

New factors in nucleotide excision repair : a study in saccharomyces cerevisiae

Dulk, B. den

Citation

Dulk, B. den. (2008, December 2). *New factors in nucleotide excision repair : a study in saccharomyces cerevisiae*. Retrieved from https://hdl.handle.net/1887/13304

Version:	Corrected Publisher's Version
License:	Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden
Downloaded from:	https://hdl.handle.net/1887/13304

Note: To cite this publication please use the final published version (if applicable).

Chapter

The Rad4 homologue YDR314C is essential for strand-specific repair of RNA polymerase I-transcribed rDNA in Saccharomyces cerevisiae

Adapted from Molecular Microbiology, Volume 56, Number 6, June 2005, pp. 1518-1526

The Rad4 homologue YDR314C is essential for strand-specific repair of RNA polymerase I-transcribed rDNA in *Saccharomyces cerevisiae*

Ben den Dulk, Jourica A. Brandsma and Jaap Brouwer

Summary

The *Saccharomyces cerevisiae* protein Rad4 is involved in damage recognition in Nucleotide Excision Repair (NER). In RNA polymerase II transcribed regions Rad4 is essential for both NER subpathways Global Genome Repair (GGR) and Transcription Coupled Repair (TCR). In ribosomal DNA (rDNA), however, the RNA polymerase I transcribed strand can be repaired in the absence of Rad4. In *Saccharomyces cerevisiae* the YDR314C protein shows homology to Rad4. The possible involvement of YDR314C in NER was studied by analyzing strand specific CPD removal in both RNA pol I and RNA pol II transcribed genes. Here we show that the Rad4-independent repair of rDNA is dependent on YDR314C. Moreover, in Rad4 proficient cells preferential repair of the transcribed strand of RNA pol I transcribed genes was lost after deletion of *YDR314C*, demonstrating that Rad4 cannot replace YDR314C. CPD removal from the RNA pol II transcribed *RPB2* gene was unaffected in *ydr314c* mutants. We conclude that the two homologous proteins Rad4 and YDR314C are both involved in NER and probably have a similar function, but operate at different loci in the genome and are unable to replace each other.

1 Introduction

Nucleotide Excision Repair (NER) is a DNA repair process capable of recognizing and removing a wide variety of helix distorting lesions, like the UV induced 6-4 photoproducts (6-4PP) and cyclobutane pyrimidine dimers (CPD). After recognition of the damage, a single strand DNA fragment containing the lesion is excised, allowing DNA synthesis using the undamaged strand as a template (de Laat *et al.*, 1999; Prakash and Prakash, 2000). The basic mechanism of NER is present in organisms ranging from *Escherichia coli* to man. The core NER proteins have been identified using an *in vitro* reconstituted system with purified proteins (Guzder *et al.*, 1995; He *et al.*, 1996; Mu *et al.*, 1996). One of the essential components of the NER reaction in *Saccharomyces cerevisiae* is the damage recognition protein Rad4. Binding of the Rad4-Rad23 complex to the damaged site initiates the recruitment of the other NER proteins that cooperatively complete the repair of the damaged DNA (Guzder *et al.*, 1998; Jansen *et al.*, 1998).

In vivo, additional proteins are required to facilitate efficient removal of lesions. Extensive studies in various organisms revealed that certain NER proteins are specifically involved in preferential repair of the transcribed strand of transcriptionally active DNA. This process is designated Transcription Coupled Repair (TCR) and, in yeast, requires Rad26, Rpb4 and Rpb9 (van Gool *et al.*, 1994; Li and Smerdon, 2002). Other proteins, like Rad7 and Rad16, are specifically involved in removal of lesions throughout the entire genome, a process referred to as Global Genome Repair (GGR). The core NER proteins, like Rad4, are essential for both GGR and TCR (Bang *et al.*, 1992; Verhage *et al.*, 1994). Previously, however, we showed that Rad4 is not essential for strand specific repair of RNA pol I transcribed rDNA, whereas all other core NER proteins, including Rad23, are indispensable (Verhage *et al.*, 1996a).

In human cells the XPC-hHR23B complex is homologous to the Rad4-Rad23 complex in *Saccharomyces cerevisiae* (Legerski and Peterson, 1992; Masutani *et al.*, 1994). In contrast to *rad4* mutants, cells devoid of XPC are completely defective in repair of RNA pol I transcribed rDNA (Christians and Hanawalt, 1994). Moreover, Rad4 and XPC differ in their contributions to GGR and TCR in RNA pol II transcribed genes. XPC cells are only defective in GGR (Venema *et al.*, 1991) whereas *rad4* cells lack both GGR and TCR (Verhage *et al.*, 1994).

The yet uncharacterized *Saccharomyces cerevisiae* protein YDR314C displays homology with established Rad4 homologues (Anantharaman *et al.*, 2001; Marti *et al.*, 2003). Moreover, analogous to Rad4, YDR314C is reported to co-immunoprecipitate with Rad23 in a large scale interaction study (Gavin *et al.*, 2002). These similarities suggest that the YDR314C gene product could be a functional Rad4 homologue.

In the fission yeast *Schizosaccharomyces pombe* two Rad4 sequence homologues were identified as well. Both homologues, designated Rhp41 and Rhp42, have been to shown to be involved in NER (Fukumoto *et al.*, 2002; Marti *et al.*, 2003). Strand specific repair analysis indicated that Rhp42 is involved in GGR whereas Rhp41 has a role in both TCR and GGR (Fukumoto *et al.*, 2002). Epistasis studies confirmed the role of Rhp41 in both NER subpathways (Marti *et al.*, 2003). However, deletion of *rhp42*⁺ in cells lacking GGR due to a mutation in the *rhp7* gene, resulted in increased UV sensitivity, whereas deletion of *rhp42*⁺ in TCR deficient *rhp26* mutants did not,

suggesting that Rhp42 is involved in TCR rather than GGR. On the other hand, transcription recovery, indicative for the efficiency of repair in transcribed DNA, was affected in *rhp41* cells but not in *rhp42* cells, contradicting the results from the epistasis analysis. Rhp41 and Rhp42 are apparently both involved in NER, but their relative contribution to GGR and TCR is not yet clear.

In *Saccharomyces cerevisiae* no function has yet been assigned to the *YDR314C* gene product. In this paper the involvement of YDR314C in NER is described. We show that YDR314C cannot substitute for Rad4 in RNA pol II transcribed regions but is essential for preferential repair of RNA pol I transcribed rDNA.

2 Results

A Rad4 homologue in Saccharomyces cerevisiae

Recently, an open reading frame in *Saccharomyces cerevisiae* was identified that shows substantial resemblance to Rad4 (Anantharaman *et al.*, 2001; Marti *et al.*, 2003). The homology between all functional Rad4 proteins is limited to the carboxyl terminal region referred to as a Rad4 protein family A (Rad4pfam-A) domain (Bateman *et al.*, 2004) (Fig. 1A). The exclusive conservation of the carboxyl terminal region suggests that the characteristics essential for NER are embedded within this domain. Indeed, for the human Rad4 homologue it was shown that the carboxyl terminal region is essential for the interactions with TFIIH, hHR23B and damaged DNA (Uchida *et al.*, 2002). The carboxyl terminal region of the yeast Rad4 homologues contains, partially overlapping the pfam-A domain, an ancient transglutaminase fold (Anantharaman *et al.*, 2001), which is also present in peptide-N-glycanases. In the Rad4 family members, however, the predicted catalytic residue is absent, suggesting that the transglutaminase fold is inactive. In contrast to the carboxyl termini, considerable diversity exists among the amino terminal regions of the Rad4 homologues. This indicates that apart from the shared function, additional functions might be present.

Interestingly, in *Saccharomyces cerevisiae* the yet uncharacterized ORF YDR314C encodes a protein containing a carboxyl terminal Rad4pfam-A domain (Marti *et al.*, 2003) (Fig. 1A,B). In addition to the sequence homology, the YDR314C gene product was, like Rad4, found to co-immunoprecipitate with Rad23 in a large-scale tandem-affinity purification (TAP) experiment (Gavin *et al.*, 2002). The sequence homology and the interaction with Rad23 indicate that YDR314C could be a genuine Rad4 homologue and consequently may have a similar function in NER. On the other hand, the UV sensitivity of *rad4* mutants is comparable to that of the other core NER mutants. Indeed, deletion of YDR314C, even in *rad4* and *rad16* mutants, does not affect sensitivity towards UV irradiation (Fig. 2A,B) or other DNA damaging and stress inducing agents (data not shown).



Figure 1

(A) Schematic representation of Rad4 homologues. The gray shaded boxes represent the conserved region that is categorized as a Rad4pfamA domain (Bateman *et al.*, 2004). The amino acid position is represented at the bottom of the figure.

Jack 373 EACCIVATE INSTRUCT SECURITY SECURITY SECURITY <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th> <th></th>								
Knp41 244	Ladi	270	EACTIVINELI	MSCOP DER	MEIDIS	LERBANYX	DIRKY PIDEC	317
Abp41 278 BEAACKANIGH FALGUL MARK ASYDIAL PH. LATHORS DID DUDY PHENET 239 DED140 288	khp4.1	264	RPEKR	RECIO SIGN	FERIPOW-DI	VTERTRIKVI	SP-KP/PV	336
APC 109	Ehp12	328	REPAIR/DI	PREQFL TEST	V2ADDR55.541	The Busid	BOUISSING:	327
TDEFINE 288	XPC.	087		KGFSUSVA85	5505UKROKK	NCS BROKASK	ES PRIDOL	991
ReA4 31.0 NONTOEX TEMPVALKT INCVENIENT APPSVACC INCVENTION See Rep11 307 SZEKINO VICTPEDING VICTPEDING VICTPEDING VICTPEDING SEE SEE </td <td>YDEJ14c</td> <td>283</td> <td>72000</td> <td>BOLTO ANI</td> <td>RUEV PT</td> <td>-100001VII</td> <td>Sharroans</td> <td>327</td>	YDEJ14c	283	72000	BOLTO ANI	RUEV PT	-100001VII	Sharroans	327
Rhp41 302 SZEKUYEN VKUPPENDE VIEWENER VKUPPENDE VIEWENER PERSONALE	Lad4	31.0	BINGERSEN.	STREPVOLKT.	I BOVRLESKL	APE3VACC	STEVERYEIA	365
Step13 120<	khp41	367	STRUCTURE	VONEPPEDAS	VIGKYRRF	EF3.88D	HLEONTY FA	353
SP2 9.22 ENERGISE UNIT/USER OUTCOMERTY NOT SECTION AND ADDRESS TO THE ADDRESS OF ADDR	Ehp12	12/1	TI200CIVETE	INVERVATING.	VYTE-DNTSF	EFEGATANET	IL-DESI AA	373
NDR14: 328 NELETERING UPTY_SPUEN NUMBER OF STATEMENT NUTALINES 328 Ladi 344 344 TENNYORE CHARNEL STATEMENT NUTALINES 413 Lapid 351 TENNYORE CHARNEL STATEMENT NUTALINES 413 Lipid 351 TENNYORE CHARNEL STATEMENT NUTALINES 413 JP2 351 TENNYORE CHARNEL STATEMENT NUTALINES 413 JP3 TENNYOR CHARNEL STATEMENT NUTALINES 413 JP3 TENNYOR CHARNEL STATEMENT NUTALINES 414 JP3 TENNYOR CHARNEL STATE ANNOLIS TENNYOR TENNYOR Lapid 414 CHARNEL STATE CHARNEL STATE ANNOLIS TENNYOR TENNYOR Lapid 139 CHARNEL STATE CHARNEL STATE TENNYOR	329-2	932	EVECTORE SE	MORECANSAAA	GOFOTCYKEN	TEP	WINENG	57.0
Ladi Kupia Med TEREFORM TERATORI SWS 25: T KUDIENDER SWITALINE KUPia Med TEREFORM SWITALINE KUPia	YDEJ14c	928	MELETRADER	ABLIEDRADO	KUNTUNKR	:12AA	DIRITING TO	363
Anp-13 351 TEACORAGE DESCRIPT YELLOUTER DEFENDATION DEFENDATION </td <td>Ladi</td> <td>366</td> <td>TERRYCORE</td> <td>BOSSAJKON-</td> <td>SKYSERE BT</td> <td>KOOK CONSTRUCT</td> <td>STITALIERS.</td> <td>413</td>	Ladi	366	TERRYCORE	BOSSAJKON-	SKYSERE BT	KOOK CONSTRUCT	STITALIERS.	413
Hep13 The TENDARY PARAMETER TOTAL SCHEDULE FOR TENARTHED FERMINATION FERMINALA C.1.1 MP3 TO DESCRIPTION FERMINATION FERMINATION FERMINATION FERMINALA C.1.1 Mail Ha Ha C.1.1 DESCRIPTION FERMINATION FERMINATION FERMINATION FERMINATION FERMINATION C.1.1 Hadd Ha	khp41	351	TEXTORYE	DROCLEY	YKILENE-RE	CEPERODARIES.	DIPERICKPO.	397
NP? 971 IEDDGAVE COMPARIZATION TUTE CF-TO ARMANILLA VIENDER UNDERVIEW 613 MA41 414 C-TELDERS SULJACHIT SUL	Ehp42	176	YENDORANE MA	DAULTER	SCR.Q-E-TP	INCENDES FD	PTEAT POOLA	121
NDR14c YC DVS PEVYZE CYC POLSES SPILOSULT STORLSWLK VLEXELAUVI (13) Ea.44 4.44 0VEIDYN DVFPGRDS SCHORNOLL SCHORNOLL SCHORNOLL	XPC	971	INCOMPANY	ICENDEVIN-	TVINECE-RD.	APPRILSTICP.	YOSP7WDREE	618
Ba34 414 E-TKIDDYE DYFCRUSS SUIF DENOUL Martin Martin SUIF DENOUL Martin Martin Suif DENOULL Martin Suif DENOULL Suif DENoull <th< td=""><td>YDRJ14c</td><td>370</td><td>DAR SEAA23</td><td>CYNEPILSIS</td><td>SPILACEURT</td><td>57291539LT</td><td>ATTEXCELTRAN</td><td>C12</td></th<>	YDRJ14c	370	DAR SEAA23	CYNEPILSIS	SPILACEURT	57291539LT	ATTEXCELTRAN	C12
Khp43 399 DYYLDENALE DELLELING BET FAILUR GET FAIL GE	Ladi	414	RTELODYS	HOVE FORDERS	BUIEDENOIL	00001210002.20	DEBOTOLVER	481
Rhp42 122 INSTANDET DESILSTAND IFE NETABLY DESILSTAND OF TAIGHT DESILSTAND OF DESILSTAND DESILSTAND <thdesilstand< th=""> DESILSTAND <thdesilstan< td=""><td>Khp41</td><td>199</td><td>DOV/SEMDATE</td><td>BACLESI 305</td><td>BRIDERSTOOL</td><td>COLUMN N</td><td>FREEMAKT</td><td>662.</td></thdesilstan<></thdesilstand<>	Khp41	199	DOV/SEMDATE	BACLESI 305	BRIDERSTOOL	COLUMN N	FREEMAKT	662.
NPP S13 XB SLEPCANING OPT TATALY ALMONINATION OPT TATALY ALMONINATION OPT TATALY ALMONINATION OPT TATALY INSTITUT STATUTE INSTITUT <t< td=""><td>Rhp42</td><td>122</td><td>TESTIMETRY</td><td>DERBLICKVP</td><td>IRE REPART</td><td>6 IF II.</td><td>IL PROPERTY.</td><td>671</td></t<>	Rhp42	122	TESTIMETRY	DERBLICKVP	IRE REPART	6 IF II.	IL PROPERTY.	671
YDR114c 13C DT ALEXCELATER FT NUMPER OTTING EPS LECTING EPS <thlecting eps<="" th=""> <thlecting eps<="" thr=""></thlecting></thlecting>	XPC	513	XB	SLEPCAKIND	OPERTNICLY.	10111127-025	DESTENSY?	660
Badd Rhpd1 462 EURISTORIES DENTED - RUL ZUMARRENAL LEGARDINE LEGARDINE LEGARDINE LEGARDINE LEGARDINE Sold Rhpd1 443 0-RECORDINE E-ADDURIAT PERSIAG 0.72 E-ADDURIAT 0.72 E-ADDURIAT ERECTOR 0.72 E-EDURIAT ERECTOR E-EDURIAT ERECTOR E-EDURIAT ERECORDIT <	YDR314c	120	II7	ATHERTALTK	PTL NOVTEI	OTTINF LPS	LECTEVERA	661
Rhp41 043 0-R2CORDIT E-N3V L.P.PTT333 SF ALE 1333 THEPADE 031 Rhp42 073 E-AD2VRIAT DEREMATE DEC.RP.VI CETERENCE THEPADE 031 XFC 063 C-AD2VAIAT DEREMATE ACCOUNT LIPETING THEPADE 051 XFC 063 C-AD2VAIRT DEREMATE ACCOUNT LIPETING 051 XFC 063 C-AD2VAIRT DEREMATE ACCOUNT LIPETING 051 XFC 063 C-AD2VAIRT DEREMATE ACCOUNT LIPETING 051 XFC 063 C-AD2VAIRT ESENCE DEREMATE 0400000000000000000000000000000000000	1444	462	DENERGYLEV	10823 EVL	200 AEPDIAD	ordia softense	Witness G. Br.	San
Rhp42 472 E-ALPONITAT FERGENATS DECORPTY CREATER FOR THE PERSIST S22 NPC 660 STMAILO YCA9	Rhp41	643	G-RACEBINT	E-N3V	LANDERVE ST	OF BRIDE WAY	SOLICENTICS	691
NPC 662 STIVALLE YC39 ALSENDENT LEPETALES A MALLSY 702 YDR114c 663 C-ADDAATTT ESENSO PUTYERD TO LEPETALES A MALLSY 702 Rad4 110 TVID-TVG	Rim42	472	E-ARPYRIAT	PERSENATS	DOC EDUCI	CREEP DESIGNATION.	S-WENESDO-	522
YDR114c 001 C-ADDATTY KINKSO PERVERT O LIFENDIAL SCHLERAR 507 Rad4 110 TAVIDA-TVG 220OH AH HIF2-DISPID DELETION 543 Rig41 192 DEMONSO OH OH OH VIEDISPID DELETION 543 Rig41 192 DEMONSO OH	XPC	562	BINAILO	YC38	AMERICANT	MERCERIAS.	2.5532.6576	702
Ra54 110 ZUVID-1796 22838 AB EIEZ-DISPEDIDED DELATIONAL 545 Rhp43 132 SUV-028AVT SZEC38 AB EIEZ-DISPEDIDED DELATIONAL 545 Rhp43 131 RMV-028AVT SZEC38 360 VLFBODS ALCOVERS 546 SKP 763 TWV-028AVT SZEC38 EIEZ-MARTS VLG-DISSD QUELWOPPE 566 SKP 763 TWV-0283	YDR314c	163	C-ANOLAIPT	KSCHS	PITA RD IC	IF SCHAIL	STATES AND	\$07
Rhp41 692 2.00707400000000000000000000000000000000	Endi	510	RATER-TYPE			tatz-Bora	NE STERA	545
Rhp41 511 E.M.Y.KRAVT ISZUSIN ELTRUATION VIGATION OLD OUT 2011 566 NP2 763 T.M.Y.GP03AAAKA HARPOIREE NELG-HEAVE OLD OF 2011 566 NP2 763 T.M.Y.GP03AAAKA HARPOIREE NELG-HEAVE OLD OF 2011 566 NP2 566 S.M.Y.H.M.KENAVE ENVIRONV VIENDAVY VIENDAVY OLD OF 2011 557 Ra54 546 SAGGLITUT T FORMULARY VIENDAVY VIENDAVY TECHTARY TOULLOOMY 557 566 Ra54 546 SAGGLITUT T FORMULARY VIENDAVY VIENDAVY TECHTARY TOULLOOMY 557 566 Ra54 546 SAGGLITUT T FORMULARY VIENDAVY VIENDAVY TECHTARY 55 TRUCTER 557 566 Ra54 546 SAGGLITUT T FORMULARY VIENDAVY VIENDAVY TECHTARY 55 566 566 Ra54 540 SAGGLITUT T FORMULARY VIENDAVY VIENDAVY TECHTARY 55 566 566 Ra54 540 SAGGLITUT T FORMULARY TECHTARY 55 570 570 566 S19 VIENDAVY 55 SAGGLITUT AND FORMULARY 55 570 570 571 S20 746 VIENDAVY 55 TO OFFILIP STATUT 500 571 573 S20 770 S20 S20 S20 570 573 <t< td=""><td>Rhu-47</td><td>392</td><td>- HW24</td><td></td><td></td><td>VLFIMDCC</td><td>AND ATERSY</td><td>518</td></t<>	Rhu-47	392	- HW24			VLFIMDCC	AND ATERSY	518
NPC 763 THEOROPSAATKB HIABPOLE E NELS-FORM ORE 0.244 763 NDRINE 566 REPUBLICAR ENVIRIENT VIENTAGED EPOLOFE DEVICES VERLEGORY 557 Rad4 546 SAGGLETET T EFERDIARY VIENTAGED EPOLOFE DEVICES VERLEGORY 557 Rad4 546 SAGGLETET T EFERDIARY VIENTAGED EPOLOFE DEVICES VERLEGORY 557 Rad4 547 SAGGLETET T EFERDIARY VIENTAGED EPOLOFE DEVICES	Rim42	521	R MY ARAVT	192XE31	I. RUARTARE	VLOG-MISSD	QHELEVE221	566
YDRIIG SCALENDER FORVENDERY VIENDRESS THEORY PET VEDLOCARY 557 Radd S40 SANGELTET INTERVENT INCOMPT DESCRIPTION S57 Radd S19 VAN-TUBER VIENDRAPT INCOMPT DESCRIPTION S56 Rhpd1 S19 VAN-TUBER VIENDRAPT INCOMPT DESCRIPTION S56 Rhpd2 S67 NDG-RUPE E FUVELEDE INTERVENT VEDLOR LANDON EN TO NERVE 558 INDONNANT INCOMPT INTERVENT S55 Rhpd3 S67 FORMAANT INCOMPT TO DEFINITE LARDON COL INFORMATION S56 Rhpd1 S67 FORMAANT INCOMPT VEDLOR VIENDRESS FORMALINE AND ALL INFORMATION Rhpd1 S67 FORMAANT INCOMPT VEDLOR VIENT S58 Rhpd1 S68 FORMAANT INCOMPT VEDLOR VIENT S58 Rhpd1 S67 FORMAANT INCOMPT VEDLOR VIENT S58 S100 784 S00 FORMANT INCOMPT VEDLOR VIENT S58 Rhpd1 S67 FORMAANT INCOMPT VEDLOR VIENT S58 S100 784 S00 FORMANT INCOMPT VEDLOR VIENT S58 S100 784 S00 FORMANT INCOMPT S58 S100 785 FORMANT S58 S100	XPC .	263	THMMSCP03-		RUNBPOLR PE	NCLO-REGYR	ONESTION 2VA	745
Rad4 346 SASGELTUET I IIIIINTARI I GUNDALTE SUPALITARIA Soft Rup41 S19 VAN-IU34 A YIIIINTARI SUPALITARIA SUPALITARIA </td <td>YDEJ14c</td> <td>100</td> <td>LARSSYLDER</td> <td>EPHVRNLDKY</td> <td>VIKELPAN</td> <td>THEFT</td> <td>VCENSOOMIV</td> <td>557</td>	YDEJ14c	100	LARSSYLDER	EPHVRNLDKY	VIKELPAN	THEFT	VCENSOOMIV	557
Rhp41 S19 VAN-IU3 A Y IDENVES Y NYHER HECHARA SS6 SS6 Rhp43 S67 ZEG-LEP G Y MECTURE L Y ZEGARLE SS6 G04 XP0 Y64 YDE-LEP G Y MECTURE L Y ZEGARLE SS6 G04 XP0 Y64 YDE-LEP G Y MECTURE L I CYOLEN LEPLING G04 YDELLAR YS8 TO DEVELOP L I CYOLEN LEPLING SS7 YS3 YDELLAR YS8 TO DEVELOP L I CYOLEN LEPLING ALMVIZIALIT ALMVIZIALIT YS3 Rup41 SS7 TS90MAR YS3 YS3 SS3 YS3 <	Ea.d4	540	SASSETTET	PREDUCART	TERONOCLUE	SEVALEAR		564
Rep41 567 FIG-TIPE G YEACOUSTS TOTALLE YESTATE TOTALLE YESTATE COL XP2 746 VD0-ENDE E FUTVLEDE H FUTVLEDE H </td <td>Rhp-12</td> <td>519</td> <td>VAN-IWRINA</td> <td>YNDIDLYVPS</td> <td>VL YEAVECR</td> <td>EXCALAN</td> <td>E</td> <td>556</td>	Rhp-12	519	VAN-IWRINA	YNDIDLYVPS	VL YEAVECR	EXCALAN	E	556
XPC 746 VDC-EUFRE E FLYYERLES ALTOVOLN LEFTING 763 YDRITHE 550 IVDC-EUFRE E FLYYERLES ALTOVOLN LEFTING 100 VDC EC. FLEYEREE 507 Re54 550 IVDC-EUFRE FLYYERLES ALTOVOLNE FLARENCE C. FLEYERDERE 507 Re54 557 TERDIARY FLYERES VOLVER IN ARVIELET ALTOTOTES. FLEYERDERE 603 Rhp41 557 TERDIARY FLYERES VOLVER IN ARVIELET ALTOTOTES. FLEYERDERE 603 Rhp41 557 TERDIARY FLYERES VOLVER IN ARVIELET ALTOTOTES. 603 Rhp43 605 TERDIARY FLYERES ALTOVER IN ARVIELET ALTOTOTES. 603 Rhp43 605 TERDIARY FLYERES ALTOVER IN ALTOTES. 603 YDEI14E 608 MONDERLEY END OF SHEVELS. 70 SHEVEL AND NEUTROPE 632 YDEI14E 608 MONDERLEYE HELD OF SHEVELS. 70 GOT Rhp41 606 EXEARVEET CILLOR TO ONE OF THE 03 Rhp41 606 EXEARVEET CILLOR TO ONE OF THE 03 Rhp41 606 EXEARVEET CILLOR TO ONE OF THE 03 Rhp41 606 EXEARVEET CILLOR TO ONE OF THE 03 Rhp41 606 EXEARVEET CILLOR TO ONE OF THE 03 Rhp42 544 ENCONTREL ALTOR FLAY OF THE 03 YDEI14E 545 LERONTHELE ALTOR FLAY OF THE CILLOR TO ONE OF THE 03 YDE114E 545 LERONTHELE ALTOR FLAY OF THE 03 ACTOR	Ehp42	567	7IG-IIP G	Y-MOCTVDS.	DEPENDINE.	YROTAKIG	E	604
YDRIIH: SAN ITOLENYER FUNDIMARY TO DEPELE LAROUTED. INFORMARE 507 Ruda SAN TOLENYER FUNDIMARY TO DEPELE LAROUTED. INFORMATION 534 Rhadi 557 TEOMARY VIECER-SY SNELLY VI SKATTANIO, IARBIDORE 633 Rhadi 557 TEOMARY VIECER-SY SNELLY VI SKATTANIO, IARBIDORE 633 Rhadi 655 TEOMARY VIECER-SY SNELLY VI SKATTANIO, IARBIDORE 633 Rhadi 655 TEOMARY TEORAGY SNELT IN FILSAMOTT INTOCHTRI 633 SPC 784 SECONDUCY I THE N-9Y SNELT IN FILSAMOTT INTOCHTRI 633 YDRIIH: 600 MENUTICES HIND NO-3RINA FR-10014 MENUTICASE Rhadi 635 DEFENDILES ACCENTEL 2 HESTERS TO 047 Rhadi 666 ENEARYNET CILLER TO DEFENDITE 533 Rhadi 666 ENEARYNET AND CAR OF HENDER 465 YDEIIH: 545 LAROUTIVLE - BENTIER 2 WYNERIA TH 635	XPC	746	YEG-EUPREE	ENNYLFLPG	KHOVDER R	LPULIER	B	763
Re34 Sas From LAPH INSCREAST WOVEFING ARMINISTIC ADDITION 634 Rha41 SS7 FROM LAPH INSCREAST WOVEFING ARMINISTIC ADDITION 634 Rha41 SS7 FROM LAPH INSCREAST WOVEFING ARMINISTIC ADDITION 633 Rha43 SS7 FROM LAPH INSCREAST WOVEFING ARMINISTIC ADDITION 633 Rha43 SS7 FROM LAPH INSCREAST ARMINISTIC ADDITION 633 ZFC 784 SD DOVEFING FROM LAPH INSCREAST CERTAINED AREADONE 632 YDEI14E SCR MONOPYLING FROM LAPH INSCREAST FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM LAPH INSCREAST FROM LAPH INSCREASE Rha41 SGR DOVEFINEL ADDITION FROM LAPH INSCREAST FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM LAPH INSCREAST FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM LAPH INSCREASE FROM STATEMENT INSCREASE Re34 S35 DOVEFINELE ADDITION FROM LAPH INSCREASE FROM STATEMENT INSCREASE Re34 S35 DOVEFINELE ADDITION FROM TO ADDITION FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM TO ADDITION FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM TO ADDITION FROM LAPH INSCREASE Re34 S35 DOVEFINELE ADDITION FROM TO ADDITION FROM LAPH INSCREASE Re34 S36 DOVEFINEL ADDITION FROM LAPH INSCREASE S36 DOVEFINEL ADDITION FROM LAPH INSCREASE S36 <tr< td=""><td>YDRJ14c</td><td>260</td><td>TTU:2012</td><td>D HELDINGKE</td><td>TREPERSE</td><td>Paktaker for</td><td>LIGATIONS</td><td>807</td></tr<>	YDRJ14c	260	TTU:2012	D HELDINGKE	TREPERSE	Paktaker for	LIGATIONS	807
Rhp41 557 T B DEAR VIE CR-MY SKELEVY SKENTELIG, IAEBIDOSDE 603 Rhp43 605 T MOREADY TOTOREASY SKELEVY SKENTELIG, IAEBIDOSDE 603 Rhp43 605 T MOREADY TOTOREASY SKENTELIG FILSADATT DITUDEDEE 603 ZPC 784 Z D D OVDET TOTORASY SKENTELIG FILSADATT DITUDEDEE 603 YDE114c 603 MOREDIANS FILD NO3 REAST FILSADATE 643 Re54 635 DEFRETHLEG A D S NTELL XAINSET FILSADATE 647 Rhp41 666 ENEARWRET CILL KAIT O S D D STARAWET CILL KAIT O S D STARAWET 647 Rhp41 666 ENEARWRET CILL KAIT O S D STARAWET CILL KAIT 643 Rhp41 666 ENEARWRET CILL KAIT O S D STARAWET CILL KAIT 643 Rhp42 644 ENEARMINET CILL KAIT ENEARMINET 645 YDE114c 645 LADOTTVLEG STATUTERA 645	J.= 24	282	-BOYERADE:	TOROTOROST	V2回V15回19回	ABVIRDER	ALDGIERED	634
Rep41 605 Total Rep41 605 Total Rep41 605 Total Rep41 605 Total Rep41 605 605 SPC 784 SDCDCVOLT Total Rep42 500 Total Rep41 605 607 SPC 784 SDCDCVOLT Total Rep41 605 Total Rep41 606 607 Rep41 606 DEFERDULES ALSONTIAL ZOTESTARS Total Rep41 606 606 Rep41 606 DEFERDULES ALSONTIAL ZOTESTARS Total Rep41 606 Rep42 64 DEFERDULES ALSONTIAL ZOTESTARS Total Rep41 606 Rep43 645 DEFERDULES ALSONTIAL ZOTESTARS Total Rep42 645 YDE114c 645 LADONTIVLE ALSONTIAL ZOTESTARS Total Rep43 YDE114c 645 LADONTIVLE ALSONTIAL Total Rep43	Rha-43	557	T B DUAK	VERDICR-RY	SKERLESVER	SERVICENDL	IABBIDOGER.	603
ZPC 784 XD/DCVONT FYRD DG-OY SERVICEST CEFERITILE ANENDATE 532 YDELINE 500 MCNECTING FILD CE-X	Etp43	605	Z M DZADAK	THE BURK- HR	ALEVIT IL	FEISADOTT	DISCRETERING P.	653
YDELLIE GON MONOPOLING AND NO2	XPC .	784	X D DOVD I	THEP DIG OY	SHEVIDEVIL	CREEKINGLE	AMENDONVIE	632
Red4 035 DEVERTURING A DEFINITION ZALE ZALESTICAS TO 047 Rhp41 506 EMEARWRET CILLER IT OFFICIALE IS 533 Rhp42 554 INCOMMENT TYD2305 IN AASID THE 04 533 Rhp42 333 REPERTURINE A DECEMPTION OF 545 YDE1146 545 INCOMPLES - SECTIONE ZALESTICAN TO 530	YDRJ16c	6CB	ACCORDENTIONS	1100 KDX	K 1141	NE BUELY	MEDTRASOL	548
Rhp41 666 ENEARNVRET CILLORG TT O HIPO VFE 1 603 Rhp42 C54 ENCOMMENT TYCO GO LEN ACCEPT THE O A COC Rhp43 C54 ENCOMMENT TYCO GO LEN ACCEPT THE O A COC Rhp43 C54 ENCOMMENT ENCOMPTO Rhp43 C54 ENCOMPTO ACCEPT THE CAC ACCEPT THE CAC Rhp44 C44 ENCOMPTO ACCEPT THE CAC ACCEPT THE CAC	J.a.54	635	DEVERSILLO	ABORDATION	2 THE SECON	TEE 047		
Rep42 054 INCOMPLET IVOCATION ACCEPTING ON A COL RPC 203 REPERZER A ON CL AX OF THE GER P 265 YDE114c 545 INCOMPLES - SECTIONER X EVEN DAW TH 580	Rhp41	666	ENEARAVRET.	CELLOKRUTT	OFFICERE	108 633		
NYC 2013 REPRESENT A DAVID AX OF THE LER PERSON YDRII4C 545 LADOTTYLLG - STUDIER ZADYLD AMA THE 580	Rhp42	654	INCOMPANY I	TYROUGHT	AND DESCRIPTION OF	OBA GAG		
YDRII4C 549 LANDYWYLG - BUSDIME ZERYNDIAU I'W 680	XPC.	833	ARRESPECT	ABONDOLBAX	GE LIEFLIGER	Page 365		
	YDEJ14c	549	THEOLESITE	- BREEDI TEK	ALCO NOT AND	THE 680		

В

(B) Alignment of the Rad4pfam-A domains of Rad4, Rhp41, Rhp42, XPC and YDR314C. Protein sequences were aligned with the clustalW program version 1.82. Similar and identical residues are boxed light and dark gray respectively.



UV survival test. Cells were grown for 3 days in YPD, diluted in water to OD_{600} values that resulted in 100-200 colonies for each of the 3 administered UV doses and for the non irradiated sample. The diluted cells were plated on YPD and irradiated with the doses indicated. The irradiated cells were grown for 3 days in the dark at 30°C, colonies were counted and survival was calculated. Survival after UV was determined and plotted as a function of the applied UV dose.

(A) UV survival of W1588 and *ydr314c* mutants (black and open circles respectively) and of *rad16* and *rad16ydr314c* mutants (black and open triangles respectively).

(B) Survival of *rad4* and *rad4ydr314c* mutants (black and open squares respectively). The values depicted in the graphs are averages of at least 3 independent experiments, error bars represent standard deviations.

CPD removal in RNA pol I transcribed rDNA

Previously we showed that the ribosomal DNA (rDNA) locus can be repaired in the absence of Rad4 (Verhage *et al.*, 1996a). The rRNA genes are present in ~150 tandemly repeated units of 9.1 kb. The densely packed rDNA is localized in the nucleolus, a membrane-free intranuclear compartment. The rRNA genes are highly transcribed, yet, depending on the growth rate, no more than 40% to 60% of the repeats is transcriptionally active (Dammann *et al.*, 1993). Each repeat consists of a 5S and 35S unit that is transcribed by pol III or pol I respectively. UV induced lesions in the rDNA locus are repaired by NER and it was shown that preferential repair of the transcribed strand occurs (Verhage *et al.*, 1996a; Conconi *et al.*, 2002; Meier *et al.*, 2002). Cells deleted for *RAD4* are still capable of repairing the RNA pol I transcribed strand of rDNA whereas repair is completely abrogated in cells lacking one of the other core NER proteins.

A plausible explanation for the Rad4-independent repair in rDNA could be that another protein fulfils the damage recognition role in NER in the RNA pol I transcribed regions. Considering the similarities of YDR314C and Rad4, we investigated the role of YDR314C in Rad4-independent repair. CPD removal from RNA pol I transcribed rDNA was analyzed in *rad4* and *rad4ydr314c* mutants using strand specific probes.

Cells lacking Rad4 are defective in CPD removal except for lesions in the RNA pol I transcribed strand, which can be repaired to approximately 50% (Fig. 3A,B) (Verhage *et al.*, 1996a). Interestingly, the Rad4-independent repair is completely abrogated when *YDR314C* is deleted (Fig. 3A,B), demonstrating that YDR314C is indeed responsible for the repair of RNA pol I transcribed rDNA in *rad4* mutants.

We subsequently examined the role of YDR314C in rDNA repair in cells containing functional Rad4. Single *ydr314c* mutants were analyzed for CPD removal in RNA pol I transcribed rDNA. Figures 3C and 3D show that in NER⁺ cells the non-transcribed strand is repaired slightly slower than the transcribed strand and that the overall repair of both strands is significantly lower compared to CPD removal in RNA pol II transcribed regions (compare Fig. 3C,D and 4A,B). After two hours, 70% of the lesions is removed from the transcribed strand and 65% from the non-transcribed strand, corresponding to our results reported earlier (Verhage *et al.*, 1996a).

In *ydr314c* mutants the percentage of removed lesions after two hours is reduced to 55% in the non-transcribed strand and 50% in the transcribed strand (Fig. 3C,D). Thus, in the absence of YDR314C a substantial amount of lesions can still be removed, albeit with lower efficiency. The slight decrease in dimer removal observed in the non-transcribed strand of rDNA might indicate that YDR314C is involved in GGR. However, the fact that GGR is completely defective in *rad4* mutants shows that YDR314C can not replace Rad4 in GGR, implying that YDR314C is not directly involved in GGR of pol I transcribed rDNA.

To investigate a possible role of YDR314C in strand specific repair, we measured the effect of a YDR314C deletion in GGR defective *rad16* cells. Due to the impaired GGR, the difference in repair-efficiency between the transcribed and non-transcribed strand is more pronounced in a *rad16* background (Verhage *et al.*, 1996b). For RNA pol I transcribed rDNA, deletion of *RAD16* does not lead to a complete defect in GGR like in RNA pol II transcribed genes, but lesion removal from the non-transcribed strand is reduced to 30%. A clear strand bias can be observed since the transcribed strand is repaired to 70% (Fig. 3E,F) (Verhage *et al.*, 1996a). Interestingly, preferential repair of the transcribed strand is completely absent after deletion of *YDR314C* in *rad16* mutants (Fig. 3E,F), even when lesion removal was analyzed after 4 hours of incubation (Fig. 3G,H). These results demonstrate that YDR314C is essential for the preferential repair of the RNA pol I transcribed strand in rDNA.

CPD removal in RNA pol II transcribed DNA

The experiments above show that Rad4 is unable to function in strand specific repair of RNA pol I transcribed rDNA, whereas YDR314C is essential for this mode of repair. Thus, Rad4 cannot replace YDR314C in rDNA repair. In RNA pol II transcribed genes on the other hand, NER is dependent on Rad4. To examine whether YDR314C can substitute for Rad4 in NER of RNA pol II transcribed genes, CPD removal from both strands of the *RPB2* gene was measured in *ydr314c* mutants. We show that the *YDR314C* deletion has no effect on the repair-efficiency (Fig. 4A,B), even when *YDR314C* is deleted in a *rad16* mutant, in which TCR is the sole mode of repair (Fig. 4C,D). These results demonstrate that YDR314C has no role in NER of the *RPB2* gene, suggesting that YDR314C is not involved in repair of RNA pol II transcribed genes in general.



Gene specific repair assay. Cells were grown in YPD, irradiated and allowed to remove lesions for the times indicated. Genomic DNA was extracted, digested with HindIII and either mock-treated or treated with T4endoV. Samples were run on an alkaline agarose gel, blotted on a nylon membrane and probed with an EcoRI-MruI rDNA fragment for either the transcribed strand (TS) or the non-transcribed strand (NTS). Fragments were visualized using a Bio-Rad Molecular Imager and fragment intensities were quantified with Quantity One (Bio-Rad). (A) Southern blots showing the removal of dimers from rDNA at various time points in *rad4* and *rad4ydr314c* mutants respectively. Time points after UV irradiation are indicated, samples mock-treated or treated with the dimer-specific enzyme T4endoV are denoted - and +, respectively. TS, transcribed strand; NTS, non-transcribed strand. (B) Graphical representation of quantified Southern blots. The percentage removed dimers as a function of time. *rad4* TS and NTS (black and open triangles respectively) and *rad4ydr314c* TS and NTS (black and open circles respectively). Values are the mean of at least three independent experiments. Error-bars indicate standard deviations. (C) As (A), but for W1588 and *ydr314c* cells. (D) As (B) but for *W1588* and *ydr314c* cells. (E) As (A), but for *rad16* and *rad16ydr314c* mutants. (G) As (E) but samples taken after 0, 120 and 240 minutes respectively.



Gene specific repair assay. Cells were grown in YPD, irradiated and allowed to remove lesions for the times indicated. Genomic DNA was extracted, digested with HindIII and either mock-treated or treated with T4endoV. Samples were run on an alkaline agarose gel, blotted on a nylon membrane and probed with an EcoRI-MruI rDNA fragment for either the transcribed strand (TS) or the non-transcribed strand (NTS). Fragments were visualized using a Bio-Rad Molecular Imager and fragment intensities were quantified with Quantity One (Bio-Rad).

(A) Southern blots showing the removal of dimers from rDNA at various time points in wildtype cells (W1588) and the *ydr314c* mutant. Time points after UV irradiation are indicated, samples mock-treated or treated with the dimer-specific enzyme T4endoV are denoted - and +, respectively. TS, transcribed strand; NTS, non-transcribed strand.

(B) Graphical representation of quantified Southern blots. The percentage removed dimers as a function of time. W1588 TS and NTS (black and open triangles respectively) and *ydr314c* TS and NTS (black and open circles respectively). Values are the mean of at least three independent experiments. Error-bars indicate standard deviations. (

C) As (A), but for *rad16* and *rad16ydr314c* mutants.

(D) As (B) but for rad16 and rad16ydr314c mutants.

3 Discussion

The YDR314C gene product shows homology to the members of the Rad4 family (Anantharaman *et al.*, 2001; Marti *et al.*, 2003) and interaction with Rad23 has been reported (Gavin *et al.*, 2002), suggesting a role for YDR314C in NER. In genome wide screens *ydr314c* mutants exhibit poor growth in medium containing nystatin or sorbitol (Giaever *et al.*, 2002). Furthermore, a synthetic lethal interaction of *YDR314C* and *CHS1* was reported (*Tong et al.*, 2004). These phenotypes might indicate involvement in processes like amino acid synthesis, osmoregulation and cell wall maintenance.

Here we show that the YDR314C gene product is responsible for Rad4-independent repair in the RNA pol I transcribed rDNA locus. Moreover, we demonstrate that YDR314C is not merely acting as a substitute when Rad4 is absent, but that preferential repair of the RNA pol I transcribed strand specifically requires YDR314C. The effect is especially evident in the GGR deficient *rad16* background, in which there is a clear difference in repair of the transcribed and non-transcribed strand. This strand bias is completely absent in *rad16ydr314c* double mutants, demonstrating that YDR314C, despite the presence of Rad4, is essential for preferential repair of the transcribed strand. The specific decrease in repair of the transcribed strand suggests that YDR314C is involved in TCR, however, we have not shown that in RNA pol I transcribed rDNA the preferential repair of the transcribed strand is dependent on active transcription. We therefore can not exclude the possibility that the YDR314C dependent repair in *rad16* cells is independent of transcription, but only occurring in the template strand.

Deletion of YDR314C has no effect on dimer removal from both strands of the RNA pol II transcribed *RPB2* gene. This suggests that YDR314C solely acts on RNA pol I transcribed regions and is unable to substitute for Rad4 in TCR of RNA pol II transcribed genes. The absence of UV sensitivity of *ydr314c* cells shows that removal of lesions from rDNA does not significantly contribute to survival. Considering that YDR314C was reported to co-immunoprecipitate with Rad23 and the fact that repair of rDNA is defective in *rad23* but not in *rad4* mutants, we assume that YDR314C functions, like Rad4, in complex with Rad23.

The two homologues Rad4 and YDR314C appear to have non-overlapping roles. Rad4 is essential for repair of both strands of RNA pol II transcribed genes and is unable to act in strand specific repair of genes transcribed by RNA pol I. YDR314C on the other hand is essential for preferential repair in RNA pol I transcribed rDNA and can not replace Rad4 in repair of RNA pol II transcribed regions. A simple explanation for the non-overlapping functions could be that Rad4 and YDR314C are prevented from travelling in and out the nucleolus respectively. However, the requirement of Rad4 for GGR of rDNA demonstrates that the inability of Rad4 to act in preferential repair of the transcribed strand of rDNA is not due to exclusion of Rad4 from the rDNA locus. Moreover, YDR314C appears not to be restricted to the nucleolus, since proteome-wide GFP localization experiments show that YDR314C are not spatially confined, we conclude that although Rad4 and YDR314C have homologous functions in analogous processes, they are unable to substitute for each other.

In Schizosaccharomyces pombe two Rad4 homologues are present as well. Involve-

ment of these proteins in repair of RNA pol I transcribed rDNA has not yet been studied. In contrast to Rad4 and YDR314C, Rhp41 and Rhp42 both seem to function, to different degrees, in GGR and TCR of RNA pol II transcribed genes (Fukumoto *et al.*, 2002; Marti *et al.*, 2003). Moreover, *rhp41rhp42* double mutants exhibit enhanced UV sensitivity compared to either single mutant, showing that the *Schizosaccharomyces pombe* Rad4 homologues have redundant functions. In addition to their role in NER, Rhp41 and Rhp42 are involved in NER dependent short-patch mismatch repair during meiosis (Marti *et al.*, 2003). A possible involvement of YDR314C and Rad4 in this type of DNA repair in *Saccharomyces cerevisiae* has not yet been investigated.

In human cells, XPC appears to be the only homologue of Rad4 since a second gene encoding a Rad4pfam-A domain containing protein is not present in the human genome (Bateman *et al.*, 2004). There are marked differences between the roles of XPC and Rad4 in NER. In *rad4* cells, repair of RNA pol II transcribed genes is completely defective whereas lesions in the RNA pol I transcribed strand of rDNA can still be removed. In human cells on the other hand, XPC is essential for repair of both strands of RNA pol I transcribed rDNA (Christians and Hanawalt, 1994) but not required for TCR in RNA pol II transcribed regions (Venema *et al.*, 1991). Here we show that in *Saccharomyces cerevisiae*, the Rad4-independent repair is explained by the involvement of YDR314C. It remains unclear how NER in humans can process lesions in the transcribed strand without XPC.

The reason why Rad4 and YDR314C are unable to replace each other at different loci in the genome is yet unknown. Possibly, differences in chromatin structure at different chromosomal positions determine the requirement for either Rad4 or YDR314C. The poorly conserved N-terminal region might harbor the properties that are necessary to perform NER at different loci in the genome. The difference in the N-termini among the Rad4 family members could also reflect additional functions of the Rad4 homologues, apart from their role in the NER reaction. Further studies are necessary to identify the factors that influence the requirement of either YDR314C or Rad4 to facilitate NER.

4 Experimental procedures

Strains and media

All experiments were conducted in the *Saccharomyces cerevisiae* W1588-4a background. The strains used in this study are listed in table 1. W1588-4a (Mortensen *et al.*, 2002) was kindly provided by R. Rothstein. Strain MGSC 471 (*rad16::hisG*) and MGSC 479 (*rad4::HisGURA3HisG*) were constructed analogous to the previously described MGSC 268 and MGSC 283 respectively (Jansen *et al.*, 2000), using a W1588-4a instead of a W303-1B background. *YDR314C* deletions were constructed by transforming target strains with a loxLEU2lox disruption cassette, created by ligating a loxLEU2lox fragment to PCR generated *YDR314C* flanking regions, using the following primers:

5'-TGGAACAGTGCTGAAAATGCGT, 5'-<u>TTCGGTGACC</u>GGTTTCAAGGTTT GACCCTTCG, 5'-<u>CATGGTTACC</u>GATTCGACGCTGTTTCGCAGAG and 5'-GGAGGCGATTCCACGTCGCTAT. Underlined sequences contain a BstEII restriction site by which the flanking regions were ligated to the loxLEU2lox sequence. Correct integration of the constructs was confirmed by Southern blot analysis. Strains MGSC 471, 537, W1588-4a and MGSC 517 were transformed with an URA3 fragment to obtain the URA3⁺ strains MGSC 578-581 respectively.

UV survival

Cells were grown for 3 days in YPD and diluted in water to appropriate OD_{600} values. The diluted cells were plated on YPD. NER⁺ cells were irradiated with 0, 20, 40 and 80 J/m², *rad16* cells with 0, 5, 20 and 35 J/m² and *rad4* cells with 0, 1, 2.5 and 4 J/m² respectively. Cells were grown for 3 days in the dark at 30°C, colonies were counted and survival was calculated. The values depicted in the graphs are averages of at least 3 independent experiments; error-bars represent standard deviations.

TT 11	4	37	•
Lable	1.	Yeast	strains
		10000	001001110

Strain	Genotype	Source
W1588-4a	MATa leu2-3,112 ade2-1 can1-100 his3-11,15	R. Rothstein
	ura3-1 trp1-1	This study
MGSC 471	rad16::hisG*	This study
MGSC 479	rad4::hisGURA3hisG*	This study
MGSC 517	<i>ydr314c::</i> loxLEU2lox*	This study
MGSC 518	<i>rad4</i> ::hisGURA3HisG <i>ydr314c</i> ::loxLEU2lox*	This study
MGSC 537	rad16::HisG ydr314c::loxLEU2lox URA3*	This study
MGSC 578	rad16::hisG URA3*	This study
MGSC 579	<i>rad16::</i> HisG <i>ydr314c::</i> loxLEU2lox URA3*	This study
MGSC 580	URA3*	This study
MGSC 581	<i>ydr314c::</i> loxLEU2lox URA3*	This study

*The remainder of the genotype is identical to that of W1588-4a

Sensitivity towards various chemical agents

Serial dilutions of stationary cells were made in water. Of each dilution 2ml was spotted on YPD or YNB plates with a concentration varying from 0 to 0.03% methyl methanesulfonate (MMS), 0 to 15 mg/ml cisplatin, 0 to 3 % dimethylsulfoxide (DMSO), 0 to 6 mM H₂O₂, 0 to 6 mM caffeine and 0 to 100 mg/ml 6-aza-uracil respectively. For the 6-aza-uracil test *URA3*⁺ cells were used. Cells were grown for 2 days at 30°C.

Repair analysis

Cells were grown in YPD to an OD_{600} of 4.0, pelleted, and resuspended in ice-cold PBS at an OD_{600} of 1.4. The cells were irradiated to 84 J/m² at a rate of 2.9 J/m²/s. The irradiated cells were pelleted, resuspended in YPD and kept at 30°C to allow repair. After 0, 30, 60 and 120 minutes cells were pelleted, resuspended in ice-cold water to stop repair, pelleted and frozen at -20°C prior to DNA isolation. DNA was isolated as described by Li and Smerdon (2002), with the following modifications. After the RNAse A+T treatment, ammonium acetate was added to a final concentration of 2.5M. The solution was kept on ice for 30 minutes. Following the removal of insoluble components by centrifugation the DNA was precipitated with ethanol. Repair of rDNA was measured as described previously (Verhage *et al.*, 1996a). Analysis of *RPB2* repair was performed as described previously (Jansen *et al.*, 2000). The Southern blots were quantified using a Bio-Rad Molecular Imager and Quantity One software. The values depicted in the graphs are the average of 3 independent experiments and the error-bars indicate standard deviations.

References

Anantharaman, V., Koonin, E.V., and Aravind, L. (2001) Peptide-N-glycanases and DNA repair proteins, Xp-C/Rad4, are, respectively, active and inactivated enzymes sharing a common transglutaminase fold. *Hum Mol Genet* **10**: 1627-1630.

Bang, D.D., Verhage, R., Goosen, N., Brouwer, J., and van de Putte, P. (1992) Molecular cloning of RAD16, a gene involved in differential repair in Saccharomyces cerevisiae. *Nucleic Acids Res* 20: 3925-3931.

Bateman, A., Coin, L., Durbin, R., Finn, R.D., Hollich, V., Griffiths-Jones, S., *et al.* (2004) The Pfam protein families database. *Nucleic Acids Res* **32 Database issue:** D138-141.

Christians, F.C., and Hanawalt, P.C. (1994) Repair in ribosomal RNA genes is deficient in xeroderma pigmentosum group C and in Cockayne's syndrome cells. *Mutat Res* 323: 179-187.

Conconi, A., Bespalov, V.A., and Smerdon, M.J. (2002) Transcription-coupled repair in RNA polymerase I-transcribed genes of yeast. *Proc Natl Acad Sci U S A* **99**: 649-654.

Dammann, R., Lucchini, R., Koller, T., and Sogo, J.M. (1993) Chromatin structures and transcription of rDNA in yeast Saccharomyces cerevisiae. *Nucleic Acids Res* 21: 2331-2338.

de Laat, W.L., Jaspers, N.G., and Hoeijmakers, J.H. (1999) Molecular mechanism of nucleotide excision repair. *Genes Dev* 13: 768-785.

Fukumoto, Y., Hiyama, H., Yokoi, M., Nakaseko, Y., Yanagida, M., and Hanaoka, F. (2002) Two budding yeast RAD4 homologs in fission yeast play different roles in the repair of UV-induced DNA damage. *DNA Repair (Amst)* **1**: 833-845.

Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., *et al.* (2002) Functional organization of the yeast proteome by systematic analysis of protein complexes. *Nature* **415**: 141-147.

Giaever, G., Chu, A.M., Ni, L., Connelly, C., Riles, L., Veronneau, S., *et al.* (2002) Functional profiling of the Saccharomyces cerevisiae genome. *Nature* **418**: 387-391.

Guzder, S.N., Habraken, Y., Sung, P., Prakash, L., and Prakash, S. (1995) Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. *J Biol Chem* **270**: 12973-12976.

Guzder, S.N., Sung, P., Prakash, L., and Prakash, S. (1998) Affinity of yeast nucleotide excision repair factor 2, consisting of the Rad4 and Rad23 proteins, for ultraviolet damaged DNA. *J Biol Chem* 273: 31541-31546.

He, Z., Wong, J.M., Maniar, H.S., Brill, S.J., and Ingles, C.J. (1996) Assessing the requirements for nucleotide excision repair proteins of Saccharomyces cerevisiae in an in vitro system. *J Biol Chem* 271: 28243-28249.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., *et al.* (2003) Global analysis of protein localization in budding yeast. *Nature* **425**: 686-691.

Jansen, L.E., Verhage, R.A., and Brouwer, J. (1998) Preferential binding of yeast Rad4.Rad23 complex to damaged DNA. *J Biol Chem* 273: 33111-33114.

Jansen, L.E., den Dulk, H., Brouns, R.M., de Ruijter, M., Brandsma, J.A., and Brouwer, J. (2000) Spt4 modulates Rad26 requirement in transcription-coupled nucleotide excision repair. *Embo J* **19**: 6498-6507.

Legerski, R., and Peterson, C. (1992) Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature* **360**: 610.

Li, S., and Smerdon, M.J. (2002) Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in Saccharomyces cerevisiae. *Embo J* **21**: 5921-5929.

Marti, T.M., Kunz, C., and Fleck, O. (2003) Repair of damaged and mismatched DNA by the XPC homologues Rhp41 and Rhp42 of fission yeast. *Genetics* **164**: 457-467.

Masutani, C., Sugasawa, K., Yanagisawa, J., Sonoyama, T., Ui, M., Enomoto, T., *et al.* (1994) Purification and cloning of a nucleotide excision repair complex involving the xeroderma pigmentosum group C protein and a human homologue of yeast RAD23. *Embo J* **13**: 1831-1843.

Meier, A., Livingstone-Zatchej, M., and Thoma, F. (2002) Repair of active and silenced rDNA in yeast: the contributions of photolyase and transcription-couples nucleotide excision repair. *J Biol Chem* 277: 11845-11852.

Mortensen, U.H., Erdeniz, N., Feng, Q., and Rothstein, R. (2002) A molecular genetic dissection of the evolutionarily conserved N terminus of yeast Rad52. *Genetics* **161**: 549-562.

Mu, D., Hsu, D.S., and Sancar, A. (1996) Reaction mechanism of human DNA repair excision nuclease. *J Biol Chem* 271: 8285-8294.

Prakash, S., and Prakash, L. (2000) Nucleotide excision repair in yeast. *Mutat Res* **451**: 13-24.

Tong, A.H., Lesage, G., Bader, G.D., Ding, H., Xu, H., Xin, X., *et al.* (2004) Global mapping of the yeast genetic interaction network. *Science* **303**: 808-813.

Uchida, A., Sugasawa, K., Masutani, C., Dohmae, N., Araki, M., Yokoi, M., *et al.* (2002) The carboxy-terminal domain of the XPC protein plays a crucial role in nucleotide excision repair through interactions with transcription factor IIH. *DNA Repair* (*Amst*) 1: 449-461.

van Gool, A.J., Verhage, R., Swagemakers, S.M., van de Putte, P., Brouwer, J., Troelstra, C., *et al.* (1994) RAD26, the functional S. cerevisiae homolog of the Cockayne syndrome B gene ERCC6. *Embo J* 13: 5361-5369.

Venema, J., van Hoffen, A., Karcagi, V., Natarajan, A.T., van Zeeland, A.A., and Mullenders, L.H. (1991) Xeroderma pigmentosum complementation group C cells remove pyrimidine dimers selectively from the transcribed strand of active genes. *Mol Cell Biol* **11**: 4128-4134.

Verhage, R., Zeeman, A.M., de Groot, N., Gleig, F., Bang, D.D., van de Putte, P., *et al.* (1994) The RAD7 and RAD16 genes, which are essential for pyrimidine dimer removal from the silent mating type loci, are also required for repair of the nontranscribed strand of an active gene in Saccharomyces cerevisiae. *Mol Cell Biol* 14: 6135-6142.

Verhage, R.A., Van de Putte, P., and Brouwer, J. (1996a) Repair of rDNA in Saccharomyces cerevisiae: RAD4-independent strand-specific nucleotide excision repair of RNA polymerase I transcribed genes. *Nucleic Acids Res* 24: 1020-1025.

Verhage, R.A., van Gool, A.J., de Groot, N., Hoeijmakers, J.H., van de Putte, P., and Brouwer, J. (1996b) Double mutants of Saccharomyces cerevisiae with alterations in global genome and transcription-coupled repair. *Mol Cell Biol* **16**: 496-502.