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Complementation of the DNA repair-deficient *swi10* mutant of fission yeast by the human *ERCC1* gene

Carsten Rödel, Tanja Jupitz and Henning Schmidt*

Institute of Genetics, Technische Universität Braunschweig, Spielmannstrasse 7, D-38106 Braunschweig, Germany

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ABSTRACT

In human cells DNA damage caused by UV light is mainly repaired by the nucleotide excision repair pathway. This mechanism involves dual incisions on both sides of the damage catalyzed by two nucleases. In mammalian cells XPG cleaves 3′ of the DNA lesion while the ERCC1–XPF complex makes the 5′ incision. The amino acid sequence of the human excision repair protein ERCC1 is homologous with the fission yeast Swi10 protein. In order to test whether these proteins are functional homologues, we overexpressed the human gene in a *Schizosaccharomyces pombe swi10* mutant. A *swi10* mutation has a pleiotropic effect: it reduces the frequency of mating type switching (a mitotic transposition event from a silent cassette into the expression site) and causes increased UV sensitivity. We found that the full-length *ERCC1* gene only complements the transposition defect of the fission yeast mutant, while a C-terminal truncated ERCC1 protein also restores the DNA repair capacity of the yeast cells. Using the two-hybrid system of *Saccharomyces cerevisiae* we show that only the truncated human ERCC1 protein is able to interact with the *S.pombe* Rad16 protein, which is the fission yeast homologue of human XPF. This is the first example yet known that a human gene can correct a yeast mutation in nucleotide excision repair.

INTRODUCTION

Nucleotide excision repair (NER) is one of the DNA repair systems of a living cell. This pathway removes dipyrimidine dimers, which are the main lesions induced by UV; it also removes other DNA alterations, such as different types of chemical adducts and crosslinks. An important feature of NER is that it induces incisions on both sides of the lesion in the damaged strand (1–3). After excision of a short oligonucleotide the gap is repaired by DNA polymerase. In humans the genetic disorder xeroderma pigmentosum (XP) is caused by a defect in one of the NER genes. Several human cell lines with deficiencies in NER have been isolated from XP patients. Cell fusion experiments led to the identification of seven complementation groups (XPA–XPG). Some NER genes were cloned by cross-complementation of UV-sensitive rodent cell lines with human DNA (4–9). *ERCC1* is one of the few NER genes for which so far no mutations have been found in humans. The ERCC1 protein seems to be essential for viability, since it was found that *ERCC1*-deficient mice die soon after birth (10).

The proteins involved in NER are very similar in all eukaryotes, from yeasts to mammals. In the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* several NER genes were isolated by functional complementation of the radiation sensitivity of the respective mutants (for a review see 11). It turned out that the amino acid sequences of the human ERCC1 protein and Swi10 from *S.pombe* show significant homologies (12). Recently it was shown that ERCC1 and XPF form a tight complex. This complex is a structure-specific endonuclease which is necessary for the 5′ incision during excision repair in mammalian cells (2). In fission yeast interaction of the two proteins Swi10 and Rad16 (the latter homologous to XPF) was demonstrated *in vivo* (13).

In addition to an increased sensitivity to UV, *swi10* mutants exhibit a reduced frequency of mating type switching (14). In homothallic strains of fission yeast the cells frequently switch their mating type by copying DNA sequences from one of two silent (i.e. non-expressed) cassettes into the expression site (15). This unidirectional transposition is similar to gene conversion. Due to mating type switching, homothallic strains are mixtures of cells of both mating types. Cells of opposite mating type are able to copulate with each other. In fission yeast the zygotes immediately undergo meiosis and develop to asci with four haploid spores. The percentage of spores in a colony can be used as a measure of the frequency of switching.

In spite of the strong conservation of excision repair proteins in eukaryotes, interspecies complementation is rare. This may be due to the fact that excision repair requires the coordinated action of >10 proteins involving complex protein–protein interactions. We demonstrate that the human ERCC1 protein is able to replace the function of the *S.pombe* repair protein Swi10. For this replacement it is necessary that ERCC1 interacts with fission yeast protein Rad16.

MATERIALS AND METHODS

Strains

All *S.pombe* strains used were derived from those originally isolated by Leupold (16). The genotypes are described in the text with the experiments. The *Escherichia coli* strain DH5α (17) was used as recipient for amplification of plasmids and for cloning experiments.

*To whom correspondence should be addressed. Tel: +49 531 391 5775; Fax: +49 531 391 5765; Email: schmidt@alpha.bio.nat.tu-bs.de

Figure 1. Constructs used for the complementation analysis. The different genes employed in the experiments are shown schematically and the results are summarized in the form of a table. pREP1 (23) is an *S.pombe* expression vector; without insert it was used as a negative control. The *swi10* and *ERCC1* cDNAs were cloned into pREP1. The hybrid genes were constructed as described in Materials and Methods. The resulting plasmids were transformed into the *swi10-154* mutant in order to determine whether they can rescue the switching defect and/or the increased UV sensitivity of the *swi10* strain. The *swi10-154* mutation is a G→A transition at the 5′ splicing site of the first intron (33; our unpublished results). The phenotype of the *swi10-154* mutant is indistinguishable from a *swi10* disruption strain (unpublished results). Two plus signs indicate correction of the defect, one plus sign indicates partial correction and a minus sign indicates no correction.

Media and general genetic techniques

The media and the standard genetic techniques for *S.pombe* are described elsewhere (18,19). Minimal medium (MMA) used to select *S.pombe* transformants was supplemented as necessary with leucine (100 mg/l) and/or uracil (50 mg/l). Media for *S.cerevisiae* were prepared as described (20).

DNA sequencing

For sequencing, the amplified PCR fragments were cloned into the pUC18 vector (21). Sequencing was performed by the dideoxynucleotide chain termination method (22) with Sequenase (US Biochemical) using synthetic oligonucleotide primers.

Construction of plasmids for complementation analysis

For expression of the human *ERCC1* gene and the fission yeast *swi10* gene in *S.pombe* the vector pREP1 (23) was used. The *swi10* coding region was amplified using the primers 5′-GAGCTCG-GATCCATTTTAGACAATGTCTGA-3′ and 5′-TCAGATGGA-TCCAAGAAATAAATCAAGTTTGT-3′, digested with *Bam*HI and ligated into a *Bam*HI-digested derivative of pREP1 in which the ATG in the polylinker was deleted. The *ERCC1* cDNA was amplified using the vector pcDE (24) with primers 5′-TGTGTTG-GATCCATATGGACCCTGGGAAGGAC-3′ and 5′-ATAACAG-GATCCTCATCAGGGTACTTTCAA-3′. After digestion with *Nde*I and *Bam*HI the *ERCC1* cDNA was ligated into *Nde*I/*Bam*HI-digested pREP1. ERCC1(1–290) was constructed using the primer 5′-AACAGGATCCTCATCAGTGCAGGACATCAAACACC-3′ for the C-terminus. The amplified *swi10* and *ERCC1* sequences were verified by sequencing. To construct the hybrid genes we first created a singular *Sal*I restriction site in the conserved region Val173–Asp174 of ERCC1 and Val115–Asp116 of Swi10 respectively by PCR-based site-directed mutagenesis without changing the amino acid sequence (25). In the second step the *Sal*I–*Bam*HI fragment with the C-terminal half of *swi10* or *ERCC1* respectively was fused with the N-terminal half of the homologous gene. This manipulation yielded the fusion genes coding for ERCC1(1–173)/Swi10(116–252) and Swi10(1–115)/ERCC1(174–297).

Determination of sporulation frequencies

The homothallic strain h^{90} *leu1-32 ura4-D18 swi10-154* was transformed by the lithium acetate method (26) with the constructs shown in Figure 1. Individual transformants were re-streaked on minimal medium (19). After 5 days the colonies were replicated on sporulation medium (MEA; 19) and incubated for a further 2 days at 30°C. From each strain 10 colonies were picked and individually suspended in saline. Then ~200 cells and spores respectively were counted in a haemocytometer. The percentage of spores was calculated as no. spores/(no. spores + no. cells).

UV inactivation curves

Quantitative UV tests (inactivation curves) were essentially as described previously (14). In short, the yeast transformants were grown in minimal medium, washed twice with saline and then resuspended to a final concentration of 10^6 cells/ml. Aliquots were irradiated with different UV doses. After appropriate dilutions, the cells were plated on complete medium. All experiments were done three times. Each set of experiments yielded reproducible results. Therefore, we used the mean values to draw the inactivation curves.

Two-hybrid experiments

The interactions of the different Swi10 and ERCC1 proteins respectively with Rad16 were analysed by cloning the respective genes in separate vectors containing either the DNA binding domain (pAS1) or the activation domain (pACT) of the GAL4 transcriptional activator (27,28). It was shown previously that the C-terminal part of Rad16 (amino acids 502–892) is sufficient for interaction with Swi10 (13). Rad16 was fused with the activation domain of GAL4, while Swi10 and ERCC1 respectively were fused with the DNA binding domain. The vectors were co-transformed into *S.cerevisiae* strain Y153 (28). If the two fusion proteins are able to interact, GAL4 activity is restored. The interaction of SNF1 and SNF4, which has already been demonstrated (27), was used as a control. The *E.coli lacZ* reporter gene is under the control of a promoter responding to GAL4 activation. For quantification β-galactosidase activity was measured using *o*-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate in

crude extracts from yeast cells. The activity in Miller units was calculated by the formula $A_{420} \times 380$/time (min) × amount of protein (mg) (29).

RESULTS

Expression of human ERCC1 in *S.pombe*

The amino acid sequence of ERCC1 has strong homology with that of the Swi10 protein from *S.pombe* (12,24). Therefore, we wished to analyse whether the human protein is functional in fission yeast. Because it was found that in human cell lines ERCC1 protein is only stable in a complex with XPF (30), we first examined whether ERCC1 protein can be stably expressed in *S.pombe*. For that we overexpressed the cDNA of human *ERCC1* from the strong *nmt1* promoter (31) in fission yeast cells. From crude extracts proteins were separated by SDS–PAGE. With the help of ERCC1-specific antibodies we could show in Western blots that ERCC1 is expressed in the yeast cells (data not shown). From this we conclude that the human ERCC1 protein is sufficiently stable in *S.pombe* in the absence of its normal partner XPF to be detectable by immunoblot analysis. This is in contrast to results found in human cell lines, where ERCC1 is unstable in extracts from XPF-deficient cells (32).

Complementation of the switching and NER defects of *S.pombe swi10* mutants

Then a *swi10* mutant was transformed with a vector containing the human *ERCC1* gene (Fig. 1). For comparison we used the *S.pombe* expression vector pREP1 (negative control) and *swi10* cDNA cloned into pREP1 (positive control). Overexpression of full-length human *ERCC1* in a *swi10* mutant showed that the defect in mating type switching was compensated for; the percentage of spores was nearly as high as in a wild-type strain (Fig. 2). However, the increased UV sensitivity of the yeast mutant was not complemented (Fig. 3a). This demonstrates that the human *ERCC1* gene is able to take over *swi10* function with regard to mating type switching but not with regard to NER. Since ERCC1 functions in NER in a protein complex with XPF, this result may indicate that ERCC1 is not able to properly interact with Rad16, which is the XPF homologue of fission yeast (13). On the other hand, it is also possible that in mating type switching and NER different enzymatic activities or protein–protein interactions are necessary.

Van Duin *et al*. (34) had shown that in human cells the C-terminus of *ERCC1* is essential for repair function. In order to test whether this region is also necessary for mating type switching, we constructed a C-terminal truncated *ERCC1* gene, ERCC1(1–290). To our surprise, the truncated *ERCC1* complemented the NER and switching defects of the *swi10* mutant (see Figs 2 and 3a).

Next we constructed two hybrid genes (Fig. 1). We fused the N-terminal half of *ERCC1* with the C-terminal half of *swi10*, yielding the hybrid ERCC1(1–173)/Swi10(116–252). The reciprocal construct was Swi10(1–115)/ERCC1(174–297). Both hybrid genes were overexpressed in a *swi10* mutant and the percentages of spores as well as the UV sensitivity were determined. Both fusion proteins were functional and were able to complement the switching defect (Fig. 2). With regard to DNA repair, ERCC1(1–173)/Swi10(116–252) fully restored the repair capacity of the *swi10* mutant, whereas Swi10(1–115)/ERCC1(174–297) restored it only partially (Fig. 3b). Based on these results we conclude that amino acid residues 1–173 of ERCC1 can completely replace the N-terminal half of Swi10.

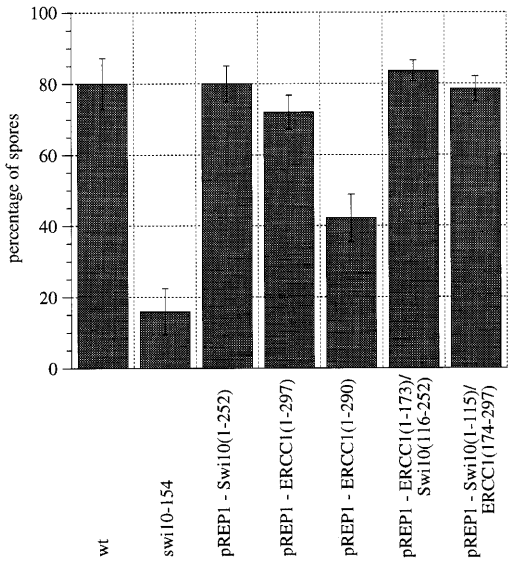

Figure 2. Complementation of the switching defect of the *swi10* mutant. The percentages of ascospores (mean values ± standard deviations) are shown for the different transformants.

Interaction of human ERCC1 with *S.pombe* Rad16

The interaction of ERCC1 with *S.pombe* Rad16 seems to be necessary for NER function. To test this assumption we examined ERCC1 and Rad16 in the two-hybrid system in *S.cerevisiae* (27). The results (Table 1) show that the truncated ERCC1 protein is able to interact with Rad16 whereas the full-length protein is not. Deletion of the last seven amino acids from the C-terminus of ERCC1 seems to change the conformation of ERCC1 in such a way that an interaction with the fission yeast Rad16 protein becomes possible. In mammalian cells the same construct is non-functional in UV repair (35). Therefore, the C-terminal part of ERCC1 might be very crucial for the three-dimensional structure of the protein.

Table 1. The truncated ERCC1(1–290) protein interacts with *S.pombe* Rad16 *in vivo*

Plasmid[a]	β-Galactosidase activity
Rad16(502–892)	<1
SNF1; SNF4	8.6
Rad16(502–892); Swi10(1–252)	45.9
Rad16(502–892); ERCC1(1–297)	<1
Rad16(502–892); ERCC1(1–290)	9.8

[a]The amino acids 502–892 of Rad16 were fused with the GAL4 activation domain, while Swi10 and ERCC1 respectively were fused with the DNA binding domain of GAL4. For details see Materials and Methods.

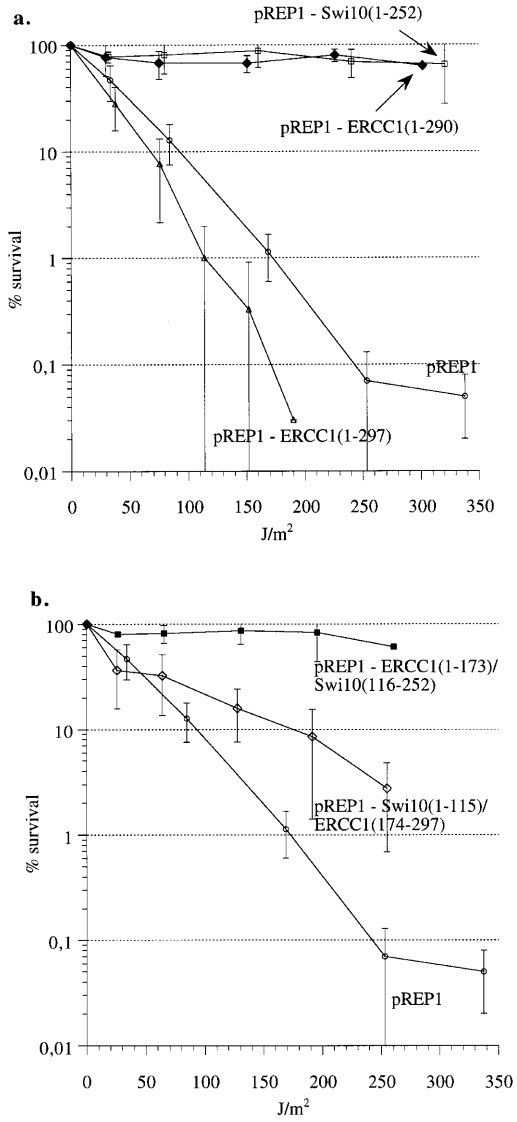

Figure 3. Reduction of the UV sensitivity of the *S.pombe swi10* mutant by overexpression of human ERCC1(1–290). UV inactivation curves of the *swi10* mutant transformed with the different plasmids (see Fig. 1) are shown. (**a**) Complete ERCC1(1–297) does not reduce the UV sensitivity of the *swi10* mutant, whereas transformants with the truncated protein ERCC1(1–290) exhibit a UV sensitivity similar to that of the wild-type. For comparison the inactivation curves are shown for strains transformed with pREP1 (expression vector without insert, no complementation) and with pREP1-Swi10 (vector with the *swi10*$^+$ cDNA, full complementation). (**b**) The hybrid protein Swi10/ERCC1 partially restores and the hybrid protein ERCC1/Swi10 fully restores the UV sensitivity of the *swi10* mutant as compared with the wild-type. Error bars represent standard errors of the means.

DISCUSSION

In fission yeast the Swi10 protein is an essential component of NER. Furthermore, it is also necessary for efficient mating type switching, a process related to gene conversion. The truncated ERCC1 protein is able to complement both defects in *swi10* mutants. However, the full-length ERCC1 protein complements only the switching defect and not the defect in repair of UV damage. How can this be explained? We assume that a weak interaction between the complete ERCC1 protein and Rad16 still takes place which, however, is not detectable in the two-hybrid system. Such a weak interaction may be sufficient for mating type switching, since this process occurs only once in a yeast cell. In contrast, UV irradiation causes many lesions in a cell for the repair of which an efficient interaction between ERCC1 and Rad16 seems to be necessary.

It is noteworthy that the ERCC1-deficient CHO mutant cell line 43-3B is highly sensitive to mitomycin C (4), a drug which causes interstrand crosslinks. Interstrand DNA crosslinks prevent strand separation and lead to a complete block of DNA replication and transcription. The hypersensitivity of ERCC1 mutants to mitomycin C indicates that ERCC1 protein may also be involved in an additional recombination repair pathway. That is in agreement with its role in mating type switching, a process which also involves recombination events.

Our results show that the human ERCC1 protein is the functional homologue of fission yeast Swi10. For NER a multi-enzyme complex consisting of several repair proteins has been postulated (36). For example, protein–protein interactions of ERCC1 with XPF and XPA have been demonstrated (37,38). We have shown that ERCC1 is able to interact with Rad16, the XPF homologue of fission yeast. In *S.pombe* a gene corresponding to human XPA is not yet known. In cells of higher eukaryotes free ERCC1 molecules are highly unstable (2); in contrast, the human ERCC1 protein is stable in *S.pombe* wild-type strains, as well as in strains with a deletion of the *rad16* gene (data not shown). Furthermore, overexpression of the full-length ERCC1 protein in a wild-type strain has no effect on repair function or on mating type switching (data not shown). Taken together, these results suggest that the (truncated) human ERCC1 protein is able to perform the different interactions which are necessary for NER and mating type switching in the heterologous yeast protein complex. To our knowledge ERCC1 is the first example of a human NER protein which functions in yeast. Analysis of the ERCC1 and Swi10 proteins in fission yeast should lead to further insights into the function of these repair proteins.

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