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Molecular cloning of *RAD16*, a gene involved in differential repair in *Saccharomyces cerevisiae*

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ABSTRACT

We have cloned the *RAD16* gene of *Saccharomyces cerevisiae* and determined its nucleotide sequence. The gene complements the UV sensitivity of a *rad16* mutant and restores the ability to repair the transcriptionally inactive *HMLα* locus that is absent in this mutant. Disruption mutants that were constructed using the cloned gene are viable and UV sensitive and show no detectable growth defect. Moreover, such a mutant is deficient for repair of the *HMLα* locus. The nucleotide sequence shows that the gene codes for a protein of 790 amino acids that has two potential zinc binding domains and shares homology with two other yeast proteins: the *RAD54* gene product involved in recombinational repair and *SNF2*, a transcription factor that possibly functions in transcription activation through an interaction with chromatin components that allows access of other factors involved in transcription. The role of *RAD16* in the repair of *HMLα* might be to change the chromatin structure of silenced genes to provide access for excision repair enzymes.

INTRODUCTION

In recent years it was shown that the efficiency of nucleotide excision repair of DNA damage induced by UV irradiation is heterogeneous for different regions of the genome (1). Preferential repair of transcribed genes as compared to inactive genes was demonstrated in cells from different eukaryotic origins (2, 3, 4). This preference could be due to the more open chromatin structure of transcribed genes. Within transcriptionally active regions in higher eukaryotic cells (5) and in *E.coli* (6) a preference for the transcribed strand within a transcription unit was shown to exist. The molecular basis of preferential repair remains to be elucidated. Sofar the evidence seems to suggest that the closed chromatin structure of inactive DNA might require additional factors that allow access of the excision repair enzymes while in a transcribed gene the template strand is preferentially repaired possibly through a mechanism that couples repair to the transcription machinery. Such a putative 'coupling-factor' was recently isolated and partially purified from an *E.coli* extract (7).

We study preferential repair of UV damage in *Saccharomyces cerevisiae* by comparing the removal of T4 endoV sensitive sites (ESS) from the two identical mating type loci *MATα* and *HMLα* that only differ in their transcriptional activity. In α-cells the *MATα* locus is actively transcribed whereas the *HMLα* locus is silenced as a result of a closed heterochromatin-like structure (8). Our earlier studies revealed that following UV irradiation of haploid α cells the active *MATα* locus is repaired preferentially to the inactive *HMLα* locus (4). Analysis of preferential repair in a large number of *rad* mutants (9) revealed that four mutants were impaired in the removal of pyrimidine dimers from the inactive *HMLα* locus. Two of these, *rad9* and *rad24*, are partially deficient in *HMLα* repair which might be related to a lack in UV-induced G2 arrest. Two other mutants, *rad16* and *rad7*, were completely impaired in *HMLα* repair. The last two mutants are partially repair-deficient members of the *RAD3* epistasis group (10). In the *rad16* mutant *HMLα* is not repaired while *MATα* is repaired although at a slower rate whereas in the *rad7* mutant *MATα* is repaired normally. Therefore the *RAD16* and *RAD7* gene products might be essential for making the chromatin of the silenced *HMLα* locus accessible for the excision repair enzymes. Phenotypically *rad16* and *rad7* cells resemble cells derived from patients carrying the human disorder xeroderma pigmentosum complementation group C in which active genes are repaired quite efficiently whereas inactive DNA is hardly repaired (11).

In this paper we describe the cloning and characterization of the *RAD16* gene. We show that the cloned gene complements the UV sensitivity of a *rad16* mutant and restores the capacity to repair the inactive *HMLα* locus. The *RAD16* protein shows interesting homologies with two other yeast proteins, *RAD54* and *SNF2*. Furthermore, some properties of *rad16* disruption mutants are described.

MATERIALS & METHODS

Strains and growth conditions

All the yeast and bacterial strains used in this study are listed in Table 1. Yeast cells were grown on complete medium (YEPD), or on YNB medium with the required growth factors at 28°C. *E.coli* strain JM101 (12) was used to propagate M13 derivatives and HB101 (13) as a host for all plasmids.

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Plasmids

For the construction of the chromosomal bank of yeast DNA and the subcloning of the *RAD16* gene the yeast centromeric plasmid YCp50 was used. The yeast episomal plasmid YEp24 (Biolabs) was used as a high copy number 2μm derived plasmid. Plasmid pUB20 which was derived from pUC19 (14) by removing the *Hind*III and *Sph*I unique sites was used to construct the *rad16* disruption mutants. For sequencing, subclones were constructed using pIC20R (15).

Transformation

For yeast transformations we used the lithium acetate method (16) except when the chromosomal bank was transformed to *rad16* cells. In that case transformation was done through electroporation according to a method kindly provided by Dr. B.Dujon (Pers. Comm.). Cells were grown in YEPD to 3×10^7 cells/ml and collected by centrifugation and pretreated for 15 min. at room temperature with 25 mM DTT. Pretreated cells were resuspended in buffer containing 270 mM sucrose, 1mM $MgCl_2$, 10 mM TrisHCl pH 7.5 at a concentration of 10^9 cells/ml. 100 ng of DNA was added per transformation. An electrical shock was given in a BIORAD capacitor in a 1 ml cuvette. (Electrical field 2250 Volt/cm, 200 Ω, capacitance 125−250 μF and time constant 15−30 mS).

DNA manipulations

In this study all restriction enzymes and polymerases were used according to the suppliers' prescriptions. DNA probes were labeled using random hexanucleotides and $\alpha[^{32}P]$dCTP (Amersham). Double strand DNA sequencing was performed using $\alpha[^{35}S]$dATP (Amersham) and T7 DNA polymerase (Pharmacia) according to the procedure proposed by the enzyme suppliers.

Construction of a yeast genomic bank

Partial *Sau*3A digested chromosomal yeast DNA was ligated into the *Bam*HI site of the centromeric plasmid YCp50. The bank was transformed to DDB7b (*rad16-1, ura3-52, trp1-289*) by electroporation. Ura$^+$ transformants were tested for UV sensitivity.

UV irradiation

Exponentially growing cells were collected by centrifugation and diluted in chilled PBS and irradiated with 254 nm UV (Philips T UV 30W) at a rate of 2 J/m^2/s. For the survival experiments appropriate dilutions of the cells were plated on YEPD agar and the survival was determined by counting the plates after 2−3 days of incubation at 28°C. For all the preferential repair experiments the cells were irradiated with 70 J/m^2 and subsequently collected by centrifugation and resuspended in YEPD and incubated for various time periods at 28°C in the dark. From these cells DNA was isolated for the determination of the removal of ESS.

Southern blot analysis showing the removal of ESS

The method used to show the removal of T4 endoV sensitive sites was described earlier (4). Briefly, genomic DNA was isolated (17) and purified on CsCl gradients (13) and digested with *Hae*II which generates a 4.0 kb *HML*α fragment and a 3.6 kb *MAT*α fragment. DNA samples were then divided into two equal parts from which one was incubated with T4 endoV and both were loaded on a denaturing agarose gel (1). After electrophoresis the DNA was transferred to Genescreen plus (NEN) and hybridized with an α-specific probe derived from plasmid pAK5 (Dr. A.Klar). The enzyme T4 endoV was isolated from *E.coli* cells that carry a plasmid containing the *DenV* gene (Dr. J.K.de Riel) according to a method described earlier (18).

Chromosomal location

Strain YNN295 (BIORAD) was used to isolate chromosomal DNA. Agarose blocks containing yeast DNA were prepared as described by van Ommen (19). Chromosomes were separated by pulsed field gradient electrophoresis using 1 cycle with pulse time 30−90 sec at 5.4 Volt/cm for 24 hours in 0.25×TBE buffer at 16°C. The gel was stained with ethidium bromide to localize the chromosome bands . The DNA was transferred to Genescreen plus (NEN) and hybridized with yeast chromosome specific probes. For chromosome V we used the 1.1 kb *Hind*III fragment containing the *URA3* gene. For chromosome II a 3.6 kb *Eco*RI fragment from plasmid pDP6 (20) that is internal to the *LYS2A* gene. Plasmid YCb7b19 which is derived from YCp50 and carries the *RAD16* containing 5.9 kb *Pvu*I fragment from YCb7b16, was linearized and used for hybridization to chromosome II (*RAD16*), IV (*CEN4* and *TRP1*) and V (*URA3*) simultaneously.

Construction of the disruption mutants

The 7.5 kb *Xho*I fragment from YCb7b19 containing the *RAD16* gene was cloned in the *Sal*I site of pUB20 to generate plasmid pUB21 that was used as the starting material for *RAD16* disruption. Plasmid pUB23 was constructed by replacing the 2.9 kb *Hind*III fragment in pUB21 (for restriction sites see figure 2) by the 1.1 kb *Hind*III fragment carrying the *URA3* gene. Plasmid pUB24 was made by replacing the 0.5 kb *Cla*I-*Sph*I fragment in pUB21 by a 1.2 kb *Cla*I-*Sph*I fragment also containing the *URA3* gene (derived from plasmid pIC20R-URA in which the 1.1 kb *Hind*III *URA3* fragment was cloned into the *Hind*III site of pIC20R (15). From both pUB23 and pUB24 the linear *Bam*HI-*Pvu*I fragment was isolated and used to transform cells from DDB5c. Ura$^+$ transformants were selected and purified. The strain carrying the *Hind*III deletion/insertion was designated DDB5c-235 and the strains carrying the *Cla*I-*Sph*I deletion/insertion DDB5c-243 and DDB5c-249.

Accession number of the *RAD16* sequence

The *RAD16* sequence was included in the GenBank/EMBL under accession number: M86929.

RESULTS

Isolation of a *rad16* complementing clone

We constructed a yeast genomic bank by ligating 10−20kb fragments from a partial *Sau*3A digest of chromosomal yeast DNA into the *Bam*HI site of the 8.1kb yeast centromeric plasmid YCp50 (21). This plasmid replicates autonomously at a low copy number due to the presence of the yeast centromere IV. We used a low copy number plasmid to avoid possible problems due to high expression of the cloned gene.

The bank was transformed into the yeast strain DDB7b (α, *rad16-1, ura3-52, trp1-289*) and Ura$^+$ transformants were checked for UV sensitivity. From 8000 Ura$^+$ transformants one UV resistant clone was isolated. This clone contained a plasmid designated YCb7b16 that carries a 14kb insert. Fig.1 shows the survival of *rad16* cells carrying YCb7b16 as compared to repair proficient cells and *rad16* cells without the plasmid. The plasmid

Table 1. Strains used in this study.

Strains	Genotype	Source
K107	*MATα, ho, gal, mal*	A.Klar
X14-2A	*MATα, ho, rad16-1, ade2-1, ade6*	YGSC
L113-2 (YS36)	*MATα, ho, his4-519, leu2-3, leu2-112, ura3-52, trp1-289*	Y.H.Steensma
DDB7b	*MATα, ho, ura3-52, trp1-289, rad16-1*	This laboratory
DDB3d	*MATα, ho, ura3-52, leu*	This laboratory
DDB5c	*MATα*, ho, ura3-52, his4-519	This laboratory
DDB5c-243	as DDB5c but *RAD16::URA3*	This laboratory
DDB5c-249	as DDB5c-243	This laboratory
DDB5c-235	as DDB5c but *RAD16::URA3*	This laboratory

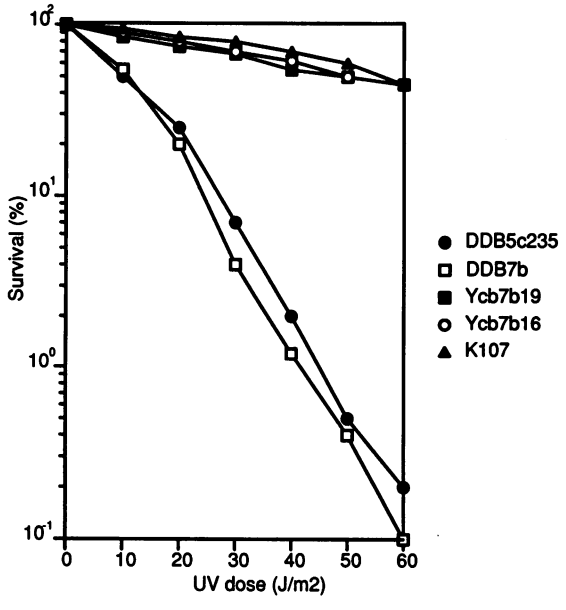

Figure 1. Survival after UV irradiation of cells from strain DDB7b (α, *ura3-52, trp1-289, rad16-1*) with and without plasmids carrying the *RAD16* gene (YCb7b19 and YCb7b16) and cells from strain K107 (α, *ho, gal, mal, Rad*$^+$). ▲ K107; □ DDB7b; ○ DDB7b with YCb7b16 (14kb insert); ■ DDB7b with YCb7b19 (5.9kb PvuI insert). Also shown is the survival curve for the disruption mutant ● DDB5c-243.

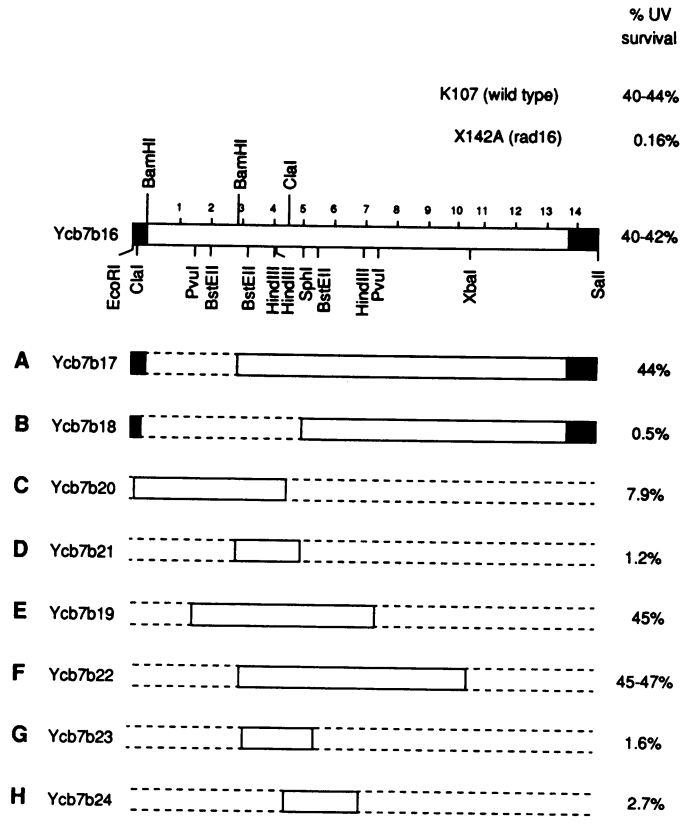

Figure 2. Restriction map of the chromosomal DNA insert in YCb7b16 conferring UV resistance to DDB7b (*rad16*). The EcoRI and SalI sites at the borders (black part) of the insert are in the tetracycline resistance gene of the vector YCp50. Shown are subclones from YCb7b16 in which various parts are deleted (dashed line) (fig.2 A−H). The UV survival of cells carrying YCb7b16 or the subclones was determined after irradiation with 60 J/m^2.

complements the UV sensitivity of *rad16* cells almost completely. A restriction map of the 14kb chromosomal insert carrying the complementing gene is given in Fig.2.

To localize the *RAD16* gene in the 14kb insert a set of deletions was constructed and tested for their ability to complement the UV sensitivity of the *rad16* mutant. The results summarized in Fig.2 show that the gene is situated to the right of the *Bam*HI site in the 5.9 kb *Pvu*I fragment. The UV sensitivity of *rad16* cells transformed with a plasmid containing this PvuI fragment (YCb7b19) is shown in Fig.1 and does not differ from that of cells containing a plasmid containing the original 14 kb insert (YCb7b16).

Earlier the yeast *RAD16* gene was mapped on chromosome II between *LYS2* and *CYC8* (22). Results obtained using pulsed field gradient electrophoresis (PFGE) were consistent with the localisation of the *rad16* complementing gene on chromosome II. (results not shown)

Restoration of repair of *HMLα*

The interesting feature of the *rad16* mutant is the absence of repair of the inactive *HMLα* locus (9). We tested whether the plasmids that complement the UV sensitivity also complement for this deficiency. We measure repair of UV induced pyrimidine dimers as the removal of T4 endoV sensitive sites by southern blot analysis using an α-specific probe that recognizes both *MATα* and *HMLα* (4). In all tests described in this paper the cells were irradiated with a dose of 70 J/m^2 which generates on average 1−2 dimers per restriction fragment containing *MATα* or *HMLα*. Figure 3 shows the results of repair experiments with strain DDB7b (*rad16*) (fig. 3A) or DDB7b carrying either plasmid YCb7b16 (14kb insert) (fig. 3B) or plasmid YCb7b19 (5.9kb *Pvu*I fragment)(fig. 3C). In the *rad16* mutant the repair of *HMLα* is clearly defective since virtually no removal of ESS from the *HMLα* fragment is observed even after prolonged repair periods (9h). In contrast, in cells transformed with either YCb7b16 or YCb7b19 the *HMLα* locus is repaired at the same rate and to the same extent as in *RAD*$^+$ cells. In conclusion, the *rad16*-complementing gene in the cloned fragment restores the ability to repair the *HMLα* locus.

The nucleotide sequence of the *RAD16* gene

The results described above show that the chromosomal insert cloned into YCb7b19 contains the *RAD16* gene. We therefore

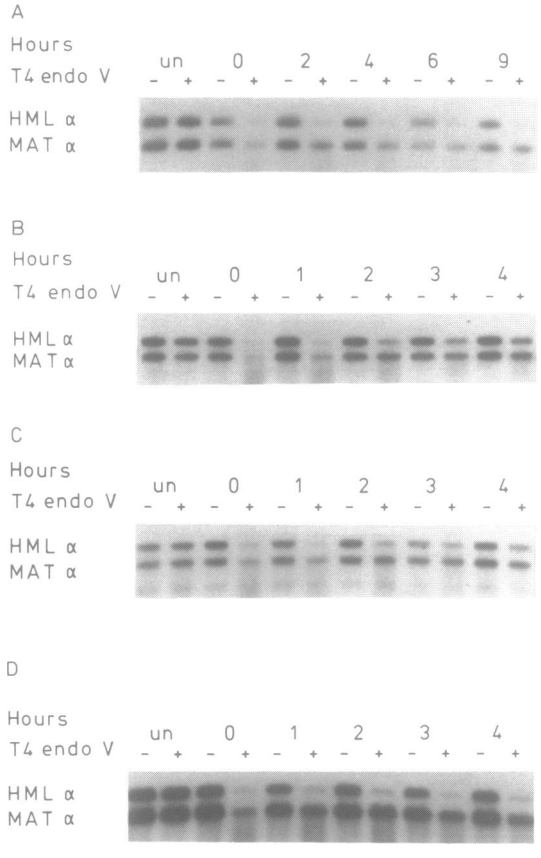

Figure 3. Southern blot analysis showing the removal of ESS from *MATα* and *HMLα*. Cells were irradiated at 70 J/m^2 and chromosomal DNA was isolated after various repair periods. After restriction with *Hae*II half of the DNA was treated with T4 endoV that incises the DNA at pyrimidine dimers. Samples were loaded on denaturing agarose gels and after electrophoresis the DNA was transferred to a membrane. The *MATα* and *HMLα* containing bands (3.6 and 4.0 kb respectively) were visualized using an α-specific probe. A: DDB7b (*rad16*), B: DDB7b with plasmid YCb7b16 (14 kb insert), C: DDB7b with plasmid YCb7b19 (5.9 kb insert), D: DDB5c-243 (disruption mutant). (+): pretreated with T4 endoV, (−): without pretreatment. Repair periods after irradiation are as depicted (hours). UN : unirradiated.

Figure 4. Nucleotide sequence of the *RAD16* gene and flanking regions and the amino acid sequence of the RAD16 protein in three-letter code. The putative 'TATA' boxes, the preceding CAAG sequence, the putative TACTAAC branchpoint for splicing and at the end of the gene the putative transcription termination signal are in bold.

decided to determine the nucleotide sequence of this fragment. The sequence of the 4235 nucleotides fragment containing the *RAD16* coding region and the 5′- and 3′-flanking regions is given in Fig. 4. The *RAD16* gene is determined by a 2373 nucleotides long open reading frame that starts with an ATG codon at position 802 (which is 1482 bp from the start of the *CYC8* gene) and ends with a TAA codon at position 3172 (which is 131 bp from the stop codon of the *LYS2* gene). That this open reading frame is indeed the *RAD16* gene was shown by the construction of disruption mutants carrying deletions/insertions in this reading frame (see below). The close proximity of the *CYC8* and *LYS2* genes confirms the position of the gene on *RAD16* chromosome II as established by physical mapping (22) and is in agreement with our PFGE analysis (see above).

Examination of the 5′ non-coding region of the *RAD16* gene revealed the presence of two possible 'TATA' boxes at positions −96 and −146 from the start codon that is preceded by the sequence CAAG (pos. −173) and a CT rich box (from pos. −174 to −236). Such a sequence has been found in a number of yeast genes that encode abundant mRNAs. (23). We also identified the consensus sequence TGA....AT rich....TTT at 64 nucleotides downstream of the stopcodon that might serve as termination signal for transcription as it was found for many yeast genes (24).

Within the open reading frame of the *RAD16* gene at position 839 from the start codon the sequence TACTAAC is present. This conserved box was identified as the branch point for splicing of yeast polymerase II transcripts (25) and is preceded by motif GTATGA (pos. 536) that has been shown to serve as the 5′ splice site in yeast messengers. The consensus sequence PyAG that serves as the 3′ splice site can be found at several positions but a sequence CAG at 8 nucleotides from the TACTAAC

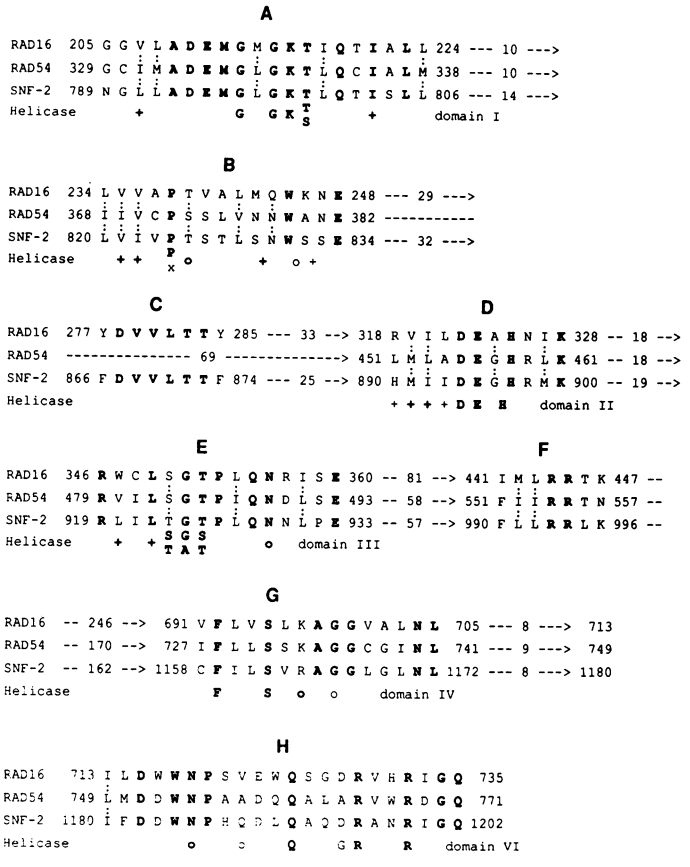

Figure 5. Homology between the *RAD16*, *SNF2* and *RAD54* gene products. Shown are the regions that have the highest homology. Underneath the protein sequences is the consensus for putative helicases (30). Bold capitals represent identical amino acids and amino acids that are similar (L-V-I-M, G-A, S-T, K-R, D-E-N-Q, F-Y-W) are marked by vertical lines. +, Hydrophobic residues. o, charged or polar residues.

branchpoint is at a proper distance and would leave the reading frame of the putatively spliced messenger intact. Preliminary results obtained with PCR on cDNA of total yeast RNA using *RAD16* specific primers, however, do suggest that at least under normal cicumstances the *RAD16* mRNA is not spliced. (results not shown).

The RAD16 protein, functional domains and homology with other proteins

The nucleotide sequence of the *RAD16* gene predicts a protein of 790 amino acids with a calculated molecular weight of 91,315 Dalton. The N-terminal part of the protein contains a high number of charged amino acids a feature that is also found in several other yeast repair genes (*RAD4*, *RAD6* and *RAD7*). At position 115−119 the sequence TKKRK is found that might serve as a nuclear localization signal (26). In the *RAD16* protein two cysteine rich regions are found that might serve as zinc-binding domains. At position 378−400 a sequence $CX_2CX_{14}CX_2C$ and at position 537−581 the sequence $CX_2CX_{11}CXHX_2CX_2CX_{16}CX_2C$. This last motif was recently found in the *RING1* gene and a computer search showed that this motif also exists in 9 other eukaryotic proteins including the human and mouse *RAG1* gene product involved in activation of V(D)J recombination, the human *RPT* protein which is the interleukin-2 receptor regulator and the yeast repair gene *RAD18* (27).

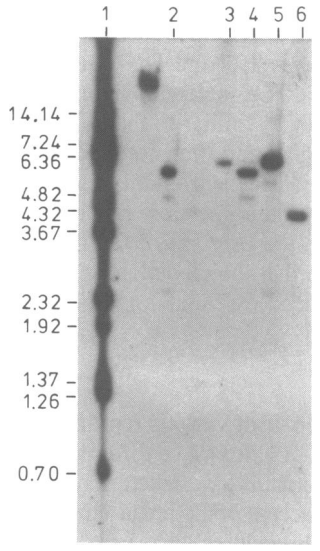

Figure 6. Southern blot analysis of disruption mutants. Total genomic DNA was isolated from a wild-type strain (DDB5c), from a rad16 mutant (X14-2A) and the *rad16* disruption mutants. The DNA samples were restricted with *Pvu*I and hybridized with 1,4 kb *Bst*EII fragment from YCb7b19 (see fig.2). Lane 1: Molecular weight standards from *Bst*EII restricted lambda DNA. Lane 2: DNA from a *rad16* mutant (X14-2A). Lane 3 and 5: DNA from the *Cla*I-*Sph*I *rad16* disruption mutants DDB5c-243 and DDB5c-249. Lane 4: DNA from the repair proficient strain DDB5c. Lane 6: DNA from the *Hin*dIII *rad16* disruption mutant DDB5c-235.

To determine whether the *RAD16* gene product has similarities with other known polypeptides we screened the EMBL databank. Homologies were found with two other yeast proteins: the *RAD54* gene product involved in recombinational repair (48.3% similarity, 25.9% identity) (28) and the transcription factor *SNF2* (51.1% similarity, 26.2% identity) (29). Most of the homology between the three proteins is found in the seven regions that are depicted in figure 5 (ABDEFGH). In addition a region was found that is only conserved between *SNF2* and *RAD16* (fig. 5C). Several of the stretches of homology share strong similarities with the different domains that together constitute the helicase motif as it was derived from a comparison of two related superfamilies of helicases (30). Therefore the three proteins are putative helicases.

Construction of *RAD16* disruption mutants

Using the cloned gene several chromosomal disruption mutants (see Materials and Methods) were constructed through homologous recombination. A southern blot analysis of chromosomal DNA isolated from such mutants clearly shows that the expected substitutions in the *RAD*16 gene have occurred (Fig. 6). In one such mutant (DDB5c235) the 2.9 kb HindIII fragment that carries the promotor region and almost the complete gene except for the C-terminal 239 bp was replaced by the *URA3* gene (fig. 6 lane 6). This mutant is viable and grows at a rate indistinguishable from the parental *Rad*$^+$ cells showing that the *RAD16* gene is not essential for growth. The disruption mutants provide the possibility to confirm the results obtained with the original *rad16* mutant that suggested that the *RAD16* gene product is essential for the repair of the inactive *HMLα* locus. For this purpose we used cells from strain DDB5c243 in which the internal 517 bp *Cla*I-*Sph*I fragment in the *RAD16* gene is replaced

by the *URA3* gene (fig. 6, lane 3 and 5). This small deletion/insertion disturbs only the ORF of the presumed *RAD16* gene and leaves the rest of the DNA sequences in the region between *LYS2* and *CYC8* intact. In figure 1 it can be seen that the survival after UV is similar to that of cells of strain DDB7b carrying the original *rad16* mutation and figure 4D shows that *HMLα* is not repaired in cells from this disruption mutant whereas *MATα* is repaired at the same slow rate completely comparable to that of *MATα* in the original *rad16* mutant (fig.3A). These results demonstrate that the cloned gene is *RAD16* and that the *RAD16* gene is indeed essential for the repair of *HMLα*.

DISCUSSION

Heterogeneity in repair of various regions of the genome might be the consequence of at least two different mechanisms. On one hand the heterochromatin-like structure might require additional proteins to provide access of the repair enzymes whereas the open structure of active chromatin allows direct repair. Within an active gene, on the other hand, the template strand might be repaired preferentially through a factor that couples excision repair to transcription (7). In yeast the silent *HML* and *HMR* loci of the mating type switch system (8) have a heterochromatin-like structure and therefore might function as a model system for studying the consequences of this structure on repair. We compared the removal of pyrimidine dimers from the inactive *HMLα* with that from the identical but active *MATα* locus. The *rad16* mutant was found to be deficient in the repair of *HMLα* whereas it can still repair *MATα* (9). The *rad16* mutant belongs to the *RAD3* epistasis group and is partially deficient in excision repair as is reflected by its moderate UV sensitivity (10). This phenotype is consistent with a specific role of the *RAD16* gene product in the repair of the inactive fraction of the chromosome. Here we report the cloning of the *RAD16* gene by complementation of the UV-sensitivity of the *rad16* mutant and some of its properties. Independently part of the gene was recently cloned and sequenced on the basis of homology with the *RAD54* gene (31). To avoid high copy problems we used the low copy plasmid YCp50 as the cloning vector but later we found out that the *RAD16* gene can also be maintained on a high copy 2 μm-derived plasmid (data not shown) without any obvious deleterious effects on the host cells. Using PFG Electrophoresis we were able to show that the complementing gene is situated on chromosome II and the sequence data revealed that the gene is in between *LYS2* and *CYC8*. These results are in agreement with the mapping data of the original *rad16* mutation (22) and affirm that the cloned gene is indeed *RAD16*. The fact that the gene not only complements the UV sensitivity of the *rad16* mutant but also restores its capacity to repair *HMLα* demonstrates that the inability to repair *HMLα* of the *rad16* mutant is indeed due to a mutation in the *RAD16* gene. These conclusions are further substantiated by the results obtained with the disruption mutant. Moreover, since the disruption mutants are viable and grow normally the *RAD16* product is not essential for growth. The disruption mutant shows no removal of pyrimidine dimers from the *HMLα* locus even after 9 hours of repair, demonstrating that *RAD16* is essential for the repair of this silent locus.

In the *RAD16* protein two cysteine-rich regions are found that could form putative Zn binding domains (32). The first domain is of the C_4 type that is also present in other repair genes like *RAD18* (33), the *E.coli* UvrA protein (34) and the FPG glycosylase protein (35). The second domain exactly matches the consensus $CCX(I,L,V)CX_{11-30}CXHX(F,I,L)CX_2C(I,L,M)X_{10-18}CPXC$ (C_3HC_4-motif) that was recently found in 9 other proteins in a variety of eukaryotic organisms including the yeast *RAD18* gene product (27). Four other proteins were recently found that carry the same motif and a synthetic peptide containing this motif is capable of zinc coordination and binds to DNA (P.S. Freemont, Pers. Comm.). Therefore these proteins including *RAD16* that match the consensus might be part of a new family of potential zinc-finger proteins with a DNA binding activity. The presence of a second Zn-binding domain next to the C_3HC_4 motif is not unique for *RAD16*. Recently it was shown that 5 members of the C_3HC_4 family carry 37 or 38 amino acids downstream of the C_3HC_4 sequence (called A-box) a second Zn-binding domain of the form CHC_2H_2 (called B-box). On this basis it was proposed that these 5 proteins form a subfamily within the C_3HC_4 group (36). Also *RAD18* carries a second Zn-binding domain downstream of the C_3HC_4 motif that, however, differs from the consensus for the B-box. In *RAD16* the second Zn-binding domain does not only differ from the consensus of the B-box but it is also upstream of the C_3HC_4 motif. The functions of the members of this family are very diverse and therefore it is difficult to speculate on the function of this type of zinc-finger in the specific role of the *RAD16* protein in repair.

The sequence of the *RAD16* gene shows homology with two other yeast genes: the *RAD54* gene involved in recombinational repair (28) and the transcription factor *SNF2* (29). The major part of the homology is found in regions that share a strong similarity with the different domains that together constitute the consensus for DNA helicases that was derived from the comparison of two related superfamilies of putative helicases (30). Members of a superfamily within the group of helicases share more homology within the helicase domains then is required by the minimal consensus for helicases. *RAD16*, *SNF2* and *RAD54* also share much more homology within the helicase domains. However, they differ clearly from the known superfamilies. Therefore *RAD16*, *SNF2* and *RAD54* might form a new family of DNA helicases.

The phenotype of the yeast *rad16* mutant resembles that of human cells derived from XP-C patients (e.g. no repair of inactive DNA). Recently a XP-C complementing clone was isolated. However the sequence of this gene seems to show no significant homology with that of *RAD16* nor does the gene contain a nucleotide binding site (domain I) essential for helicase activity (R. Legerski, Pers. Comm.).

The homology of *RAD16* with the recombination gene *RAD54* could suggest that *RAD54* plays a similar role in recombination repair as *RAD16* does in excision repair. One could also envisage that recombination in non-active heterochromatin-like structures requires proteins that alter the chromatin structure to permit access of the recombination enzymes. In support of this it was shown that recombination is stimulated by transcription (37) a process that leads temporarily to a more open structure of the chromatin. Arguing against an identical role as *rad16* is, however, that the *rad54* mutant is fully sensitive for X-rays (38) in contrast to the *rad16* mutant that is only moderately UV sensitive. The homology of *RAD16* with the transcription factor *SNF2* is very interesting with respect to its possible mechanism of action. *SNF2* (also called *SWI2*) was originally found to be involved in derepression of the *SUC2* gene that codes for invertase (39) but appeared to be also involved in the regulation of several other yeast genes like *HO* (40) and in transcription from the transposable element Ty1 (41). It was found that mutations in *SIN1* relieve the requirement for

SNF2 of the transcription of *HO* (42). Recently *SIN1* was sequenced and it appeared to share homology with the human *HMG1* gene (43). Some of the properties of the *SIN1* gene product are in agreement with a role as a high mobility group protein. Also deletions in the *HTA1-HTB1* locus that codes for the H2A and H2B histone proteins suppress a *snf2* mutation (44). On the basis of these results it was hypothesized that *SNF2* might function as a transcription factor that alters the chromatin structure thereby allowing transcription. The results presented in this paper show that *RAD16* is essential for the repair of *HMLα* as we had suggested before on the basis of the phenotype of the *rad16* mutant. The homology of *RAD16* with *SNF2* suggests that both gene products might play a similar role in changing chromatin structure: *SNF2* to allow transcription and *RAD16* to allow repair of heterochromatin-like structures.

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