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CLONING OF HUMAN REPAIR GENES BY GENOMIC DNA TRANSFECTION

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Summary. - *General aspects of strategies for the isolation of mammalian DNA repair genes using DNA mediated gene transfer to repair deficient mutant cells are discussed with emphasis on the possibilities and limitations of the technique as well as potential problems and pitfalls. A summary is given of results obtained thusfar using this approach.*

Riassunto (Clonaggio di geni umani della riparazione del DNA con la tecnica della trasfezione di DNA genomico). - *In questo articolo sono presentati gli aspetti generali riguardanti le strategie in uso per l'isolamento di geni di mammifero della riparazione del DNA che prevedono trasferimento di DNA genomico in linee cellulari mutanti. Le possibilità e le limitazioni di queste tecniche saranno particolarmente discusse. Verrà inoltre presentato un riassunto dei risultati finora ottenuti seguendo questo tipo di approccio.*

Introduction

In prokaryotic and yeast systems many mutations can be corrected by the introduction of wild type donor DNA. In general, the molecular cloning of the transferred sequences can be readily achieved with standard molecular cloning techniques, provided that this DNA is linked to a selectable marker or some other kind of *tag*. Important conditions for application of this powerful strategy to the mammalian system have been worked out by Szybalska and Szybalski [1] who pioneered the transfer of genes to cultured animal cells and by Graham and van der Eb [2] who considerably improved the efficiency of this process by introducing the calciumphosphate DNA co-precipitation technique. Transfer of mammalian genes is hampered by the large size and the high complexity of mammalian genomes compared to prokaryotes and yeast. This means that much more recipient cells have to be transfected to cover in total the equivalent of one genome. Moreover, the efficiency of transferring intact functional gene copies decreases with increasing gene size. Furthermore, molecular rescue of the transferred gene from the genome of a mammalian transformant requires more sophisticated cloning techniques compared to lower organisms. Notwithstanding these limitations inherent to mammalian genomic DNA transfection, many laboratories have successfully adopted this approach in the last decade for the isolation of a variety of mammalian genes with different functions.

It is evident that genomic DNA transfer to repair deficient cells is - at present - the most straightforward approach to clone human DNA repair genes.

In the first part of this chapter general aspects of genomic DNA transfer to mammalian cells are discussed. The subsequent sections focuss on results obtained in genomic transfections with human cells of DNA repair deficiency syndromes and *in vitro* mutagenized repair defective rodent cells. Further details on the methodology and strategy of genomic transfections have been described in several recent review papers [3-5].

The strategy of genomic DNA transfections

In Table 1 a summary is presented of the mammalian genes isolated by genomic DNA transfections as extracted from the literature. The TK, HPRT and APRT genes have been cloned using mutant mouse L-cells whereas normal L-cells were employed to isolate genes encoding human cell surface proteins like CD4, CD8, nerve growth factor receptor and the transferrin receptor [6-20] (Table 1). In the latter experiments antibodies against the gene product of interest have permitted the rapid identification of transformants either by the fluorescent activated cell sorter (FACS) or a rosette assay. In case of the T-cell surface proteins a subtractive cDNA cloning procedure was followed to recover the transfected genes from primary transformants. Syrian hamster BHK (baby

Table 1. - *Overview of mammalian genes that have been isolated following DNA mediated gene transfer with genomic DNA*

Gene	Donor DNA	Recipient cell	Cotransfected DNA	Selection (*)	Cloning (*) strategy (*tag*)	Gene size	Ref.
NGF-receptor	human	Ltk$^-$	pTK	HAT, Rosette	Alu	15-30 kb	6
Transferrin receptor	human	Ltk$^-$	pTK	HAT, FACS	Alu	31 kb	7, 8
Thymidine kinase	chicken	Ltk$^-$	pBR322	HAT	plasmid rescue	2 kb	9
	human	Ltk$^-$	-	HAT	Alu	5-15 kb	10-12
HPRT (*)	human	LA9 hprt$^-$	-	HAT	Alu	± 30 kb	13
APRT (*)	human	Ltk-aprt$^-$	pBR322	azaser./aden.	plasmid rescue	< 7 kb	14
T8 (Leu-2)	human	Ltk$^-$	pTK	HAT, Rosette	subtr. cDNA cl.	< 13 kb	15
Lyt-2	mouse	Ltk$^-$	pTK	HAT, FACS	subtr. cDNA cl	5 kb	16-18
T8 (Leu-2)	human	Ltk$^-$	pTK	HAT, FACS	subtr. cDNA cl.	?	19
T4	human	Ltk$^-$	pTK	HAT, Rosette	subtr. cDNA cl.	30 kb	20
Cell cycle: G1	human	BHKts11	-	39.5 °C	Alu	?	21
RCC-1	human	BHKtsBN2	-	39.5 °C	Alu	± 30 kb	22
CCG-1	human	BHKtsBN462	-	39.5 °C	Alu	25-30 kb	23
Cell cycle: G1	human	BHKtsBN51	-	39.5 °C	Alu	7-8 kb	24
XRCC-1	human	CHO EM-9	pSV2gpt	MPA, chldUrd.	Alu	?	25-27
ERCC-1	human	CHO 43-3B	pSV3gptH	MPA, UV, MMC	linked to gpt	15-17 kb	28
ERCC-2	human	CHO UV5	pSV2gpt	MPA, UV	Alu	± 20 kb	29
ERCC-3	human	CHO 27-1	pSV3gptH	MPA, UV	Alu	35-40 kb	(a)
(Proto)-oncogenes	human	NIH/3T3	pSVgpt pSVneo	MPA, oncogenic G418, transformation	Alu	5-40kb	30-31

(*) HPRT: hypoxanthine phosphoribosyltransferase; APRT: adenine phosphoribosyltranserase; azaser./aden: azaserine/adenine; HAT: medium containing hypoxanthine, aminopterine and thymidine; FACS: fluorescent activated cell sorter; MPA: mycophenolic acid; subtr. cDNA cl.: subtractive cDNA cloning.
(a) G. Weeda, personal communication.

hamster kidney) ts-mutants appeared to be a suitable source for the cloning of several cell cycle control genes [21-24] (Table 1) and the isolation of human DNA repair genes has been accomplished using ultraviolet light (UV)-sensitive Chinese hamster ovary (CHO) mutants [25-29] (Table 1 and below). In the field of cancer research the pre-neoplastic NIH/3T3 mouse fibroblast has become the standard recipient for the transfer of transforming sequences from tumor DNA of many sources [30]. This has facilitated the isolation of a diverse assortment of activated oncogenes. The first to be identified were alleles of the c-*ras* oncogenes which had acquired a transforming potential by single point mutations [31]. An increasing group of oncogenes that are picked up by the NIH/3T3 assay harbors large abnormalities, primarily rearrangements which in a number of cases are also found back in the original tumor DNA but in some instances seem to have arisen during the transfection process [32].

Although differences exist between the strategies followed to clone the genes listed in Table 1, the basic outline of the approach encompasses a number of common steps: a) transfection of high molecular weight genomic DNA to the recipient cells by the calciumphosphate method or alternative techniques; b) selection of cells with the desired genetically transformed phenotype; c) segregation of irrelevant cotransfected DNA by a second round of transfection using DNA of a primary transformant; d) application of recombinant DNA techniques to recover the transfected gene from the DNA of a secondary transformant.

From the available data it can be inferred that the efficiency of genomic DNA transfer depends on a variety of factors which are discussed below.

Recipient cells

Obviously, naturally occurring mutants or *in vitro* mutagenized cells are likely candidates to be used in genomic transfections. However, it is apparent from the cloning of several genes encoding cell surface proteins that also normal cells can be utilized provided that they do not express by themselves the phenotype selected for. Recipient cell lines should be stable with respect to the property selected for or otherwise have a reversion frequency which is considerably lower than the transfection efficiency of the gene of interest.

Until now only a limited number of recipient cell strains, all of rodent origin have been used successfully in genomic transfections (Table 1). To our knowledge until now no reports have documented the cloning of genes after genomic DNA transfer to human cells. Especially cell lines from patients with DNA repair syndromes have been utilized extensively in many genomic DNA transfections in different laboratories [33]. However, with one notable exception ([34] see also below),

unequivocal evidence for the generation of *bona fide* transformants retaining one specific correcting gene is even lacking. One explanation for the inefficient gene transfer experienced with human (and also some rodent) cell lines is the very small quantities of exogenous sequences that in general are integrated by these cells compared to the rodent lines mentioned above [35, 36]. In addition, factors such as transfection frequency and degree of scrambling of exogenous DNA molecules also determine the suitability of a given cell line for genomic transfections. It is of interest to know to what extend these parameters are genetically determined. Recent experiments of Shiomi *et al.* [37] suggest that the property of "transfection frequency" is dominant and can be conferred to a specific cell by cell fusion with an X-irradiated good recipient cell line. Obviously, it is of considerable importance to investigate this further because in this manner any interesting mutant could be turned into one which is also valuable for gene cloning.

The transfected donor DNA

Currently, in most transfection protocols both in the first and second round of transfection genomic DNA is cotransfected with a dominant marker gene *Ecogpt* or *agpt* to provide a preselection for the small proportion of cells that are competent to integrate DNA. In order to "follow" the transfected sequences it is preferable to use genomic DNA of a heterologous species. In case of human DNA the abundant presence of dispersed *Alu* repeats [38] provides a natural *tag* which can be used to detect the presence of transfected DNA in primary and secondary transformants of e.g. rodent origin and most importantly it facilitates the recovery of transfected sequences from a recombinant DNA library of secondary transformant DNA.

The cloning of transfected genes is seriously hampered when after a second round of transfection no physical linkage of the gene of interest with either repetitive DNA or any other kind of *tag* can be detected. Absence of detectable transfected sequences on Southern blots of secondary transformants probed with species specific repeats leaves two possibilities open. First, the transfected gene is very poor in repetitive elements and hence difficult to visualize on Southern blots. This possibility was put forward by Albino *et al.* [39] and Dulhanty and Withmore [40] to explain their transfection results. Second, the secondary "transformant" does not contain any transfected DNA and is a revertant. A way to get around this problem is to try to establish physical linkage between the gene of interest and a dominant marker copy. In the secondary transfection this requires first a selection on the dominant marker gene copies present in the genomic DNA of a primary transformant. If subsequent co-selection on the gene of interest yields secondary transformants there is compelling evidence for a physical linkage between the dominant marker and the transfected gene. The cloned marker copy can then serve as a *tag* for the isolation of the transferred gene that must be located in close vicinity of this dominant marker gene [28].

Because exogenous DNA is subject to scrambling in the cell during the transfection process, gene size is an important factor determining efficiency of gene transfer in genomic transfections. On the average one double stranded break is induced every 5-15 kilobase (kb) in transfection experiments with various cell lines [35, 41]. However, in this respect also considerable differences between individual cell strains have been noted [41]. The largest gene isolated thus far by DNA mediated gene transfer is ki-*ras* (38 kb) [42]. However, the recently cloned *ERCC6* gene appears to reside on a fragment of 105 kb (C. Troelstra, personal communication) and could well shift the upper limit for a transfectable gene size to the 100 kb range.

Chromosome mediated gene transfer or interspecific cell fusions are alternative means to introduce large pieces of DNA into recipient cells [43-45]. However, these procedures have the disadvantage that for gene cloning purposes too much DNA is transferred, as a result of which the use of repetitive DNA as a probe to isolate the sequences of interest becomes virtually impossible.

To circumvent the problems associated with a large gene size or poor ability of recipient cells to incorporate large amounts of foreign DNA, transfection of cDNA expression libraries might be considered as a useful alternative. Okayama and Berg [46, 47] have designed an efficient cDNA cloning procedure in which the cDNA is inserted in an oriented manner in between the strong SV40 large T promoter and an SV40 derived poly(A) signal. Transfection of a total cDNA library to mouse hypoxanthine phosphoribosyltransferase (HPRT) deficient cells yielded wild type transformants which was shown to be the result of transfected full lenght HPRT cDNA clones [48]. However, no examples exist of studies in which human recipient cells were successfully transfected by a total cDNA library followed by the cloning of the cDNA. Factors critically determining the rate of success with this cDNA transfection approach are the level of transcription and the size of the mRNA (which indirectly influence the fraction of functional cDNA copies in the library). Another unknown parameter concerns the possibility that the gene of interest requires a strictly regulated expression, which is obviously not provided by the regulatory elements on the cDNA expression vector. Finally, when more than one cDNA is necessary, because of alternative splicing of the gene to be cloned, the cDNA approach will not work either.

Integrity of transformants

In general, in genomic transfection experiments a relatively small number of transformants is obtained, which does not allow statistics to be used to discrimi-

nate between the frequency of real transformants and of revertants. Moreover, reversion frequencies are often determined in independent experiments not of the same scale and under different conditions as used for the transfection experiments. A number of criteria can be applied to distinguish unequivocally between real transformants and revertants. *Bona fide* transformants can be identified by: a) detection of transfected genomic DNA in secondary transformants, preferably identification of common transfected DNA fragments in the genome of independent secondary transformants as determined by Southern hybridization using species specific repeat probes; b) cosegregation or physical linkage of the phenotype selected for with a dominant marker gene copy as assessed e.g. in successive rounds of transfection; c) isolation of unique transfected sequences from the DNA of a secondary transformant and their presence in independent other primary or secondary transformants (co-inheritance analysis).

It is essential that the status of (secondary) transformants is established with respect to one or more of these criteria before transfection of a specific gene is claimed or its cloning initiated.

Genomic transfections to human DNA repair deficient cells

A large number of permanent human cell lines from DNA repair deficiency syndromes are available [49] that can be used for genomic transfections. The hypersensitivity of these cells to DNA damaging treatments in principle provides relatively easy means of selecting corrected transformants. Many efforts to correct the excision repair defect in xeroderma pigmentosum (XP) cells have been unsuccessful [33]. Mock transfections with XP group A cells have yielded UV-resistant clones with a frequency of 10^{-7} [50, 51]. In most cases these apparent revertants were obtained after multiple UV irradiations suggesting that the reversion of the XP mutation was the result of UV-induced mutagenesis. The fact that the XP-A mutation is revertible to normal, indicates that the genetic defect in the XP-A lines examined is most likely a point mutation. Recently, Cleaver *et al.* [52] have produced a number of XP-A revertants by treatment of XP12RO-SV40 cells with ethyl-methylsulfonate and an accumulated UV dose of 60 J/m^2. However, it appeared that in this revertant (6-4) photolesions were repaired normally in contrast to pyrimidine dimers. This may suggest that the XP-A protein has a variable affinity for different kinds of DNA adducts. Comparison of the UV-selection regimes used, suggests that the chance of inducing XP-A revertants is high for low UV-doses administered over long periods of time and very low when cells are exposed to high doses over a short time interval. Using the latter selection system we have never obtained XP-A revertants in transfection experiments involving in the order of 10^9 cells. Furthermore, with a very similar type of UV-regime Tanaka *et al.* [34] were able to isolate two genuine UV-resistant primary transformants in an impressive series of transfection experiments, using mouse genomic DNA. In these experiments no revertants were identified. In subsequent rounds of transfection with genomic DNA of one "primary" a single secondary transformant was obtained that had retained mouse sequences. Preliminary data have shown that two isolated overlapping lambda clones with mouse DNA from the secondary transformant were able to confer a repair proficient phenotype to the XP2OS-SV40 cells with a high efficiency [34]. These data demonstrate that fragments of the mouse homologue of the XP-A gene have been isolated. Since apparently the encoded murine protein is functional in human cells the nucleotide sequence conservation between the murine and human XP-A gene probably will be sufficient to use mouse probes for cloning the human gene. In cell fusion studies the mouse chromosome 4 was found to correct the XP-A defect [53]. It will be of interest to see whether the sequences isolated by Tanaka *et al.* [34] also map to this murine chromosome. The persistance displayed by Tanaka *et al.* in transfecting XP-A cells notwithstanding their low transfection frequency for the XP-A correcting gene provides the first example of gene cloning following DNA mediated gene transfer to human cells.

Other attempts to clone XP-genes (including XP-A) using genomic DNA transfection have not (yet) reached the stage in which rigorous proof is obtained for genomic secondary transformants according to the criteria defined above. Also alternative strategies involving cell hybridization [54, 55] or cDNA transfection [56, 57] have not yet resulted in cloning of the complementing genes. Unfortunately, the same holds for all (often extensive) efforts to isolate genes involved in other human repair syndromes such as ataxia telangiectasia (AT) [58] and Fanconi's anemia (FA) [59, 60]

DNA repair deficient rodent cells

In recent years a large number of rodent mutant cells with increased sensitivity to DNA damaging agents have been isolated by *in vitro* mutagenesis [49, 61, 62]. In most cases UV and ionizing radiation have been used as the selective agents after treatment with mutagens like ethyl-methanesulfonate and ethylnitrosourea. Many of the laboratory induced mutant cells are phenotypically resembling the cellular hypersensitivity to DNA damage manifested in human DNA repair syndromes. However, until now there are no direct indications that these naturally occurring mutations are represented in *in vitro* induced rodent mutant cells. A defective incision step of the excision repair process underlies the UV-sensitivity of a number of CHO mutants which is very similar to

XP [63]. Hence, it is conceivable that one or more of the ten XP mutations overlaps with the UV-sensitive rodent collection. Several mitomycin-C (MMC)-sensitive CHO mutants are in a number of respects reminiscent of the FA phenotype and recently, γ-ray sensitive CHO cells have been isolated that show a lack of inhibition of DNA synthesis after γ-ray exposure, which is a consistent feature of AT cells (Zdzienicka *et al.*, submitted for publication). Therefore, in general, rodent repair mutants are not only valuable tools for studying mammalian DNA repair, but may also facilitate identification of hereditary repair defects in human cells.

A large number of mutants exhibits a cross-sensitivity to a variety of DNA damaging agents [62]. This suggests that common steps might be involved in the repair of single strand breaks, monofunctional adducts and DNA cross-links. To shed more light on this apparent complexity it is important that complementation tests are performed to establish the actual number of different mutated genes in the collection of mutants isolated in various laboratories.

The presently isolated UV-sensitive mutants of Chinese hamster origin have been classified in 7 complementation groups [64-67]. Recent data on the characterization of those complementation groups and the human genes that correct the defect in corresponding rodent cells are summarized in Table 2 [64, 66-72]. Four complementation groups have been described for UV-sensitive mouse lymphoma lines [69] and recently evidence was reported for overlap between the mouse and Chinese hamster classifications. In fusion experiments, mouse group 1 cells failed to complement CHO group 5 [72]. Moreover, the repair defect in both groups could be corrected by a gene on human chromosome 13 [72, 73]. The US31 mutant which is the sole representative of mouse group 3 complements the first 6 Chinese hamster groups [27]. Fusion experiments with the recently described seventh hamster group [67] will have to reveal whether mouse group 3 represents the eight rodent UV-sensitive complementation group.

With respect to the potential use of these UV-sensitive cell lines in genomic transfections aimed at isolating complementing human genes it is important to note that considerable differences in transfectability exist. The mouse lymphoma mutants have a very low transfection frequency [37] and are very unsuitable for this approach. V79 Chinese hamster cells were much less efficient DNA "uptakers" than CHO-9 and AA8 cells which also makes these cells less favourable for genomic DNA transfer [5].

Chromosomal localization of ERCC-*genes*

By cell fusions between UV-sensitive rodent mutants and wild type human cells proliferating repair proficient hybrids could be generated. Karyotyping of these hybrids allowed the chromosomal assignment of the complementing human genes that have been designated as *ERCC* genes (excision repair cross complementing rodent repair deficiency). In this way the human *ERCC1* to *ERCC5* genes, which complement the first 5 CHO complementation groups, have been mapped on human chromosomes 19, 19, 2, 16 and 13 respectively (Table 2). For *ERCC1* and *ERCC3* these data have been confirmed by Southern blot analysis of panels of human/rodent hybrids with probes of the cloned genes [74, 75] (G. Weeda, Leiden, personal communication). Surprisingly, the human gene *XRCC1* which corrects the double strand break repair defect in CHO mutant EM9 was also localized on chromosome 19 [76], which means that 3 out of 6 CHO mutants are corrected by a gene on the same human chromosome. Several other

Table 2. - *Complementation groups of UV-sensitive CHO mutants and chromosomal localization and other properties of the human* ERCC-*genes that correct the CHO repair defect as found by cell fusion experiments and gene cloning by DNA mediated gene transfer*

Compl. group	Mutant(s)	Parental line	UV^s	MMC^s (> 5x)	Ref.	Gene name *(a)*	Human chrom.	Ref.	Cloned (y/n)	Gene/mRNA size (kb)		Ref.
1 (*)	43-3B; UV20	CHO9; AA8	+	+	64, 68	ERCC-1	19	70	y	15-17	1.1	28
2	UV5	AA8	+	-	64	ERCC-2	19	71	y	20	2.5-3	29
3	27-1; UV24	CHO9; AA8	+	-	64, 68	ERCC-3	2	72	y	35-40	3	*(b)*
4	UV41	AA8	+	+	64	ERCC-4	16	71	n	?	?	
5	UV135; Q31	AA8; L5178Y	+	-	64, 69	ERCC-5	13	72	n	?	?	
6	UV61	AA8	+	?	66	ERCC-6	?		y	?	?	*(c)*
7	VB11	V79	+	?	67	ERCC-7	?		n	?	?	

(*) At a recent UCLA meeting (Taos, USA, January 1988) it was decided to rename complementation group 1 and 2 respectively in order to match the numbering with the complementing human genes.
(a) ERCC: excision repair cross complementing.
(b) G. Weeda, Leiden, personal communication.
(c) C. Troelstra, personal communication.

markers for this chromosome map on Chinese hamster chromosome 9 which is thought to be hemizygous in CHO cells [77]. Therefore, the monosomic state of hamster chromosome 9 might explain the apparent non-random chromosomal distribution of mutations that account for the different CHO complementation groups.

Isolation of ERCC-*genes by genomic DNA transfer*

In several laboratories genomic DNA transfection experiments have been used or are in progress to isolate human *ERCC*-genes complementing the CHO repair defects. In general, UV is used as the selective agent for the isolation of primary transformants. However, mutants belonging to group 1 and 4 display a marked cross-sensitivity to mitomycin C (MMC) [78] which also could be used as a selective drug.

The human *ERCC1* gene which complements CHO group 1 is the first human DNA repair gene that was isolated by genomic DNA transfer [28]. In a primary transfection total human DNA was partially digested with *PstI* and after ligation to the selectable marker *Ecogpt* transfected to 43-3B cells. Repair proficient primary transformants were obtained by selection in medium containing mycophenolic acid (MPA) and MMC or UV. In a secondary transfection a linked transfer of MPA and MMC resistance could be achieved. This allowed screening of a cosmid library of a secondary transformant, with the *Ecogpt* as a probe, which yielded the isolation of a functional *ERCC1* gene close to one of the *Ecogpt* copies [28]. The UV5 mutant, belonging to group 2, has been successfully employed for the cloning of the *ERCC2* gene [29] and recently also the isolation of human *ERCC3* was achieved after gene transfer to the 27-1 mutant (group 3) [79]. Molecular analysis revealed that tertiary UV135 (group 5) transformants had retained human sequences [80] and independent primary and secondary UV61 (group 6) transformants were found to harbor identical, transfected, unique human sequences (C. Troelstra, personal communication) indicating that the cloning of the *ERCC5* and *ERCC6* genes is in an advanced state.

Except for the strategy used for *ERCC1* in all other cases total human DNA was transfected and human *Alu* probes were used for analysis of transformants and isolation of transfected sequences from recombinant DNA libraries. In contrast, it seems that efforts to isolate *ERCC4* are hampered due to the apparent low abundance of human repetitive sequences in the *ERCC4* gene as a result of which transfected human sequences can not be detected in DNA of secondary transformants [81, 82]. Transfection of the isolated human *ERCC1* and *ERCC2* genes to representative cell lines of all 6 CHO complementation groups yielded repair correction only in group 1 and 2 mutants respectively [29, 83]. Furthermore, it was found that introduction of *ERCC1* in 43-3B cells gives close to wild type levels of all repair parameters examined (cross-sensitivity to other agents, UV-induced mutagenesis, DNA incision as measured by UV-endo assay and preferential repair) [84, 85]. Comparable data were obtained for UV5 cells with the cloned *ERCC2* gene [29]. Hence, it is reasonable to assume that *ERCC1* and *ERCC2* are the human homologues of the mutated Chinese hamster repair genes.

With respect to the characterization of the cloned *ERCC*-genes it is of prime interest to investigate whether there is a relationship with the human DNA repair syndromes. Southern blot analysis has revealed that the *ERCC1* gene is not deleted or grossly rearranged in all nine XP (A to I) and the Cockayne's syndrome (CS) and FA complementation groups A and B. Furthermore, Northern blot experiments revealed correct transcription of *ERCC1* in all nine XP groups examined (Van Duin *et al.*, submitted for publication). Introduction of *ERCC1* into representative cell lines of all XP and CS complementation groups did not confer DNA repair proficiency or UV-resistance. Taken together these data strongly suggest that *ERCC1* is most likely not the mutated gene in XP and CS cells examined (Van Duin *et al.*, submitted for publication).

The *ERCC2* and *ERCC3* gene have recently been cloned. The until now limited data on the molecular characterization of both genes are summarized in Table 2.

The ERCC1 *gene*

A functional human *ERCC1* cDNA which corrects the CHO group 1 mutation has been isolated and using human probes also a biologically active mouse cDNA was obtained [75, 86]. The mammalian *ERCC1* protein has a predicted molecular weight of 32-33 kDa. A putative DNA binding domain and nuclear location signal have been tentatively assigned to the deduced protein based on comparison with consensus amino acid sequences of functional domains in other proteins [87]. Currently, experiments are in progress to lay hand on the *ERCC1* protein which permits *in vitro* analysis of *ERCC1* functions.

Interestingly, the mammalian *ERCC1* protein appeared to exhibit significant homology to the yeast excision repair protein *RAD10* and parts of the *E. coli* excision repair proteins *uvrA* and *uvrC* [75, 88, 89]. The similarity between the different repair proteins is schematically depicted in Fig. 1. The homology with the bacterial proteins concerns relatively short regions which may be indicative of a similar domainal function. However, the homology with *RAD10* includes half of the yeast protein and one third of the mammalian repair protein. Therefore, it seems likely that *ERCC1* and *RAD10* have a similar function in DNA excision repair. The postulated *ERCC1* DNA binding domain coincides with the most homologous region indicating that this protein part may represent the functional centre of *ERCC1/RAD10*. It seems likely to assume that

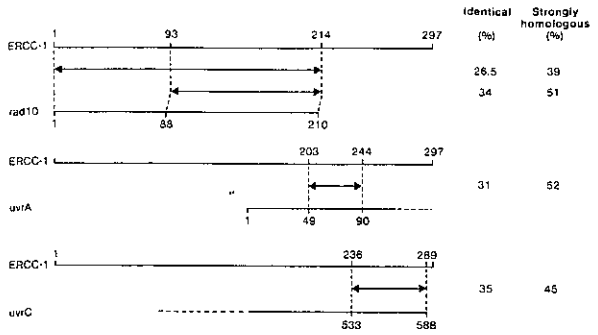

Fig. 1. - Schematical presentation of the amino acid (AA) homology between *ERCC1* and *RAD10*, *uvrA* and *uvrC*. Left column: perfect AA homology. Right column: conserved AA homology.

ERCC1 and *RAD10* have evolved from a common ancestral gene. The complete genomic organization of the human *ERCC1* gene and part of the mouse gene have been established [86, 90]. The architecture of the human *ERCC1* gene region is shown in Fig. 2. The human *ERCC1* gene has a length of 15-17 kb and consists of 10 exons of which the first exon is non-coding (open box), the eigth exon (hatched) subject to alternative splicing and the last exon (coding part:black, non-coding:open) to differential polyadenylation which yields transcripts of 1.0, 1.1 and 3.4 kb. The significance of both the alternative splicing and 3' end processing is unknown. It was found that the large mRNA is primarily located in the nucleus (Van Duin *et al.*, submitted for publication) which might suggest that they represent intermediates in RNA processing. Northern blot experiments have revealed a relatively low *ERCC1* expression level in a variety of human cells and different mouse organs and stages of development. UV-exposure or MMC-treatment of HeLa cells did not induce *ERCC1* transcription [90]. The *ERCC1* promoter was found to be devoid of known promoter elements and therefore might represent a class of eukaryotic promoters that are responsible for a low constitutive rate of transcription.

Surprisingly, an opposite transcription unit was found to be located in the 3' *ERCC1* region of mouse and man (see also Fig. 2). Characterization of a partial

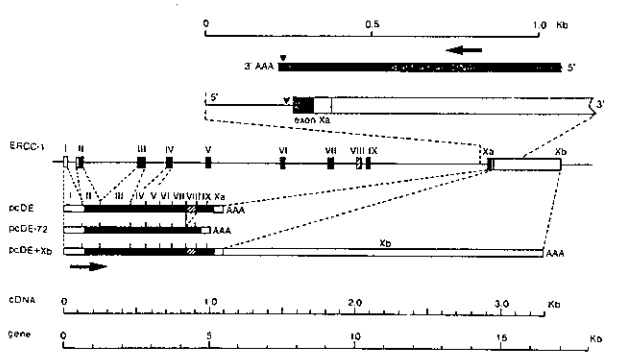

Fig. 2. - Architecture of the human ERCC1 gene (see text for explanation).

cDNA of the human antisense gene (designated *ASE1*, antisense *ERCC1*) and genomic sequencing indicated that this opposite oriented transcript of 2.6 kb is overlapping with *ERCC1* exon X and terminating in the ninth intron (Van Duin *et al.*, submitted for publication). This is the first example of naturally occurring gene overlap in the human genome.

From the presently available data it is clear that the antisense gene product is not required for correction of the CHO mutation by *ERCC1* since *ERCC1* cDNA constructs were fully competent in this respect. The function of the *ASE1* gene and its relationship with DNA repair remains to be established.

Concluding remarks

Correction of the genetic defect in UV-sensitive cells by DNA mediated gene transfer is the most direct route to achieve the cloning of the complementing gene. As it turns out the isolation of UV-sensitive CHO mutants has been of great importance in this regard. These rodent mutants have far better transfection properties compared to their human XP counterparts [35]. However, as has been reported by Tanaka *et al.* [34], genomic transfections with XP cells are not impossible. Based on the data reported by the Japanese group it can be expected that detailed information on the XP-A gene will be available soon.

In several laboratories genomic DNA transfections into UV-sensitive CHO mutants are in progress with the aim to isolate human *ERCC*-genes. This has resulted in the cloning of the *ERCC1* gene [28] while recently the human *ERCC2* and *ERCC3* genes have been isolated [29, 79]. Genomic transfections with representative cell lines of the other CHO groups are in an advanced state. Therefore, it seems likely that the cloning of the remaining *ERCC*-genes will be achieved in the next few years.

It is evident that further isolation of rodent repair mutants deserves a high priority. Considering the differences that exist among different cell lines in terms of DNA uptake in gene transfer experiments [35, 36] it is preferable to isolate new mutants from suitable parental cells. The pattern of hemizygozity of such lines largely determines the spectrum of mutants that can be generated. In order to avoid saturation of the hemizygous parts of the genome of one particular line with mutated repair genes and repeated isolation of the same mutants whereas other (diploid) loci are underrepresented it is advisable to start mutagenesis experiments with rodent cell lines other than CHO, which presumably harbor other patterns of hemizygosity. Using the Chinese hamster lung fibroblast line V79 Zdzienicka *et al.* [67] have recently isolated the seventh UV-sensitive rodent complementation group.

At present only for the *ERCC1* protein the amino acid sequence has been deduced from the nucleotide sequence of the cloned cDNA [75]. One of the most remarkable results of the experimental work on *ERCC1* is its significant homology with the yeast *RAD10* and *E. coli uvrA* and *uvrC* proteins [75, 88, 89]. The interesting finding that the *ERCC1* amino acid sequence has significant homology with yeast and bacterial excision repair proteins suggests that functional aspects of DNA excision repair are well conserved during evolution. Assuming that this will also hold for DNA repair mechanisms as a whole it will be of interest to investigate whether the other yeast repair genes also have homologous human counterparts. Therefore, it seems an attractive approach to recrute yeast repair genes in order to use them as probes to clone homologous genes in higher organisms. If no direct homology with mammalian sequences can be detected a step by step approach with intermediate species like *Drosophila* might be followed to gradually ascent the evolutionary ladder.

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