ERCC-3* mutant CHO 27-1 was mixed with human XP-B extract (Fig. 3, lower). Mixing of XP-B and CHO 27-1 extracts led only to the dilution of the residual synthesis in the CHO 27-1 extract, and no combination gave an activity greater than that achieved by 200 µg of CHO 27-1 extract protein alone. These *in vitro* results are thus consistent with genetic complementation experiments with intact fibroblast cells that have demonstrated that the *ERCC-3* gene is equivalent to the *XP-B* gene (33). In other experiments (not shown), complementation could be achieved by mixing extract from XP-A cells with extract from either CHO 43-3B or CHO 27-1 cells.

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Complementation of DNA Repair with CHO Cell Extracts

It is possible that this residual synthesis arises because the ERCC-1 and ERCC-3 products in the particular mutant alleles used have activity that is reduced, but not abolished. This seems unlikely because measurements with cells show that the residual repair activity as estimated by unscheduled DNA synthesis is as low or lower in UV20 or UV24 cells as it is in CHO 43-3B or CHO 27-1 cells (38, 41, 42). Additionally, both UV4 and UV24 mutants were isolated after treatment of AA8 cells with the DNA-intercalating compound ICR-170, an agent that almost exclusively causes frameshift mutations, and such mutations often abolish protein function. We believe that the residual synthesis arises in UV-irradiated DNA from a process independent of ERCC-1 or ERCC-3.

We were interested to determine whether the residual repair synthesis in extracts from UV-sensitive mutants was confined to UV-damaged DNA or whether it occurred with other types of damaged DNA as well. The latter appears to be the case. For example, when plasmid containing AAF adducts was used in reactions, a background of residual DNA synthesis was found with UV24 extracts and a lower background with CHO 43-3B extracts, reflecting the results obtained with UV-irradiated DNA (Fig. 6). Thus, the “background” DNA synthesis in the UV-sensitive mutant extracts seems to be a general phenomenon that is not confined only to UV-irradiated DNA.

One possibility is that residual damage-dependent repair synthesis in mutant extracts is initiated by an S1 nuclease-type enzyme that adventitiously introduces nicks in regions of damaged DNA where the double helix is partially destabilized (43, 44).

**DISCUSSION**

The results presented here show that extracts from the mutagen-sensitive complementation groups 1 and 3 of CHO cells are deficient in repair synthesis in cell-free extracts. This demonstrates that both the ERCC-1 and ERCC-3 gene products are required for the reaction *in vitro* with damaged plasmid DNA. Restoration of repair synthesis by biochemical complementation can be achieved by mixing extracts from the two groups with one another, and interspecies complementation can take place between extracts from human and CHO cells.

The amount of damage-dependent repair synthesis catalyzed by extracts from the repair-proficient parental CHO-9 and AA8 cell lines is at least as great as that seen with human cell extracts in terms of the total femtomoles of nucleotides incorporated per reaction. Previous experiments using enzymatic photoreactivation of DNA indicated that repair synthesis *in vitro* principally represents repair of (6-4)pyrimidine dimer photoproducts (18, 22), and so the present data are consistent with measurements showing that CHO and human cells in culture remove (6-4)photoproducts from DNA at similar rates (45).

These experiments place restrictions on the possible func-
Complementation of DNA Repair with CHO Cell Extracts

... of the ERCC-1 and ERCC-3 products in DNA repair. In the cell-free extracts, the plasmid DNA substrates are not replicated or transcribed. Thus, it is unlikely that the major function of either ERCC-1 or ERCC-3 is to couple DNA repair with replication or to act as a transcriptional or translational regulator. Furthermore, since very little of the plasmid substrate is assembled into chromatin during incubations with whole cell extract under conditions favorable for repair synthesis (24, 27, 46), the principal function of ERCC-1 or ERCC-3 is probably not in a chromatin disassembly process to allow access for other repair enzymes. In fact, it has recently been reported that when damaged plasmid DNA is first efficiently packaged into chromatin and then incubated with repair-proficient whole cell extracts, repair synthesis is severely inhibited (24). A formal possibility would be that one of the gene products is a post-translational regulator for another repair gene product, for example a protein kinase (47). However, the protein sequences predicted by the DNA sequences of the two ERCC genes suggest that both polypeptides interact directly with DNA.

In the case of ERCC-1, the predicted sequence shows a centrally located region of amino acids having a high degree of conservation with the predicted sequence of the *Saccharomyces cerevisiae* excision repair protein RAD10. Yeast cells with a defective RAD10 gene are defective in the incision of DNA after ultraviolet irradiation (48). It has been noted that the conserved sequence could form a DNA-binding domain of the helix-turn-helix type (28, 49). Thus, it seems likely that ERCC-1 associates directly with DNA during the excision repair process. A striking feature of CHO complementation group 1 mutants is their extreme hypersensitivity to DNA cross-linking agents in comparison to most other complementation groups. Repair of DNA interstrand cross-links is thought to involve a recombinational strand-exchange step in other organisms (50–52), and so it is possible that ERCC-1 is also involved in a recombinational repair of DNA damage in mammalian cells. A possible recombinational role seems even more likely in view of the recently discovered involvement of RAD10 in mitotic recombination in yeast (53).

A limited term, acidic sequence similarity of ERCC-1 to both the *E. coli* UvrA and UvrC DNA excision repair proteins has been noted (28, 54, 55). However, it has been pointed out that any similarity of ERCC-1 to UvrC is of doubtful significance because the relevant region of UvrC is not conserved between the otherwise closely related *E. coli* and *Bacillus subtilis* UvrC polypeptides, even though the latter two proteins can functionally substitute for one another (56).

For ERCC-3 protein, the most informative hint of function is provided by DNA sequence data that show the occurrence of sequential domains conserved among a family of DNA and RNA helicases (33). This feature is shared in common with other DNA repair genes, including *ERCC-2* (10) and *ERCC-6,* and with the RAD3 protein, a known ATP-dependent helicase (57).

Elucidation of the precise roles of the ERCC-1 and ERCC-3 proteins in nucleotide excision repair of DNA must await the purification of these proteins in active form. The work presented here suggests a realistic way to assess the activity of these proteins. The ability of CHO cell extracts to complement one another to allow DNA repair in *vitro* should permit purification of the proteins from appropriate sources following the sort of procedure recently used to successfully purify active XP-A protein (27). In preliminary experiments, we have found that fractionated extracts from cultured HeLa cells or calf thymus can complement group 1 and 3 extracts. We are currently attempting to complement these extracts with protein produced by expressing the corresponding cDNAs in bacteria.

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Complementation of DNA Repair with CHO Cell Extracts