Evidence for a repair enzyme complex involving ERCC1 and complementing activities of ERCC4, ERCC11 and xeroderma pigmentosum group F


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Communicated by D.Bootsma

Nucleotide excision repair (NER), one of the major cellular DNA repair systems, removes a wide range of lesions in a multi-enzyme reaction. In man, a NER defect due to a mutation in one of at least 11 distinct genes, can give rise to the inherited repair disorders xeroderma pigmentosum (XP), Cockayne's syndrome or PIBIDS, a photosensitive form of the brittle hair disease trichothiodys trophy. Laboratory-induced NER-deficient mutants of cultured rodent cells have been classified into 11 complementation groups (CGs). Some of these have been shown to correspond with human disorders. In cell-free extracts prepared from rodent CGs 1–5 and 11, but not in a mutant from CG6, we find an impaired repair of damage induced in plasmids by UV light and N-acetoxy-acetylaminofluorene. Complementation analysis in vitro of rodent CGs is accomplished by pairwise mixing of mutant extracts. The results show that mutants from groups 2, 3, 5 and XP-A can complement all other CGs tested. However, selective non-complementation in vitro was observed in mutual mixtures of groups 1, 4, 11 and XP-F, suggesting that the complementing activities involved somehow affect each other. Depletion of wild-type human extracts from ERCC1 protein using specific anti-ERCC1 antibodies concomitantly removed the correcting activities for groups 4, 11 and XP-F, but not those for the other CGs. Furthermore, we find that 33 kDa ERCC1 protein sediments as a high mol. wt. species of ~120 kDa in a native glycerol gradient. These results strongly suggest the presence of a pre-existing enzyme complex in mammalian cell extracts, harbouring at least the products of the ERCC1 and ERCC4 genes. This complex also carries complementing activities of XP group F and rodent CG11. We postulate that the complex, like the one in Saccharomyces cerevisiae involving the RAD1 and RAD10 proteins (the latter being the homologue of ERCC1), functions in both NER and recombinational repair.

Key words: Chinese hamster mutant/complementation analysis/damaging agents/nucleotide excision repair/repair complex

Introduction

Nucleotide excision repair (NER) is a DNA repair system that has evolved in all living organisms for the removal of lesions inflicted on DNA by mutagenic radiations (notably UV light) or numerous chemical agents. It is a multi-enzyme process involving specific recognition and dual incision of the damaged strand, followed by lesion removal, gap-refilling and strand ligation (Grossman and Yeung, 1990; Selby and Sancar, 1990; Hoeijmakers, 1991). In man, genetic defects in NER of UV-induced DNA damage give rise to the severe disorders xeroderma pigmentosum (XP), Cockayne's syndrome (CS) or PIBIDS. Besides hypersensitivity of the skin to sunlight and progressive neurological dysfunction, a dramatically increased risk of skin cancer (XP) or developmental abnormalities (CS) entail the clinical hallmarks of these disorders (Cleaver and Kraemer, 1989; Nance and Berry, 1992). PIBIDS is a photosensitive form of the brittle hair disorder trichothiodystrophy (Lehmann, 1987). Somatic cell hybridization studies have revealed extensive genetic heterogeneity: in XP, at least seven NER-defective complementation groups (named A to G) exist whereas in CS two (CS-A and CS-B) have been identified so far (Lehmann, 1982; Vermeulen et al., 1991) and two complementation groups have been found in PIBIDS patients (Stefanini et al., 1992, 1993).

In addition to these naturally occurring mutants, a large collection of laboratory-induced, UV-sensitive, NER-defective mutants have been obtained from cultured rodent cells. By restoration of UV resistance in cell hybrids, 11 complementation groups (CGs) were discerned among these mutant cell lines (for recent overviews see Thompson et al., 1988; Busch et al., 1989; Stefanini et al., 1991; Zdzienicka et al., 1991; Riboni et al., 1992; Collins, 1993). Cross-sensitivity to chemicals producing bulky DNA lesions is a feature of the UV-sensitive mutants in all groups. However, representatives from groups 1 and 4 are unique with regard to the property of extreme sensitivity of DNA crosslinking agents, such as mitomycin C (Busch et al., 1989).

A number of excision repair cross-complementing (ERCX) genes, capable of correcting the NER defects in the rodent CGs have been isolated using gene transfer strategies. Characterization has provided clues to the function of their gene products and their involvement in human disease (for reviews see Hoeijmakers and Bootsma, 1990; Friedberg, 1992; Hoeijmakers, 1993). For instance, based on sequence motifs, both the genes ERC2 and ERC3 are expected to encode proteins with helicase activity. These genes are now known to be involved in XP-D and XP-B, respectively (Weeda et al., 1990; Flejter et al., 1992; Weber, cited in Lehmann et al., 1992). A third putative helicase, encoded by ERC6, is required for preferential NER of actively transcribed DNA segments and is defective in CS group B (Troelstra et al., 1992). The ERC5 gene was recently found to be responsible for XP-G (O'Donovan and Wood, 1993; Scherly et al., 1993). The ERCC1 protein is not associated with the defect in any of the XP, CS or PIBIDS groups (Van Duin et al., 1989). On the other hand, the XPAC protein is affected in XP-A (Tanaka et al., 1989), but in none of
the rodent CGs 1–7 or CG 11 (our unpublished results).

As another outcome of the characterization of NER genes it has become clear that they are highly conserved in evolution. Thus, homologues for most cloned mammalian NER genes could be identified in lower eukaryotes, particularly in yeast. As examples, the ERCC1, 2, 3 and 5 genes share extensive sequence homology at the protein level with the RAD10, RAD3, RAD25/SSL2 and RAD22 genes of Saccharomyces cerevisiae, respectively (Van Duin et al., 1986; Weber et al., 1990; Gulyas and Donahue, 1992; Park et al., 1992; Scherly et al., 1993). Therefore, it is likely that the basic features of the NER mechanism are conserved from yeast to man as well and that the homologues have functional equivalence. Both yeast and mammalian studies indicate that some NER genes play a role in other cellular processes. For instance, RAD1 and RAD10 are required for mitotic recombination (Schiestl and Prakash, 1988, 1990) and recently a direct involvement of the ERCC3 protein in transcription initiation was found (Schaeffer et al., 1993).

A cell-free in vitro assay of mammalian NER is another important tool to elucidate the functions of the NER gene products at the enzymological level. The in vitro system measures repair synthesis in UV-irradiated plasmid DNA mediated by isolated human cell extracts (Wood et al., 1988). The assay can mimic in vitro complementation analysis of XP extracts (Hansson et al., 1991; Coverley et al., 1992) and can detect repair of bulky lesions induced by chemical agents (Hansson et al., 1989; Sibghat-Ullah et al., 1989; Sibghat-Ullah, 1990). It has served to assess the topology of the early endonucleolytic step of human NER (Huang et al., 1992), the involvement in NER of additional protein factors such as PCNA and SSB/RP-A (Coverley et al., 1991, 1992; Nichols et al., 1992; Shivi et al., 1992) and the purification of XPAC protein from calf thymus (Robins et al., 1991).

Here, we report the results of a systematic study of NER in UV-sensitive mutants of various rodent CGs using the in vitro assay. In attempting in vitro complementation analysis, we encounter selective absence of correction, when cell extracts from representatives of groups 1, 4, and 11 are mixed. Results from antibody depletion experiments and sedimentation analysis indicate that these specific patterns of in vitro non-complementation can be explained by the presence of a high mol. wt repair enzyme complex, comprising at least the products of the ERCC1 and ERCC4 genes. In addition, the factors able to complement XP group F and CG11 are associated with this complex.

Results

**In vitro repair activity in Chinese hamster cell extracts**

Two cell-free extracts, prepared from wild-type Chinese hamster cells (CHO9) and from a NER-deficient strain belonging to CG1 (43-3B), were compared with respect to their ability to mediate repair synthesis in vitro using UV-irradiated plasmid DNA (450 J/m²) as a substrate. As shown in Figure 1A, UV-dependent repair synthesis was observed in vitro, with only a very slight incorporation of 32P-labelled dATP in the non-damaged plasmid. With these substrates no significant difference in repair synthesis was detected between a wild type (CHO9) and a CG1 mutant extract (43-3B).

Besides pyrimidine dimers and 6-4 photoproducts UV induces oxidative thymine species as a minor UV lesion, which is efficiently recognized by a NER-independent endonuclease activity present in human extracts. Plasmids can be cleared from such damage by treatment with the Escherichia coli Nth protein (Wood et al., 1988). Using a ‘cleaned’ UV-irradiated substrate [UV(nth)] a clear difference between the repair activities of wild-type CHO9 and mutant 43-3B extracts became apparent (Figure 1B). Similar results were detected with plasmids damaged by N-acetoxy-2-acetylaminofluorene (AAF) (Figure 1C), which causes almost exclusively N-(guanine-8-yl) adducts (Landegent et al., 1984). Apparently, the endonuclease-triggered activity is very potent in CHO extracts, necessitating the use of AAF or UV(nth) substrates to measure NER activity. The observed levels of repair synthesis of human and Chinese hamster repair-proficient protein extracts, when expressed as fmol 32P incorporated per ng plasmid, were in the same range and linearly dependent on the amount of extract added (data not shown).

As in CG1, a clear defect in in vitro NER is present in the tested rodent CG2, 3, 4, 5 and 11, as well as in XP-A and XP-F (Table I), in agreement with their inability to incise UV damage in vivo. In an extract derived from CG6 a normal response was observed, in line with the near normal

![Fig. 1](image-url)
level of unscheduled DNA synthesis and incision in cultured cells (Thompson et al., 1988). Some variation of residual activity in separate extracts of these NER-deficient mutants was found, ranging from 15 to 30% of the wild-type response. We conclude that NER can be detected readily in vitro using rodent cell-free extracts and that this repair requires the active presence of the products of the ERCCI, 2, 3, 4, 5 or 11 genes, in addition to those of XPAC and XPFC.

**In vitro complementation analysis**

Since in vitro complementation with human extracts can be achieved by mixing representatives of different CGs of XP (Wood et al., 1988), it is expected that mutant extracts from various rodent CGs should complement each other as well. To determine the complementation patterns of the various Chinese hamster CGs in more detail, CG1, 2, 3, 4, 5 and 11 were tested, together with human CGs XP-A and XP-F. In view of the normal levels of repair activity in vitro, CG6 could not be used for analysis.

First, extracts from CG1 and 2 or CG1 and 3 were combined in various proportions and in vitro NER was measured. A substantial, ratio-dependent increase of repair synthesis was found with maximal repair activity around a mixing ratio of 1:1 (Figure 2). Restoration of repair synthesis was observed in the damaged plasmids only. Some variation in different experiments was experienced, but the presence of correction was unequivocal.

Then, proportional mixing experiments were conducted with other CGs. The results from a number of typical tests are shown in Figure 3 and the complete data set is summarized in Table II. Clear complementation was observed in any mutual combination of CG2, 3, 5 and XP-A and in combination with extracts from CG1, CG4 and CG11. Unexpectedly, pairwise mixing of representatives of CG1, CG4 or CG11 did not yield levels of repair synthesis significantly different from those in the deficient extracts alone (Figure 3). Similarly, XP-F extracts failed to complement CG1, 4 and 11, whereas clear correction was observed with CG2 and CG3. This pattern of non-complementation was consistently observed using independent isolates and with different representatives of CG1 and CG4 (Table II). These results indicate absence of correction in all combined extracts of CG1, CG4, CG11 or XP-F.

**Apparent molecular weight of ERCCI1 protein on glycerol gradients**

The consistent pattern of non-complementation in vitro between groups 1, 4, 11 and XP-F suggests that the corresponding proteins somehow affect each other; the presence of a defect in one has an effect on the activities of the others. Such a situation may occur, for instance, when these gene products form a protein complex in vivo, of which the subunits cannot exchange under the conditions of the in vitro assay. To investigate this possibility, a NER-proficient HeLa extract was size-fractionated on a 10–30% glycerol gradient. To minimize effects of buffer and salt the gradient was run in the conditions of the repair assay together with internal mol. wt markers. The presence of ERCCI1 protein in gradient fractions was monitored by SDS–PAGE and immunostaining with a specific anti-ERCCI1 antiserum (for characterization of the antiserum see below). ERCCI1 runs on SDS gels with an apparent mol. wt of 39 kDa, which is larger than the calculated mol. wt of 33 kDa. This is probably due to the proline-rich N-terminus, since truncated ERCCI1 missing the N-terminal segment migrates at the
predicted position (data not shown). The profile of the glycerol gradient depicted in Figure 4 showed that, under native conditions, the protein sediments as a uniform band between the 67 and 149 kDa mol. wt markers, peaking on the position corresponding to ~120 kDa. Although nonspecific aggregation of the ERCC1 protein cannot be completely excluded, the overall protein patterns analysed on SDS–PAGE did not show any indication of this. These results are consistent with the idea that ERCC1 resides in a complex.

**ERCC1 protein produced by E.coli fails to correct CG1 extracts in vitro**

Human ERCC1, overproduced in E.coli as a ubiquitin–ERCC1 fusion protein, was tested for in vitro complementation. Details of the overexpression constructs will be published elsewhere (M.H.M.Koken, H.Odijk, M.Van Duin, M.Fornérod and J.H.J.Hoeijmakers, in preparation). The ubiquitin moiety can be removed either by a specific ubiquitin lyase (UBP1 protein) from S.cerevisiae (Tobias and Varshavsky, 1991) or by ubiquitin-specific lyase activity present in Manley-type extracts incubated under the conditions of the repair assay (Figure 5A, lanes 1–6). The free full-length ERCC1 protein migrated at 39 kDa in SDS–PAGE (Figure 5A) at the same position as ERCC1 protein synthesized in a reticulocyte lysate in vitro (Figure 5C, lane 6).

Purified cleaved ERCC1 protein or fusion protein (either crude or partially purified) was added in excess to CG1 extracts. In none of these cases was significant correction observed. The presence of inhibiting factors in the ERCC1 protein preparation was ruled out by adding these proteins also to repair-competent extracts. It appears that in these in vitro conditions ERCC1 protein on its own cannot participate in the repair reaction.

**Characterization of anti-ERCC1 antibodies**

To study the possibility that ERCC1 is involved in a complex, we needed to do experiments at the enzymological level. Therefore a polyclonal antiserum was raised in rabbits against the ubiquitin–ERCC1 protein and affinity-purified on a column carrying immobilized fusion protein. The antisera were characterized in the following ways. First, Western blot analysis visualized a protein band with the exact mol. wt of ERCC1 on SDS–PAGE in crude lysates and Manley-type extracts of HeLa cells (Figure 5D, lanes 4, 5, 7 and 8). This band was not recognized by the preimmune serum (Figure 5D, lanes 1 and 2) and the reaction could be competed by partially purified ERCC1 fusion protein.

| Table II. Summary of in vitro complementation dataa |
|---------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| CG– 1 | Strain | XP-F | XP-A | 11 UVs1 | 5 UV135 | 4 UV41 UV47 | 3 27-1 | 2 UV5 |
| 1 | 43-3B | – | + | – | + | – | – | + |
| 2 | UV5 | – | – | – | + | – | – | + |
| 3 | 27-1 | + | – | – | + | + | + | + |
| 4 | UV41 | + | – | – | + | – | – | + |
| 5 | UV135 | – | – | – | – | – | – | – |
| 11 | UV51 | – | – | – | – | – | – | – |
| XPA | XP12RO | + | | | | | | |

*+, complementation; –, non-complementation; nd, not determined.
No cross-reaction was observed with ubiquitin or with the ERCC1 protein from bovine or Chinese hamster origin (Figure 5D, lanes 6 and 9). Secondly, the antiserum was able to immunoprecipitate 35S-labelled ERCC1 protein, synthesized in vitro by reticulocyte lysate (Figure 5C, lanes 2-5). Finally, a complete abolition of UV-induced unscheduled DNA synthesis was observed after micro-

\[ \text{M=25} \quad \text{M=67} \quad \text{M=147} \]

Fig. 4. Mol. wt determination of ERCC1 by sedimentation analysis. Active HeLa extract was layered, together with mol. wt standards, on a non-denaturing glycerol gradient and sedimented. The markers are chymotrypsinogen A (25 kDa), albumin (67 kDa) and aldolase (147 kDa). Gradient fractions were analysed on SDS-PAGE to determine the marker positions and immunoblotted to assess the position of ERCC1.

injection of the antibodies into repair-proficient human fibroblasts, whereas the preimmune serum did not have any effect (data not shown). These results indicate that the antibodies are able to react specifically with ERCC1 in vivo as well as in vitro.

**ERCC1 antiserum depletes a repair-competent HeLa extract from ERCC1, 4, 11 and XPFC**

In view of the species specificity of the antiserum, a NER-competent extract from HeLa cells was used to study the effect of removal of ERCC1 protein in the in vitro repair reaction. To this end antiserum was immobilized on protein A-Sepharose beads prior to incubation with the HeLa extract. After removal of the beads by centrifugation the supernatant exhibited a significantly decreased in vitro NER activity (Figure 6A). No such effect was detected when preimmune serum was used even at a 50× higher concentration (Figure 6B). We conclude that ERCC1 protein can be removed from a human wild-type extract using specific antibodies, resulting in a lowered repair activity comparable with the level of a CG1 mutant.

The HeLa extract depleted of ERCC1 (HeLa-ERCC1) was

\[ \text{A} \quad \text{1 2 3 4 5 6 7 m} \]

\[ \text{B} \quad \text{1 2 3 4} \]

\[ \text{C} \quad \text{1 2 3 4 5 6} \]

\[ \text{D} \quad \text{1 2 3 4 5 6 7 8 9} \]

Fig. 5. Production of ubiquitin-ERCC1 fusion protein and characterization of anti-ERCC1 antibodies. (A) SDS-PAGE analysis of crude soluble lysate of ERCC1-overproducing E.coli incubated with increasing amounts of HeLa Manley-type extract. Lanes 1–6, lysate reacted for 2 h with 0, 15, 30, 45, 60 or 75 μg of HeLa extract; lane 7, 75 μg of HeLa extract alone; lane m, mol. wt markers: phosphorylase b (97 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20 kDa) and α-lactalbumin (14 kDa). (NB: the released ubiquitin of 8 kDa has run off the gel.) (B) SDS-PAGE analysis of lysate from ERCC1 fusion protein, partially purified by 33% saturated ammonium sulfate precipitation. Lanes 1–2, not treated with lysis; 0.7 and 35 μg of lysate; lanes 3–4, pre-treated with purified UBI1-lysate, 0.7 and 35 μg of lysate. (C) SDS-PAGE and autoradiography of immune-precipitated [35S]ERCC1 translated in vitro. Lane 1, preimmune serum, undiluted; lane 2–5, crude immune serum, diluted 1×, 10×, 100× and 1000×, respectively; lane 6, input amount of [35S]ERCC1. (D) Specificity of ERCC1 antiserum on immunoblots of different cell extracts. Lanes 1–3, preimmune serum; lanes 4–6, crude immune serum; lanes 7–9, affinity-purified anti-ERCC1 antibodies; amounts used are equivalent to lanes 4–6. The extracts analysed are total HeLa cell sonicate (lanes 1, 4, and 7). Manley-type extracts of HeLa (lanes 2, 5 and 8) or CHO9 (lanes 3, 6 and 9).
Fig. 6. Depletion of HeLa extract using anti-ERCC1 antibodies. Serum was immobilized on protein A-Sepharose beads; after incubation with repair-competent HeLa extract and centrifugation, the supernatant was tested for repair activity in vitro. (A) 10 µl of serum was used for depletion of 150 µg HeLa extract. Lane 1, undepleted extract (100 µg); lane 2, extract treated with empty protein A beads (90 µg); lane 3, extract depleted with pre-immune serum (80 µg); lane 4, extract depleted with immune serum (110 µg). (B) Various concentrations of sera were used to deplete 150 µg of HeLa extract. Lanes 1–4, anti-ERCC1 serum (50, 15, 5 and 1 µl respectively); lanes 5–8, pre-immune serum (50, 15, 5 and 1 µl respectively).

then tested for the ability to correct NER-deficient Chinese hamster mutants in mixing experiments. As expected, no complementation was detected when HeLa-ERCC1 was mixed with a representative of CG1. However, when an extract of CG2, CG3, CG5 or XP-A was added to HeLa-ERCC1 a significantly higher repair synthesis was observed (Figure 7A and C), indicating that the antibodies cannot remove the correcting activities of these groups. In contrast, HeLa-ERCC1 failed to restore repair in mixtures with extracts of CG4, CG11 and XP-F (Figure 7B, A and D), showing that in addition to ERCC1 protein, correcting activities of CG4, CG11 and XP-F are concomitantly removed from the wild-type extract. Both crude and affinity-purified antisera were equally capable of depleting HeLa extract from ERCC1, 4, 11 and XPFC.

Discussion

In vitro repair studies in Chinese hamster NER-deficient mutants

In agreement with findings of others (Biggerstaff and Wood, 1992) we have observed that extracts isolated from cultured Chinese hamster cells can support NER of DNA damage in vitro. DNA substrates with a well-defined and narrow damage spectrum can be used in this assay, provided they are extensively purified. Clearance of UV-damaged plasmids from oxidative thymine species by pretreating with bacterial Nth protein proves to be an absolute requirement when Chinese hamster cell extracts are used, due to an apparently high endonuclease III-like activity in these cells.

The NER deficiency of CG1, 2, 3, 4, 5 and 11 in vitro agrees with the low unscheduled DNA synthesis levels (Van Duin et al., 1988) and low incision capabilities of these cell strains in culture. In CG6, which is only moderately sensitive to UV, the repair activity is in the range of NER-proficient extracts. The ERCC6 gene is defective in CS-B (Troelstra et al., 1992), where preferential repair of actively transcribed strands is impaired, but overall genome repair, which is responsible for the bulk of repair synthesis, is unaffected (Venema et al., 1990). The normal in vitro repair rates are likely to reflect overall repair, since transcription is not taking place in the reaction conditions. Our finding that, in vitro, human and hamster repair activities are similar, contrasts with the low activity of overall repair in cultured rodent cells in comparison with human cells (Bohr and Hanawalt, 1987; Lommel and Hanawalt, 1991). It follows that the factors responsible for this difference do not play a role in the in vitro reaction, perhaps because they are related to chromatin structure.

Extracts of CG2, 3 and 5 are able to complement each other and representatives of all other CGs tested. This suggests that the proteins which are missing or defective in these CGs are available in the complementing extracts and can freely interact to ensure restoration of normal repair activity. The findings that XP-F can correct CG2 and CG3 and that XP-A complements CG3 and CG5 show that human and Chinese hamster repair proteins are sufficiently conserved and interchangeable in vitro. This is in line with the correction in vivo by transfection of human genes into the Chinese hamster mutants.

Evidence for a repair enzyme complex

Mixtures of CGs 1, 4, 11 and XP-F do not show restoration of repair activity in vitro, whereas they do in vivo after cell hybridization. Since non-complementation occurred with independent mutants from CG1 and CG4, this unexpected phenomenon is unlikely to be related to an allele-specific behaviour. Our data indicate that the normal gene products needed for correction are either unable to exchange in vitro or are not present in the mixtures. Such a situation can occur
when these correcting proteins are part of a pre-existing stable complex. Depletion of a wild-type extract using specific anti-ERCC1 antibodies not only results in the loss of CG1 complementing activity, but concomitantly removes the activity which is capable of correcting CG4 and CG11 or even XP-F. This strongly suggests that such a complex does exist in wild-type extracts. This is further supported by the finding that human ERCC1 protein, which has an apparent mol. wt of 39 kDa in denaturing SDS gels, sediments in glycerol gradients as a much larger species.

The absence of complementation in some combinations indicates that the in vitro assay cannot replace classical cell fusion as a system for complementation analysis. To explain these non-complementation patterns in vitro, several mechanisms can be envisaged. (i) Reconstitution of a complex from its components may not be possible under in vitro conditions. An inactive complex harboring a tightly bound defective protein in one mutant extract will be unable to complement another mutant, in which the same complex is crippled by the presence of another impaired component. (ii) Alternatively, or in addition, dependent on the type of mutation in the cell strain, the complex may be required for stability of its components in vivo. In that case cell-free extracts from CG1 lacking ERCC1 would be also deficient in ERCC4 and the other proteins, and vice versa. Indirect support for this idea comes from studies with 41D, a CG1 cell strain containing an amplified correcting human ERCC1 gene. Whereas at DNA and mRNA levels ERCC1 is amplified in this strain 100- to 1000-fold, the amount of ERCC1 protein is enhanced only by a factor of three and many degradation products become apparent on immunoblots with anti-ERCC antibodies. In addition, micro-injection into human fibroblasts of an excess of ERCC1 protein, over-produced in E.coli, results in unusually rapid (<1 h) degradation in vivo as judged by immunofluorescence (our unpublished results). These data suggest that ERCC1 protein on its own may not be very stable in the cell, which is in agreement with our observation that addition of an excess of purified ERCC1 protein fails to restore repair activity in CG1 extracts.

Involvement of the xeroderma pigmentosum group F gene product

Extracts from a human XP-F cell strain fail to complement Chinese hamster extracts of CG1, CG4 and CG11 in vitro. This pattern of non-complementation is group specific, as efficient correction of repair is found with other CGs. In addition, the XP-F extract is not able to correct the ERCC1-depleted HeLa extract, indicating that the XPFC-correcting activity is also part of the postulated complex. Well-controlled cell fusions in vivo between human XP-F cells and Chinese hamster NER-deficient mutants, being complicated by poor interspecies fusion and chromosomal loss in the resulting hybrids, have not been done so far, so there is no in vivo correlation of our in vitro results. Direct involvement of ERCC1 in XP-F is ruled out by the inability of the ERCC1 gene to correct XP-F cells in vivo (Van Duin et al., 1989). Therefore, ERCC4 and ERCC11 are possible candidates for the gene defective in XP-F. This would represent another overlap between Chinese hamster mutants and human repair disorders. Alternatively, XPFC might also encode a distinct protein, which may be less likely in view of gradient sedimentation data.

The relation between ERCC4 and ERCC11

The complex between the ERCC1 and ERCC4 proteins also contains the correcting activities of CG11 and XP-F. The latter two mutants do not show the extreme sensitivity to DNA crosslinking agents characteristic for representatives of CG1 and 4. However, absence of mitomycin C sensitivity has also been noted in some ERCC1 mutants, whereas the sensitivity to UV has been retained (J Van de Berg and J.H.J.Hoeijmakers, unpublished results). It is possible that such relatively mild mutations are present in CG11 and XP-F as well. Since the strain UV51 is the sole representative of CG11 isolated so far, one has to consider also the theoretical possibility that the complementation in cell hybrids of CG11 with CG1 or CG4 mutants is of the intra-allelic type. Our (unpublished) observation that transfected ERCC1 cDNA is unable to correct UV51 cells definitely rules out involvement of ERCC1. On the other hand, complementation has been shown after cell fusion with UV51 and the two independent CG4 mutants UV47 (Hata et al., 1991) and UV41 (D.Busch, personal communication), which makes intra-allelic complementation less likely. Although such patterns of complementation have never been observed in the numerous fusions between either Chinese hamster or human (XP) repair-deficient mutant cells, a definitive answer must await the isolation of the ERCC4 or ERCC11 gene and its transfection into the reciprocal mutants.

Possible functions of the complex

The homologue of ERCC1 in S.cerevisiae is the RAD10 gene (Van Duin et al., 1986). This protein can form a very stable complex with the RAD1 gene product, both in vivo and in vitro (Bailly et al., 1992; Bardwell et al., 1992), explaining the similar phenotypes of the yeast RAD1 and RAD10 mutants, having a defect in NER as well as in mitotic recombination (Schiestl and Prakash, 1988, 1990). Also ERCC1 and ERCC4 mutants share unique features, such as extreme sensitivity to crosslinking agents. Therefore, it is conceivable that the RAD1 - RAD10 complex and the mammalian repair complex identified here are equivalent and have similar functions. The studies in yeast do not exclude the possibility that additional proteins are part of the RAD1 - RAD10 complex, which is relevant in view of our findings that ERCC11 and/or XPFC correcting activities also appear to be present in the mammalian complex. In this idea, the yeast RAD1 gene becomes a good candidate for being the homologue of one of the mammalian genes ERCC4, ERCC11 or XPFC.

Combining these data, we postulate that the mammalian enzyme complex has a dual function, as has its homologue in yeast. Deficiency of the NER function results in a 5- to 10-fold increased sensitivity to UV light and other chemical agents inducing bulky DNA adducts. Such a sensitivity is also found in XP-A cells, where NER is fully impaired due to the complete inactivation of the XPAC protein (Tanaka et al., 1990). On top of the moderate sensitivity for crosslinks (such as found in XP-A cells), the additional participation of ERCC1 and ERCC4 proteins in a recombinational repair pathway might explain the extreme (80- to 100-fold) sensitivity of the corresponding mutants to crosslinking agents. Obviously, interstrand crosslinks may require additional recombinational steps for their removal.


Received on April 14, 1993; revised on May 17, 1993.