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Expression of the cDNA for the beta subunit of human casein kinase II confers partial UV resistance on xeroderma pigmentosum cells

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Summary

An immortalized xeroderma pigmentosum cell line belonging to the complementation group D (XP-D) was transfected with a normal human cDNA clone library constructed in a mammalian expression vector. Following UV-irradiation–selection, a transformant having a stable, partially UV-resistant phenotype was isolated. A transfected cDNA of partial length was rescued from the transformant's cellular DNA by *in vitro* amplification, using expression-vector specific oligonucleotides as primers in a polymerase chain reaction (PCR). Expression of this cDNA complemented the UV sensitivity of the XP-D cell line to the UV-resistance levels characteristic of the primary transformant. The nucleotide sequence of the cDNA was determined. The deduced protein identified the cDNA as encoding for the beta subunit of casein kinase II (CKII- β). Similar to the effect exerted by the truncated CKII- β cDNA, expression of a cDNA clone encompassing the complete translated region of CKII- β leads to XP-D cells partially resistant to UV-irradiation. However, transfection of CKII- β cDNA could also partially complement the UV-sensitivity of a xeroderma pigmentosum cell line belonging to group C (XP-C). Analysis by Southern, Northern and RNAase mismatch cleavage techniques did not reveal any functional defect in the CKII- β gene of cell lines derived from either 7 XP-D or 10 XP-C families. We therefore consider it unlikely that either the XP-D or the XP-C DNA repair deficiency is associated with a defect in the beta subunit of casein kinase II. Nevertheless, our findings suggest the possibility that the cell's response to DNA damage is modulated by CKII-dependent protein phosphorylation.

Xeroderma pigmentosum (XP) syndrome is a generic term for a closely related group of autosomal recessive diseases which constitute today the best studied mutations in DNA repair in hu-

mans. XP is manifested by early onset of severe photosensitivity of the exposed regions of the skin and eyes, a very high incidence of skin cancer and frequent neurological abnormalities. The primary defect in most XP patients is an inability to perform the first step (incision) of excision-repair of UV-induced lesions in DNA (Cleaver, 1968). Genetic studies involving cell fusion have identi-

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fied to date 8 complementation groups, termed XP-A through XP-H, within the excision-deficient class of XP patients (Friedberg, 1985). A claim for the existence of a 9th complementation group has recently been retracted (Bootsma et al., 1989). Exceptionally, the XP 'variant' (XP-V) was thought to be defective in post-replication repair, while having normal levels of excision repair. However, employing a cell-free extract system, it was recently reported that XP-V cells are also deficient in excision repair (Wood et al., 1988), suggesting that at least 9 gene products are involved in excision repair. It is noteworthy that the full clinical manifestations of XP may not be solely attributable to DNA-repair defects. This was initially claimed by Lambert and Lambert (1985), and their argument was recently refined by Lehmann and Norris (1989), who propose that the XP syndrome constitutes recessive mutations at two independent loci, one controlling DNA repair, the other regulating natural killer cell activity.

Because of its complexity, little has been elucidated about the mechanism or genetic control of excision-repair in man, or for that matter in higher eukaryotes in general. Approaches that have been employed in attempts to identify those genes or gene products that are defective in the XP syndrome include: stable phenotypic complementation of mutant cells by gene transfer; transient phenotypic complementation by microinjection; use of permeabilized cell systems; and use of *in vitro* systems.

One of the major difficulties in the use of DNA-mediated transfer for the molecular cloning of those DNA-repair genes which are defective in XP patients, is that human cells, in the absence of continuing selective pressure, retain significantly lower amounts of transfected DNA than do rodent cells (Colbere-Garapin et al., 1987; Hoeijmakers et al., 1987; Mayne et al., 1988; Lohrer et al., 1988). Nevertheless, the molecular cloning of a mouse DNA which partially complements the XP-A defect (Tanaka et al., 1989) and of a hamster DNA which partially restores UV resistance to XP-D cells (Arrand et al., 1989) have recently been reported. Two human genes, ERCC-1 (Westerveld et al., 1984) and ERCC-2 (Weber et al., 1988) were cloned following complementation of

CHO excision-repair mutants groups 1 and 2 (new nomenclature), respectively. However, neither of these two genes can complement any of the XP genetic groups.

Our earlier studies have shown that transfection with a human cDNA clone library incorporated in a mammalian expression vector produced a UV-resistant (UV^R) phenotype (Teitz et al., 1987) and DNA excision-repair proficiency (Stark et al., 1989) in an immortalized XP-C cell line. Rescue by polymerase chain reaction identified a cDNA whose product specifically complemented the XP-C recipient cell line to the same UV-resistance levels typical of the secondary transformant from which the cDNA was rescued (Teitz et al., 1990). We now report that, using a similar approach, the UV sensitivity of an immortalized XP-D cell line was partially complemented. This step was followed by rescue and molecular cloning of a cDNA whose expression increased the UV survival of XP-D as well as XP-C derived cell lines. DNA sequencing followed by comparative sequence analysis revealed that the complementing cDNA is the product of the human gene for the beta subunit of casein kinase II.

Materials and methods

Cell culture, transfection, selection of transductants and UV-irradiation

Conditions for cell growth were as outlined previously (Teitz et al., 1987). Except for the SV40-immortalized XP-C GM2096-SV3 and XP-D GM434-MH3 cell lines (Canaani et al., 1986; Wood et al., 1987; respectively) all other cell lines were obtained from the Human Genetic Mutant Cell Repository (Camden, NJ). Details of transfection with the expressible cDNA library and the selections that followed are outlined in Results. All the other transfections were performed by a modification of the calcium phosphate protocol, using 20–30 µg plasmid DNAs per 5×10^6 cells (Chen and Okayama, 1987). To increase the efficiency of stable co-transfer of genes, a 10-fold molar excess of the tested plasmid over the *neo*-containing plasmid was employed in each of the co-transfections. Selection for G418-resistant colonies was carried out as previously described (Canaani and Berg, 1982). In every transfection, at

least 40 independent transfectants were isolated, individually grown and assayed for UV-resistance as described (Teitz et al., 1987). Each transfection experiment was repeated at least twice and usually 3 times.

In vitro amplification of cDNA containing sequences

The polymerase chain reaction was done in 100 μ l reaction volume, starting with 1 μ g of total chromosomal DNA as outlined (Kogan et al., 1987; Chehab et al., 1987). The three 30-mer oligonucleotide primers used are SV40 DNA sequences which constitute part of the pcD vector, as follows: 5'-TCCCTCGAGGGATCCCCTG-GTTCTTTCCGC-3' (primer 1, has the antisense polarity and flanks the cDNA on the 3'-end); 5'-CCCGGATCCGGTGGTGGTGCAAATCAA-AGA-3' (primer 2); and 5'-TTGACAGCTTAT-CATCGATAAGCTTGGTGG-3' (primer 3). Primers 2 and 3 both have the sense polarity and are located 5' to the cDNA, as shown in Fig. 3.

40 cycles of amplification were performed manually with the addition of 4 units *Taq* DNA polymerase (New England Biolabs) every 10 cycles. Each cycle consisted of 1 min denaturation at 92°C, 1 min annealing at 55°C and 5 min extension at 63°C. The first denaturation step lasted for 7 min and the last extension step took 10 min. The products of the amplification were analyzed by electrophoresis on 1.4% agarose gel.

Northern and primer extension analysis

Total cellular RNA was prepared from either cultured cell lines or peripheral blood samples, according to Cathala et al. (1983). Electrophoresis of total RNA (20 μ g per lane) through agarose-formaldehyde gels and transfer to nitrocellulose paper (S and S BA 85) was carried out as described by Antoniou et al. (1986). These Northern blots were probed with a ^{32} P uniformly-labeled antisense RNA synthesized with T3 RNA polymerase from a Bluescript KS vector containing a p50 derived cDNA insert (Melton et al., 1984).

To map the 5'-termini of the CKII- β mRNA, the primer extension method was employed (Calzone et al., 1987). The template for the reverse transcription was total RNA (20 μ g) extracted from the SV40-transformed normal fibroblast cell line GM637. The oligonucleotide primer (30 ng)

was an antisense 30-mer located between nucleotide numbers 73 and 44 of the cDNA displayed in Fig. 6, and having the sequence: 5'-CAGAAGAA-TTCATTGCCACGGAGCCCACAG-3'. 300 units of M-MLV reverse transcriptase (BRL) were employed, with 2.5 μ l of [α - ^{32} P]dATP (3000 Ci/mmol, 10 mCi/ml) as internal label, in a 25 μ l reaction volume. Following elongation for 90 min at 42°C, the reaction products were extracted by phenol, precipitated by ethanol and, following re-suspension, were separated on a 6% denaturing polyacrylamide gel.

RNAase A cleavage analysis

Several overlapping DNA fragments derived from the CKII- β cDNA clone p16 were subcloned into the Bluescript KS vector within the polylinker region. Single-stranded radioactive RNA probes were synthesized from 1 μ g of these linearized DNA plasmids, using 10 units of T3 RNA polymerase (Stratagene) in a protocol similar to that formulated by Melton et al. (1984). Total cellular RNA (20 μ g) from each of the indicated cell lines was hybridized to a ^{32} P uniformly-labeled antisense RNA ($5-9 \times 10^5$ c.p.m.) for 16 h at 45°C, essentially as described by Winter et al. (1985). Hybridized RNAs were digested at 30°C with RNAase A (40 μ g/ml) and RNAase T1 (2 μ g/ml) for 60 min and then electrophoresed through denaturing polyacrylamide gels. After drying, the gels were exposed for autoradiography overnight.

Results

Complementation of the UV-sensitive (UV^S) phenotype of an XP-D cell line

The recipient used in the gene transfer experiments was the GM434-MH3 cell line generated by Wood et al. (1987), by immortalization of the XP-D GM434 fibroblasts with the SV40-containing plasmid pSV-7 (Colby and Shenk, 1982). This cell line was co-transfected with mpSV2-*neo* (Canaani and Berg, 1982) and a human cDNA library from SV40-transformed normal human fibroblasts (GM637), constructed in the pcD mammalian expression vector (Okayama and Berg 1983). Transfection of about 2.8×10^6 cells was accomplished using the calcium phosphate precipitation method as modified by Chen and

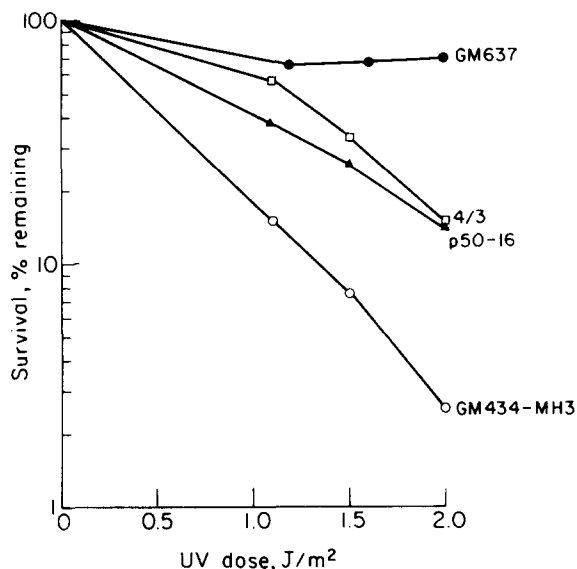


Fig. 1. UV-sensitivities of SV40-transformed fibroblasts from a normal control (GM637), an XP-D patient (GM434-MH3), and two transformants of the latter: 4/3, derived by transfection with a human cDNA clone library, and p50-16, generated by transfection with plasmid p50 (PCR-rescued insert in pcD vector). UV survival was measured as described by Teitz et al. (1987).

Okayama (1987) with an estimated 20-fold molar excess of the cDNA library (pcDGM637) over the selectable marker-containing plasmid. Transfection was followed by selection for G418 resistance (provided by the co-transfected *neo* gene). The efficiency of transformation to G418 resistance was 6.6×10^{-3} . The surviving colonies were trypsinized, replated and then selected for UV resistance by exposure to several consecutive UV-irradiation dosages at intervals of 1–2 days. The UV dose employed (4 J/m^2) is lethal to the GM434-MH3 recipient cells but causes little damage to normal human fibroblasts. From the initially transfected cell population there was only one surviving colony with a stable UV^R phenotype, indicating a minimal efficiency of transformation to UV-resistance of 3.5×10^{-7} . UV survival measurements (Fig. 1) showed that this transformant (4/3) had acquired partial but significant UV-resistance which was stable and at least 5-fold higher than that of the XP-D recipient cell line GM434-MH3. Control experiments with the GM434-MH3 cell line transfected by mpSV2-

neo plasmid alone, followed by selection for G418 and UV-radiation resistance, did not give rise to any UV^R colonies.

Transfected vector sequences in the chromosomal DNA of the UV^R XP-D transformant

Hybridization to Southern blots of low-molecular-weight DNA present in a Hirt extract of transformant 4/3 failed to detect any sequences (less than one copy per cell) unique to the transfecting pcD vector. However, the high-molecular-weight DNA of clone 4/3 contained transfected pcD DNA sequences. Fig. 2A shows Southern blots of *Hind*III-cleaved chromosomal DNA derived from the XP-D recipient GM434-MH3 cell line (lane 1) and its transformant 4/3 (lane 2), after hybridiza-

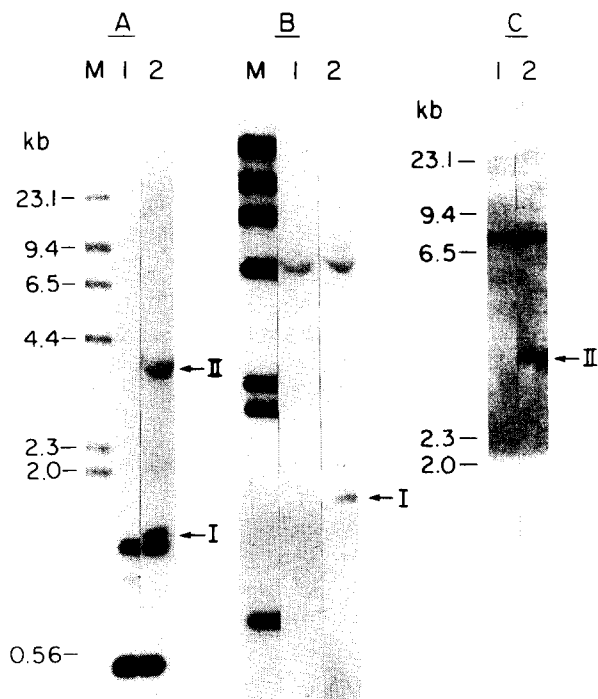


Fig. 2. DNA sequences homologous to the pcD vector and to rescued transfected cDNAs in *Hind*III-cleaved high-molecular-weight DNA of an SV40-immortalized XP-D GM434-MH3 cell line, and its transfected UV^R cell line 4/3 (indicated as 1 and 2, respectively). The southern blots were hybridized to the following ³²P-labeled probes: (A) the pcD's *Xho*I–*Pst*I fused introns 0.165-kb fragment (see Fig. 3); (B) *Hinc*II–*Pst*I 0.17-kb fragment from pL1's cDNA; (C) the 0.2-kb *Hae*III fragment from the PCR-rescued p50's cDNA. The arrows indicate the position of transfected DNA sequences I and II. The marker (M) is a *Hind*III-cleaved lambda phage DNA.

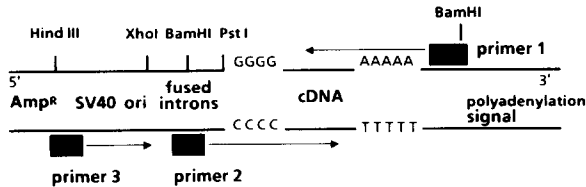


Fig. 3. Schematic representation of the pcD vector around the cDNA region, and the location of the oligonucleotide primers used for the *in vitro* amplification by the polymerase chain reaction (marked by filled boxes with the arrow indicating the direction of new DNA synthesis). Primer 1 has the antisense polarity while primers 2 and 3 both have the sense polarity. The pcD plasmid vector has been described in detail elsewhere (Okayama and Berg, 1983).

tion to ^{32} P-labeled SV40 fused intron DNA sequences (this pcD vector-derived segment, shown in Fig. 3, is not present in the co-transfecting mpSV2-*neo* plasmid). Two *Hind*III-cleaved SV40 DNA fragments, corresponding to 0.65–0.86 and 0.86–0.945 SV40 map units, derived from the immortalizing plasmid pSV-7, were present as multiple copies in the XP-D GM434-MH3 recipient cell line (Fig. 2A, lane 1). However, the high-molecular-weight DNA of transformant 4/3 contained two additional fragments, indicated as bands I and II (Fig. 2A, lane 2) which hybridized to the pcD-derived SV40 DNA probe. This suggests that, if no DNA rearrangements impair the linkage between cDNA and its flanking vector sequences, cell clone 4/3 has only two transfected cDNA molecules integrated into its genome.

Rescue and molecular cloning of a cDNA which confers UV-resistance upon an XP-D cell line

Initially, we rescued an integrated pcD-cDNA-like molecule from 4/3 cells following their fusion with COS cells (Breitman et al., 1982). The cDNA insert of this plasmid (pL1) hybridized to a transfected DNA fragment previously indicated as band I (Fig. 2B, lane 2). When expressed in GM434-MH3 cells, however, this cDNA had no biological activity. Sequence analysis of pL1 cDNA demonstrated the presence of multiple termination codons for translation in all three reading frames (our unpublished data). Thus, the cDNA within plasmid pL1 represents either a pseudogene or the 3' untranslated region of an mRNA.

In order to rescue the integrated cDNA sequences represented in band II in Fig. 2A, we attempted to amplify them *in vitro* (Saiki et al., 1985) using pcD-vector-specific oligonucleotides as primers for the polymerase chain reaction. Three oligonucleotides have been synthesized to serve as primers for the *Taq* DNA polymerase (Saiki et al., 1988); two of them are localized 5' and one 3' to the cDNA (Fig. 3). Each primer is a 30-mer containing several recognition sites for restriction endonucleases, thus allowing for subsequent cloning of the amplified DNA segment. Fig. 4 shows the products of *in vitro* amplification, using as a template genomic DNA of GM434-MH3 cells (lane a) and genomic DNA of transformant 4/3 (lanes b and c). When primers 1 and 2 were used with the *Taq* DNA polymerase, several fragments were amplified from the XP-D parental cell line (Fig. 4, lane a) while primarily one pronounced DNA

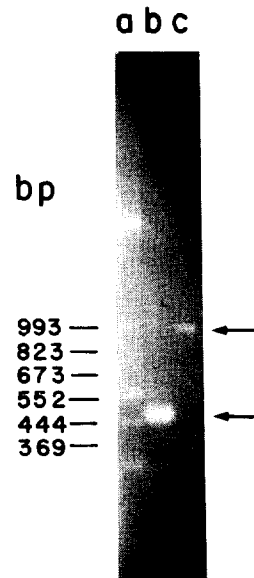


Fig. 4. Agarose gel electrophoresis of PCR-amplified DNA from control GM434-MH3 genomic DNA (a) and the UV^R transfectant 4/3 chromosomal DNA (b and c). DNA was amplified with *Taq* DNA polymerase with primers 1 and 2 (a and b) and with primers 1 and 3 (c). Following 40 cycles of amplification, 15- μ l aliquots from the amplification mixtures were subjected to electrophoresis on 1.4% agarose gel. The gel was stained with ethidium bromide and photographed under UV light. The arrows point to the major DNA fragments amplified from the 4/3 genomic DNA. The marker is SV40 DNA cut by *Bst*NI restriction endonuclease.

fragment with an approximate size of 500 bp was amplified from the transformant 4/3 (Fig. 4, lane b, lower arrow). Southern blot hybridization, performed under stringent conditions, revealed that this DNA fragment contained the portion of the SV40-fused introns sequence bounded by the *Bam*HI and *Pst*I restriction endonuclease sites displayed in Fig. 3 (data not shown). When the PCR reaction on 4/3 genomic DNA was performed with primers 1 and 3, a distinct DNA fragment was observed (Fig. 4, lane c, upper arrow) of a size about 450 bp larger than the one recovered earlier (Fig. 4, lane b). Since this increase reflects the distance between primers 2 and 3 (Fig. 3), it seems reasonable to assume that the same cDNA was amplified in both cases. The products of an in vitro amplification reaction with primers 1 and 2, using genomic DNA of clone 4/3 as a template, were isolated, digested with *Bam*HI restriction endonuclease and separated by agarose gel electrophoresis. The mobility of the major amplified DNA segment showed no detectable change following *Bam*HI cleavage. It was extracted from the gel and cloned in the pcD vector between the *Bam*HI restriction endonuclease sites shown in Fig. 3.

A plasmid (p50) containing the amplified cDNA inserted in the proper orientation for expression was co-transfected with a pRSV-*neo* plasmid (Gorman et al., 1983) into the XP-D GM434-MH3 cell line. Following selection for G418 resistance, individual transformants were picked out, grown to mass culture and tested for acquisition of UV resistance by UV-survival measurements (Teitz et al., 1987). Fig. 1 shows a typical survival curve of one of the stable UV^R transformants (p50-16); it is significantly more UV-resistant than cells of the parental XP-D GM434-MH3 line, with resistance levels similar to those exhibited by the primary transformant 4/3. The frequency of stable UV^R clones among the G418-resistant p50 transformants was 10%. This cotransfer value is relatively low, compared to corresponding values in rodent cells, but reflects the extreme instability of transfected DNA in human cells (Hoeijmakers et al., 1987; Colbere-Garapin et al., 1987; Mayne et al., 1988; Lohrer et al., 1988). Hybridization of a ³²P-labeled insert from p50 cDNA to *Hind*III-cleaved GM434-MH3 (Fig. 2C, lane 1) and 4/3

genomic DNA's (Fig. 2C, lane 2) revealed that this cloned cDNA insert is homologous to the transfected cDNA designated as band II (Fig. 2A, lane 2). We therefore concluded that the expression of the cloned cDNA contained within p50 is responsible for the UV-resistant phenotype observed in transformant 4/3.

DNA sequencing and RNA analysis

Sequencing of the in vitro amplified DNA segment in p50 revealed the expected nucleotide sequences of primers 1 and 2, the pcD sequences between primers and the cDNA, and a 277 base-pair cDNA insert which includes a poly(dA) tail of 35 bases. This cDNA has three open reading frames, but none of them has an initiating ATG codon. The first reading frame has an internal ATG codon, followed by a sequence encoding 20 amino acid residues. Alternatively, the same reading frame (encoding 33 amino acids) can potentially be expressed as part of a fused protein initiating at an ATG codon located 60 nucleotides upstream from the cDNA's start site, in the SV40 16S intron sequence. Such a transcript can still use the SV40 late 19S intron for splicing (Okayama and Berg, 1983). To assess the size of the corresponding mRNA in normal human fibroblasts and XP derived cells, Northern blot analysis of total RNA was carried out. A 0.2 kb *Hae*III segment from the cDNA of p50 was subcloned into Bluescript KS plasmid vector, permitting the generation of a uniformly ³²P-labeled antisense RNA probe (Melton et al., 1984).

As shown in Fig. 5A, hybridization of this probe to Northern blots containing total RNA from immortalized normal human fibroblasts GM637 (lane 1) or XP-D GM434-MH3 fibroblasts (lane 2) revealed a single species of mRNA, of a size close to 1.0 kb. Analysis of RNA from 6 additional XP-D families failed to reveal any significant change in this particular mRNA's size or abundance, in comparison to normal counterparts (data not shown).

In order to isolate a longer cDNA clone, the p50 derived 0.2 kb *Hae*III segment was uniformly labeled by random primer extension (Feinberg and Vogelstein, 1983) and used to screen the original pcD-GM637 human cDNA library (Okayama and Berg, 1983). This led to the recovery of a

plasmid clone (p16) with an insert of about 850 bp. Several overlapping restriction fragments covering the insert in p16 were subcloned into Bluescript KS vector, and sequenced on both strands, using the dideoxynucleotide chain termination method. The complete sequence of the cDNA insert in p16, excluding the oligo(dG) linker to the pcD vector and the poly(dA) tail, is shown in Fig. 6. This sequence, of 793 bp, contains only one large open reading frame encoding a deduced protein of 215 amino acid residues, initiated by an ATG codon located five base-pairs from the 5' end (Fig. 6). The predicted protein is

in the same frame as the first of the three possible open reading frames of p50 cDNA. Therefore, the putative polypeptide encoded within p50 cDNA to which we alluded previously constitutes the carboxy terminal part of this predicted protein. Inspection of the 3'-region of p16 cDNA shows an untranslated region of 170 bp (Fig. 6). The poly(dA) tail is preceded, 15 nucleotides upstream, by the common polyadenylation signal AATAAA. The cDNA clone present within p16 probably does not represent the full-length copy of the corresponding mRNA. This was suggested initially by the revelation by sequence analysis of a putative nontranslated leader of only five base pairs (Fig. 6).

To obtain a better estimate of the size of this mRNA, a primer extension experiment was carried out (Fig. 5B). The template for the reverse transcriptase was total RNA extracted from GM637 cells, and the oligonucleotide primer was an antisense 30-mer located between nucleotide numbers 73 to 44 in Fig. 6. Two extension products were seen following separation on a sequencing gel (Fig. 5B). Their estimated length of 180 and 175 bases puts the 5' termini at 107 and 102 nucleotides upstream of the 5'-end of the recovered cDNA clone. This suggested that the normal mRNA consists of about 900 bases, excluding the post-transcriptionally-added poly(A) tail. This mRNA may have more than one initiation site clustered in one region of the gene, as found for quite a number of eukaryotic mRNAs. Alternatively it is also possible that the shorter extension product in Fig. 5B results from premature termination by the reverse transcriptase.

The cDNA responsible for UV-sensitivity-complementation codes for the human CKII- β subunit

To determine whether the cloned cDNA is homologous to other eukaryotic or prokaryotic genes, a computer search of the Genbank database was carried out. Significant homology was found with the nucleotide sequence of the *Drosophila* casein kinase II beta subunit (Saxena et al., 1987). The deduced 215 amino acid sequence of the *Drosophila* protein was aligned in a gap-free manner with the predicted 215 amino acid human sequence, displaying 88% sequence homology. Moreover, of the 209 amino acid residue sequence

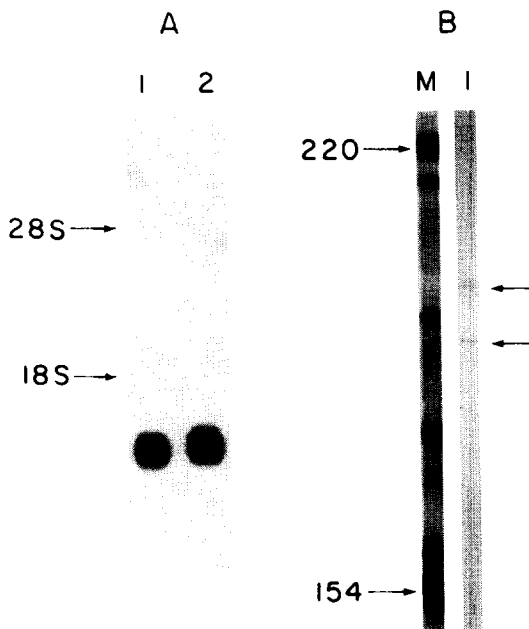


Fig. 5. Characterization of the mRNA represented in plasmid p50. 20 μ g of total RNA from the SV40-transformed normal fibroblasts GM637 (A1 and B1) and the XP-D, GM434-MH3 (A2), were analyzed by northern blot (A) and primer extension methods (B). The probe for the Northern blot was a radioactively labeled antisense RNA synthesized with T3 RNA polymerase from a linearized Bluescript KS vector containing the 0.2-kb p50 *Hae*III fragment. The positions of the 18S and 28S rRNA are indicated. Extension of an antisense 30-mer primer located near the 5'-end of the cDNA shown in Fig. 6 is displayed in lane 1 of (B). The template was total RNA extracted from the normal GM637 cell line; extension was performed with M-MLV reverse transcriptase. The extension products were separated on a 6% acrylamide-urea gel and are indicated by the two arrows. Two of the fragments derived from pBR322 DNA cut by *Hinf*I, which served as a radioactive marker, are shown in lane M of (B).

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          45
TG AAG ATG AGC AGC TCA GAG GAG GTG TCC TGG ATT TCC TGG TTC TGT GGG CTC CGT GGC AAT GAA TTC TTC TGT GAA GTG GAT GAA GAC
   MET Ser Ser Ser Glu Glu Val Ser Trp Ile Ser Trp Phe Cys Gly Leu Arg Gly Asn Glu Phe Phe Cys Glu Val Asp Glu Asp

90          (A)          135          (C)
TAC ATC CAG GAC AAA TTT AAT CTT ACT GGA CTC AAT GAG CAG GTC CTT CAC TAT CGA CAA GCT CTA GAC ATG ATC TTG GAC CTG GAG CCT
Tyr Ile Gln Asp Lys Phe Asn Leu Thr Gly Leu Asn Glu Gln Val Phe His Tyr Arg Gln Ala Leu Asp Met Ile Leu Asp Leu Glu Pro

180          225
GAT GAA GAA CTG GAA GAC AAC CCC AAC CAG AGT GAC CTG ATT GAG CAG GCA GCC GAG ATG CTT TAT GGA TTG ATC CAC GCC CGC TAC ATC
Asp Glu Glu Leu Glu Asp Asn Pro Asn Gln Ser Asp Leu Ile Glu Gln Ala Ala Glu Met Leu Tyr Gly Leu Ile His Ala Arg Tyr Ile

270          315
CTT ACC AAC CGT GGC ATC GCC CAG ATG TTG GAA AAG TAC CAG CAA GGA GAC TTT GGT TAC TGT CCT CGT GTG TAC TGT GAG AAC CAG CCA
Leu Thr Asn Arg Gly Ile Ala Gln Met Leu Glu Lys Tyr Gln Gln Gly Asp Phe Gly Tyr Cys Pro Arg Val Tyr Cys Glu Asn Gln Pro

360          405
ATG CTT CCC ATT GGC CTT TCA GAC ATC CCA GGT GAA GCC ATG GTG AAG CTC TAC TGC CCC AAG TGC ATG GAT GTG TAC ACA CCC AAG TCA
Met Leu Pro Ile Gly Leu Ser Asp Ile Pro Gly Glu Ala Met Val Lys Leu Tyr Cys Pro Lys Cys Met Asp Val Tyr Thr Pro Lys Ser

450          495
TCA AGA CAC CAT CAC ACG GAT GGC GCC TAC TTC GGC ACT GGT TTC CTT CAC ATG CTC TTC ATG GTG CAT CCC GAG TAC CGG CCC AAG AGA
Ser Arg His His His Thr Asp Gly Ala Tyr Phe Gly Thr Gly Phe Pro His Met Leu Phe Met Val His Pro Glu Tyr Arg Pro Lys Arg

540          585
CCT GCC AAC CAG TTT GTG CCC AGG CTC TAC GGT TTC AAG ATC CAT CCG ATG GCC TAC CAG CTG CAG CTC CAA GCC GCC AGC AAC TTC AAG
Pro Ala Asn Gln Phe Val Pro Arg Leu Tyr Gly Phe Lys Ile His Pro Met Ala Tyr Gln Leu Gln Leu Gln Ala Ala Ser Asn Phe Lys

630          675
AGC CCA GTC AAG ACG ATT CGC TGA TTC CCT CCC CCA CCT GTC CTG CAG TCT TTG ACT TTT CCC TTC TTT TTT GCC ACC CTT TCA GGA ACC
Ser Pro Val Lys Thr Ile Arg TER

720          765
CTG TAT GGT TTT TAG TTT AAA TTA AAG GAG TCG TTA TCG TGG TGG GAA TAT GAA ATA AAG TAG AAG AAA AGG CC

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Fig. 6. Nucleotide and encoded amino acid sequence of human CKII- β cDNA. The sequence of the coding strand of the cDNA insert of p16 is shown, oriented in the 5' to 3' direction. The deduced amino acid sequence of the largest open reading frame is indicated below the nucleotide sequence. The termination codon for translation of the deduced protein is indicated as TER and the polyadenylation signal is underlined. The polymorphic nucleotides revealed by RNAase A mismatch cleavage (Fig. 7) and determined by DNA sequencing are shown in brackets above the sequence of p16's cDNA.

of the bovine lung CKII- β , determined by protein sequencing (Takio et al., 1987), a continuous stretch of 208 residues is identical to the predicted human enzyme sequence. In comparison with the bovine protein sequence the predicted human sequence is longer by three amino acid residues at the amino terminus and by two residues at the carboxy end. The position of the initiating methionine was putatively assigned (Fig. 6), based on comparison with the bovine sequence on the one hand and the fact that the first 9 amino terminal residues were MSSSEEVSW in both *Drosophila* and the deduced human enzyme on the other. This assignment is supported by the presence of a purine (adenosine) at position -3, three nucleotides upstream from the ATG codon. According to Kozak (1986), this is the most conserved feature of the CC_G^ACCAUGG consensus sequence for translation initiation in eukaryotes. The calculated molecular weight of the native unmodified human beta subunit is 24914 dalton. This value is close to the value of 25000 dalton estimated for the human enzyme (Boivin and Garland, 1979). Initially, we reported our findings in

Canaani et al. (1989). Following the completion of this work, the molecular cloning and sequencing of a full-length cDNA encoding the CKII- β subunit has been reported (Jakobi et al., 1989) confirming both our putative assignment of the amino terminus and the deduced amino acid sequence for the beta subunit.

Extent and specificity of phenotypic complementation

Transfection of the cDNA segment for CKII- β contained within p50 conferred only partial UV resistance upon GM434-MH3 XP-D cells. To test whether increased resistance could be obtained by expression of the native CKII- β protein, the same XP-D cell line was co-transfected by p16 DNA and pRSV-*neo* DNA. The UV-resistance levels of several of the G418^R transfectants were very similar to those displayed by clone p50-16 (Fig. 1) harbouring p50 DNA sequences (data not shown).

To determine the specificity of the UV-sensitivity-complementation, p50 DNA was co-transfected with pRSV-*neo* DNA into the XP-C recipient cell line GM2096-SV3 (Canaani et al., 1986).

TABLE 1
UV-RESISTANCE OF XP-C TRANSFORMANTS

Clone ^a	Transfecting DNA	Fraction of cells surviving UV dose	
		0.9 J/m ²	1.5 J/m ²
GM637 (normal)	—	1.0	1.0
GM2096-SV3 transformants			
134-2	pRSV-neo	0.08	0.003
131-4	pRSV-neo + p50	0.30	0.075
131-15	pRSV-neo + p50	0.18	0.040

^a GM637 is a normal human fibroblast cell line transformed with SV40. GM2096-SV3 is an SV40-transformed line of XP-C fibroblasts.

Following selection for G418 resistance, individual transformants were isolated and tested for UV survival. As shown in Table 1, whereas clone 134-2 generated by transfection with pRSV-*neo* alone retained the typical UV-sensitivity of the parental XP-C cell line, two transformants co-transfected with p50 DNA (131-4 and 131-15) acquired elevated UV-resistance. In comparison to the levels of UV-resistance exhibited by normal human cells (GM637) this complementation is only partial (Table 1).

DNA polymorphisms at the *CKII-β* locus

The increased UV resistance conferred upon XP-D and XP-C derived cell lines following transfection with an expressible cDNA for the human *CKII-β* enzyme, opened up the formal possibility of a defect in the *CKII-β* gene in one or both of these genetic groups. To test this possibility, Southern blot analysis of genomic DNA samples from 7 XP-D families was performed with *CKII-β* cDNA as a probe. This screening failed to detect any DNA rearrangement or large deletion. Likewise, Northern blot analysis of RNA did not reveal any change in size or abundance of *CKII-β* mRNA derived from cell lines belonging to 7 XP-D families and 10 XP-C families versus those taken from normal individuals (data not shown).

In a search for more subtle mutations, the ribonuclease A mismatch cleavage method (Winter et al., 1985; Myers et al., 1985) was used to analyze the *CKII-β* mRNA from normal, XP-D and XP-C affected individuals. The degree of

RNAase A cleavage depends on both the specific mismatch and the flanking sequences. Some mismatches are totally resistant to cleavage, while others are cleaved with less than 100% efficiency. Thus, only 30–50% of possible single base mispairings can be identified by this method. When RNA from the SV40-transformed normal human fibroblast cell line GM637 was hybridized to the 482-bp antisense *CKII-β* RNA (containing also distal Bluescript vector sequences), the expected 398-nucleotide sequence was protected (Fig. 7, lane 2). When RNA from six cell lines established from XP-C affected individuals (Fig. 7, lanes 1, 3, 4) and XP-D affected family members (Fig. 7, lanes 5, 6, 7) was tested, two shared the normal pattern of protected full-length RNA of 398 bases (Fig. 7, lanes 4, 5). In contrast, the RNA from the other four individuals exhibited two additional protected fragments of 256 and 142 bases (lanes 1, 3, 6, 7) that add up to the length of the fragment normally protected.

The RNA from the XP-C cell line GM2096-SV3 displayed, in addition to the former, another RNAase A cleavage site resulting in protected fragments of 286 and 112 bases (Fig. 7, lane 1). Results obtained with two other overlapping probes from *CKII-β* cDNA unambiguously localized the RNAase cleavage sites to 142 and 112 base pairs downstream from the *CKII-β* cDNA clone 5'-terminus. This suggested the occurrence of either a small deletion, or a single base pair mismatch, at the site of ribonuclease A cleavage. Using the RNAase A mismatch cleavage method, the former mutation was found in 5 out of 12 XP-C families, and in 1 out of 7 XP-D families (lanes 6 and 7 in Fig. 7 represent an XP-D mother and son, respectively), but also in 7 out of 32 apparently healthy individuals. In contrast, out of 68 families tested (mostly normal individuals) the putative mutation at 112 bp was found only in the XP-C patient GM2096 (Fig. 7, lane 1). The nature of the putative mutations was established through sequencing of DNA fragments cloned following amplification by the polymerase chain reaction from either chromosomal DNA or RNA which had been reverse transcribed.

The mutation at 142 bp constitutes a transition of T to C, while the one at 112 bp is a transversion of T to A. However, as shown in Fig. 6, both

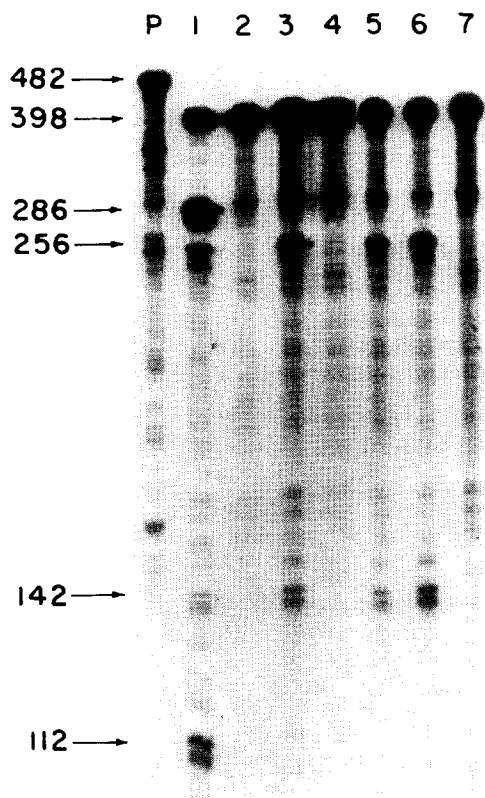


Fig. 7. RNAase mismatch cleavage patterns of CKII- β mRNA transcripts from normal, XP-D and XP-C cell lines. The radioactively labeled probe is an antisense RNA synthesized with T3 RNA polymerase from a linearized Bluescript KS vector containing the 0.4-kb *Pst*I–*Nco*I p16 derived cDNA fragment. For RNAase A cleavage analysis, the probe (482 bp long, as shown in lane p) was hybridized to 20 μ g of total cellular RNA from the following cell lines: GM637 representing normal (lane 2); the XP-C's GM2096-SV3, GM02249C, GM02248A (lanes 1, 3 and 4) and the affected XP-D GM02485A, the XP-D heterozygote mother, GM02473A, of patient GM02253D (lanes 5, 6 and 7). The sizes of the protected fragments indicated by the arrows were determined following DNA sequencing. A control lane which included carrier tRNA hybridized to the probe did not display any protected fragments and is not shown in the figure.

mutations occur at a third base in a codon and do not result in a change of the encoded amino acid, tyrosine, in the former, and leucine, in the latter.

Discussion

Transfection of an immortalized XP-D cell line with an expressible cDNA library led to partial

complementation of the UV-sensitivity of the recipient cells. Rescue by the polymerase chain reaction, followed by cloning and expression in XP cells, identified the cDNA which is responsible for the partial UV resistance acquired by the XP-D primary transformant 4/3. DNA sequencing has revealed that this cDNA encodes the beta subunit of human casein kinase II. The CKII is a ubiquitous messenger-independent serine/threonine kinase, localized in both the cytoplasm and the nucleus (reviewed by Krebs et al., 1988). The mammalian enzyme is isolated as an $\alpha_2\beta_2$, $\alpha'_2\beta_2$ or $\alpha\alpha'\beta_2$ tetramer in which the α and α' are closely related subunits of 37–44 kd that possess catalytic activity. The β subunit (24–28 kd) is the regulatory subunit. In vitro studies have shown that the CKII catalytic subunit by itself had very low activity; addition of an equimolar quantity of the β subunit resulted in a dramatic increase in enzyme activity (Cochet and Chambaz, 1983). Using the synthetic peptide RRREEETEEE as a substrate unique for CKII (Sommercorn and Krebs, 1987) we have recently shown that extracts prepared from XP-D cells transfected with pcD-CKII- β cDNA acquired a higher activity of CKII than the non-transfected parental cells (Teitz, Naiman and Canaani, unpublished results).

However, the phenotypic complementation exerted by expression of the CKII- β cDNA is not specific to group D, as transfection of p50 DNA could also partially complement the UV sensitivity of an XP-C cell line. Transfection of either XP-D or XP-C immortalized cell lines with the co-transfecting plasmid pRSV-*neo* DNA by itself had no effect on the UV survival of the recovered transformants. Using Southern, Northern and RNAase mismatch cleavage analysis, we were unable to detect any functional defect in the CKII- β gene of cell lines derived from either 7 XP-D or 10 XP-C families.

We do not know the molecular mechanism whereby increased UV survival has been obtained. This problem is particularly difficult to tackle as CKII has multiple substrates in vivo, most of which are still unknown at this stage. It was recently shown that microinjection of a purified CKII, at physiological concentrations, into *Xenopus laevis* oocytes, inhibited their progesterone-induced maturation. This observation suggested that

CKII participates in the natural arrest of oocytes at the G2 phase of the cell cycle (Mulner-Lorillon et al., 1988). It is a well established fact that there are cell cycle-dependent fluctuations in the sensitivity of cultured mammalian cells to UV-irradiation. Sensitivity, measured as either the loss of colony-forming ability or the proportion of unrepaired thymine dimers, is highest in the M and S phases and lowest in the G2 phase of the cell cycle (Erikson and Szybalski, 1963; Sinclair and Morton, 1965; Rauth and Whitmore, 1966; Djordjevic and Tolmack, 1967). Taken together with the increase of CKII activity in XP-D 4/3 primary transformants mentioned above, it might be concluded that the XP-D and XP-C transfectants expressing higher levels of CKII- β are more resistant to UV-irradiation because they have acquired a prolonged G2 phase. Whether CKII has any role in the mitotic delay which is induced by many DNA-damaging agents remains an open question. Recently, Weinert and Hartwell (1988) showed that the RAD9 gene controls the X-ray irradiation-induced G2 arrest in *Saccharomyces cerevisiae*. On the basis of the results of this study, we consider it unlikely that either the XP-D or XP-C DNA repair deficiency is associated with a defect in the beta subunit of casein kinase II. However, our results clearly suggest the possibility of modulation of the cell's response to DNA damage by CKII-dependent protein phosphorylation.

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