DNA REPAIR 8 (2009) 40–50

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/dnarepair

Yeast Elc1 plays an important role in global genomic repair but not in transcription coupled repair

*Danielle LeJeune, Xuefeng Chen, Christine Ruggiero, Shannon Berryhill, Baojin Ding, Shisheng Li**

Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA 70803, United States

ARTICLE INFO

Article history:
Received 30 May 2008
Received in revised form
28 August 2008
Accepted 28 August 2008
Published on line 7 October 2008

Keywords:
Elc1
Nucleotide excision repair
Rad7
Rad16
Rad26
Rpb9
Saccharomyces cerevisiae

ABSTRACT

Transcription coupled repair (TCR) is a nucleotide excision repair (NER) pathway that is dedicated to repair in the transcribed strand of an active gene. The genome overall NER is called global genomic repair (GGR). Elc1, the yeast homolog of the mammalian elongation factor elongin C, has been shown to be a component of a ubiquitin ligase complex that contains Rad7 and Rad16, two factors that are specifically required for GGR. Elc1 has also been suggested to be present in another ubiquitin ligase complex that lacks Rad7 and Rad16 and is involved in UV-induced ubiquitylation and subsequent degradation of RNA polymerase II. Here we show that *elc1* deletion increases UV sensitivity of TCR-deficient cells but does not affect the UV sensitivity of otherwise wild type and GGR-deficient cells. Cells deleted for *elc1* show normal NER in the transcribed strand of an active gene but have no detectable NER in the non-transcribed strand. Elc1 does not affect UV-induced mutagenesis when TCR is operative, but plays an important role in preventing the mutagenesis if TCR is defective. Furthermore, the levels of Rad7 and Rad16 proteins are not significantly decreased in *elc1* cells, and overexpression of Rad7 and Rad16 individually or simultaneously in *elc1* cells does not restore repair in the non-transcribed strand of an active gene. Our results suggest that Elc1 has no function in TCR but plays an important role in GGR. Furthermore, the role of Elc1 in GGR may not be subsidiary to that of Rad7 and Rad16.

1. Introduction

Nucleotide excision repair (NER) is a conserved DNA repair process that is capable of removing a large variety of helix-distorting lesions including UV-induced cyclobutane pyrimidine dimers (CPDs) and 6–4 photoproducts [1]. NER has traditionally been grouped into two pathways: transcription coupled repair (TCR) and global genomic repair (GGR) [2]. TCR is dedicated for repairing the transcribed strand (TS) of active genes and generally occurs faster than GGR, which removes lesions throughout the genome [2].

While the mechanism of TCR is relatively well understood in *Escherichia coli* [3], the detailed biochemical mechanism of this repair process remains largely elusive in eukaryotes [4–6]. It is generally thought that a stalled RNA polymerase at a DNA lesion serves as the initial signal for TCR [2]. In mammalian cells, Cockayne syndrome complementation group A (CSA) and B (CSB) proteins are required for TCR [7–10]. In *Sac-*

Abbreviations: NER, nucleotide excision repair; TCR, transcription coupled repair; GGR, global genomic repair; Pol II, RNA polymerase II.

* *Corresponding author*. Tel.: +1 225 578 9102; fax: +1 225 578 9895.
E-mail address: shli@vetmed.lsu.edu (S. Li).

1568-7864/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2008.08.010

charomyces cerevisiae, Rad26 [11], the homolog of human CSB, and Rpb9 [12–15], a non-essential subunit of RNA polymerase II (Pol II), have been shown to mediate two subpathways of TCR.

The process of GGR in mammalian cells depends on xeroderma pigmentosum complementation group C (XPC) [16,17] and damage-specific DNA-binding proteins (DDBs) [18]. In yeast, GGR has been shown to rely on Rad7 and Rad16, which show no significant sequence or structural similarity to XPC [19]. Rad7 and Rad16 form a stable heterodimeric complex termed nucleotide excision repair factor 4 (NEF4) [20]. Rad16 is a member of the Swi2/Snf2 family of ATPases, and the Rad7/Rad16 complex binds specifically and preferentially to UV damaged DNA in an ATP-dependent manner [20]. The precise roles of these proteins remain unclear. One suggestion is that the Rad7/Rad16 complex acts as an ATP-dependent motor which translocates along the DNA in search of damage, and upon encountering a lesion, ATPase activity is inhibited, stopping the enzyme [21]. This stalled complex may serve to remodel and open damaged chromatin, thereby facilitating recruitment of other repair proteins and access to the lesion [22,23]. Contrary to the supposition that Rad7 and Rad16 are involved the early steps of NER, including DNA damage recognition and stimulation of incision at damage sites, it has also been posited that the Rad7/Rad16 complex instead participates in the subsequent postincision events of oligonucleotide excision and repair synthesis [24].

Yeast Elc1 is a homolog of mammalian elongin C which forms a heterotrimeric complex with elongins A and B [25–29]. In mammalian cells, the elongin A, B and C complex increases the rate of transcription by suppressing Pol II pausing [28,29]. However, in yeast, only elongins A (Ela1) and C are present, and there is no evidence of a role for this complex in transcriptional stimulation [30]. The yeast Elc1 has been shown to be a component of a ubiquitin ligase that contains Rad7 and Rad16, and is responsible for regulating the levels of Rad4 protein in response to UV damage [31]. It was later found that this ubiquitin ligase complex also contains Cul3, and plays an important role in ubiquitination and subsequent degradation of Rad4 [32]. The ubiquitination of Rad4, but not its subsequent degradation, was shown to facilitate NER [32]. It has also been suggested that Elc1 is a component of another ubiquitin ligase complex, which contains Ela1, Cul3, and Roc1 and is responsible for the polyubiquitylation and subsequent degradation of Pol II in response to DNA damage [33,34].

We sought to determine the roles of Elc1 in different pathways of NER, and found that Elc1 has no function in TCR but plays an important role in GGR. Furthermore, we present evidence that the role of Elc1 in GGR is not subsidiary to that of Rad7 and Rad16.

2. Materials and methods

2.1. Yeast strains and plasmids

Wild type yeast strains Y452 (*MATα ura3-52 his3-1 leu2-3 leu2-112*) and BJ5465 (*MATa ura3-52 trp1 lys2-801 leu2Δ1 his3Δ200 pep4::HIS3 prb1Δ1.6R can1 GAL*) were obtained from Dr. Louise Prakash and the American Type Culture Collection, respectively. All deletions were made in these backgrounds and confirmed by PCR analysis using procedures described previously [14].

Strains with their genomic *RAD7* and *RAD16* genes tagged with three consecutive FLAG sequences (3 × FLAG) were created using PCR products amplified from plasmid p3FLAG-KanMX, as described previously [35]. PCR primers were designed to include about 20 bases complementary to the tagging cassette and approximately 50 bases complementary to the gene of interest. These primers were used to amplify the dictated segment using PCR and subsequently transformed into the appropriate yeast strains. The correct integration of the tagged sequences was confirmed by PCR.

The plasmid overexpressing 3 × myc tagged Rad16 was created using vector pESC-URA (Stratagene). The vector contains divergent *GAL1-10* promoters, and genes inserted downstream of the promoters can be highly induced by galactose. Two consecutive myc tag sequences were inserted in-frame downstream of the vector's native single myc sequence to create a vector for overexpressing 3 × myc tagged proteins under the control of the *GAL1* promoter. The *RAD16* gene coding sequence was amplified by PCR and inserted in-frame upstream of the 3 × myc sequences (between the XmaI and SalI sites) to create plasmid pRAD16-3 M.

The plasmid overexpressing 3 × FLAG tagged Rad7 was created using another modified version of pESC-URA. Two consecutive FLAG sequences were inserted in-frame downstream of the vector's native single FLAG sequence to create a vector for overexpressing 3 × FLAG tagged proteins under the control of the *GAL10* promoter. The coding sequence of the *RAD7* gene was amplified by PCR and inserted in-frame upstream of the 3 × FLAG sequences (between the SpeI and ClaI sites), yielding plasmid pRAD7-3F.

The plasmid simultaneously overexpressing 3 × FLAG tagged Rad7 and 3 × myc tagged Rad16 (pR16R7) was created by replacing the SpeI–PacI sequence (encompassing the FLAG sequence) in plasmid pRAD16-3 M with the entire RAD7-3 × FLAG segment (between the SpeI and PacI sites) from pRAD7-3F.

2.2. UV sensitivity assay

Yeast cells were grown at 30 °C in YPD medium (2% peptone, 1% yeast extract, 2% glucose) or minimal medium containing 2% galactose to saturation, and sequential 10-fold dilutions were made. The diluted samples were spotted onto YPD or YPG (2% peptone, 1% yeast extract, 2% galactose) plates. When the spots had dried, the plates were irradiated with different doses of 254-nm UV light. The plates were incubated at 30 °C for 3–4 (on YPD plates) or 5–7 (on YPG plates) days in the dark prior to being photographed.

2.3. UV irradiation, repair incubation, and DNA isolation

Yeast cells were grown at 30 °C in minimal medium containing 2% glucose or galactose to late log phase ($A_{600} \approx 1.0$), harvested, and washed twice with ice-cold water. The washed cells were resuspended in ice-cold 2% glucose (for glucose cultures) or 2% galactose (for galactose cultures) and irra-

diated with 80 J/m^2 of 254 nm UV light. One-tenth volume of a stock solution containing 10% yeast extract and 20% peptone was immediately added to the irradiated cell suspension. The cells were incubated at 30 °C in the dark and aliquots were collected at different time points. Total DNA was isolated from the collected cells using a glass beads method [14].

2.4. NER analysis of UV-induced CPDs

The gene fragments of interest were 3′ end labeled with [α-^{32}P]dATP using a procedure previously described [36,37]. Briefly, ~1 μg of genomic DNA was digested with restriction enzyme(s) to release the fragment of interest and incised at CPDs with an excess amount of purified T4 endonuclease V (Epicentre). Excess copies of biotinylated oligonucleotides, which are complementary to the 3′ end of the fragments to be labeled, were mixed with the sample. The mixture was heated at 95 °C for 5 min to denature the DNA and then cooled to an annealing temperature of around 50 °C. The annealed fragments were attached to streptavidin magnetic beads (Invitrogen) and the other fragments were removed by washing the beads at the annealing temperature. The attached fragments were labeled with [α-^{32}P]dATP (PerkinElmer) and resolved on sequencing gels. The gels were dried and exposed to a Phosphoimager screen (BioRad). The signal intensities at gel bands corresponding to CPD sites were quantified using Quantity One software (BioRad).

2.5. UV-induced mutagenesis

Cells were cultured to stationary phase in YPD medium. To select for canavanine resistant (Can^R) mutants, 100 μl of saturated culture from each strain was spread on minimal medium plates containing 60 mg/L of canavanine. Plates were irradiated at various doses of 254 nm UV light, and Can^R colonies were counted after 5–10 days of incubation in the dark at 30 °C. To measure the numbers of viable cells at the different UV doses, serial 10-fold dilutions of each saturated culture were spread onto minimal medium plates lacking canavanine. The plates were irradiated with the same doses of UV as those for selecting Can^R mutants and incubated in the dark at 30 °C before colonies were counted. Mutation frequencies were calculated by dividing the number of canavanine resistant colonies by the number of viable cells.

2.6. Western blot

Yeast cells were cultured in minimal medium to late log phase ($A_{600} \approx 1.0$) and pelleted by centrifugation. The cell pellet from a 5 ml culture was resuspended in 500 μl of 15% TCA and broken by vortexing them with acid washed glass beads. The proteins in the cell lysates were pelleted by centrifugation at 20,000 × *g* for 15 min at 4 °C. The protein pellet was washed with ice-cold 80% acetone and dissolved in 2 × sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel loading buffer [38]. The insoluble cell debris was removed by centrifugation.

Proteins in the whole cell extracts were resolved on an SDS-PAGE gel and transferred onto a polyvinylidene difluoride membrane (Immobilon-P; Millipore). 3 × FLAG tagged proteins were probed with an anti-FLAG antibody M2 (Sigma). As a loading control, Rpb1, the largest subunit of RNA polymerase II, was probed with antibody 8WG16, which specifically recognizes the C-terminal heptapeptide repeats of Rpb1 [39]. Blots were incubated with SuperSignal West Femto maximum-sensitivity substrate (Pierce), and the protein bands were detected using a chemiluminescence scanner (VersaDoc Imaging System; BioRad).

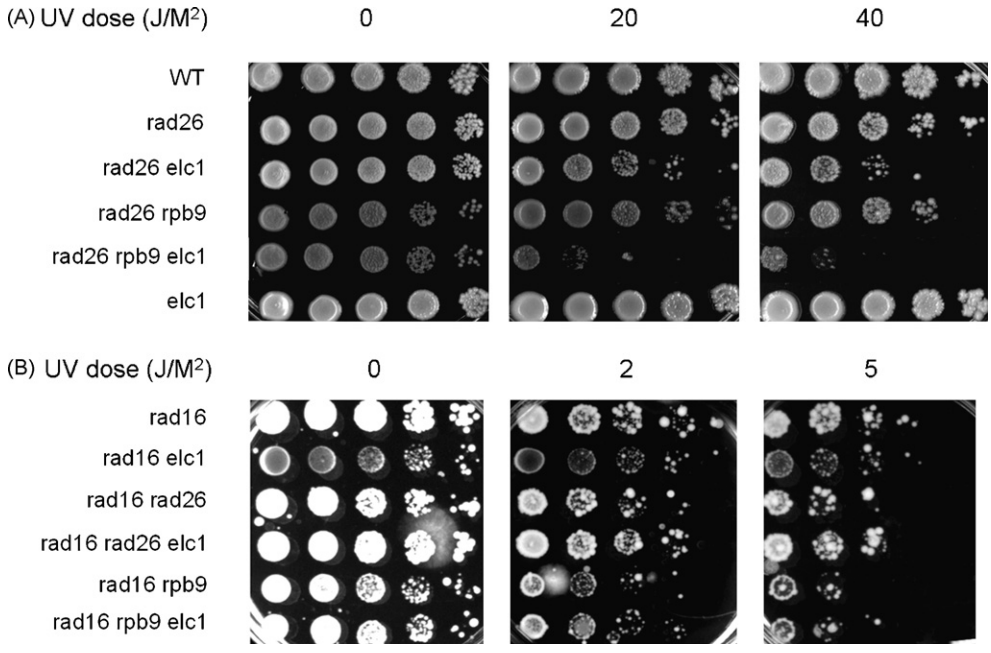

Fig. 1 – Effect of the *elc1* deletion on UV sensitivity of otherwise wild type and GGR- and TCR-deficient cells. Sequential 10-fold dilutions were made, spotted onto YPD plates, and irradiated with different doses of 254-nm UV light.

3. Results

3.1. UV sensitivities of elc1 mutants

It has been shown that *ELC1* is epistatic to *RAD7/RAD16*, and *elc1* deletion increases UV sensitivity of *rad26* cells [33]. In agreement with the previous report, *elc1* deletion did not enhance the UV sensitivity of otherwise wild type and GGR-deficient *rad16* cells (Fig. 1). The deletion also did not increase the UV sensitivity of *rad16 rad26* cells, where the Rpb9 mediated TCR is functional, and *rad16 rpb9* cells, where the Rad26 mediated TCR is operative [14,15]. This further indicates that *ELC1* is epistatic to *RAD16*. However, the deletion significantly increased the UV sensitivities of *rad26* cells, which are partially deficient in TCR (the Rpb9 mediated TCR is still active), and

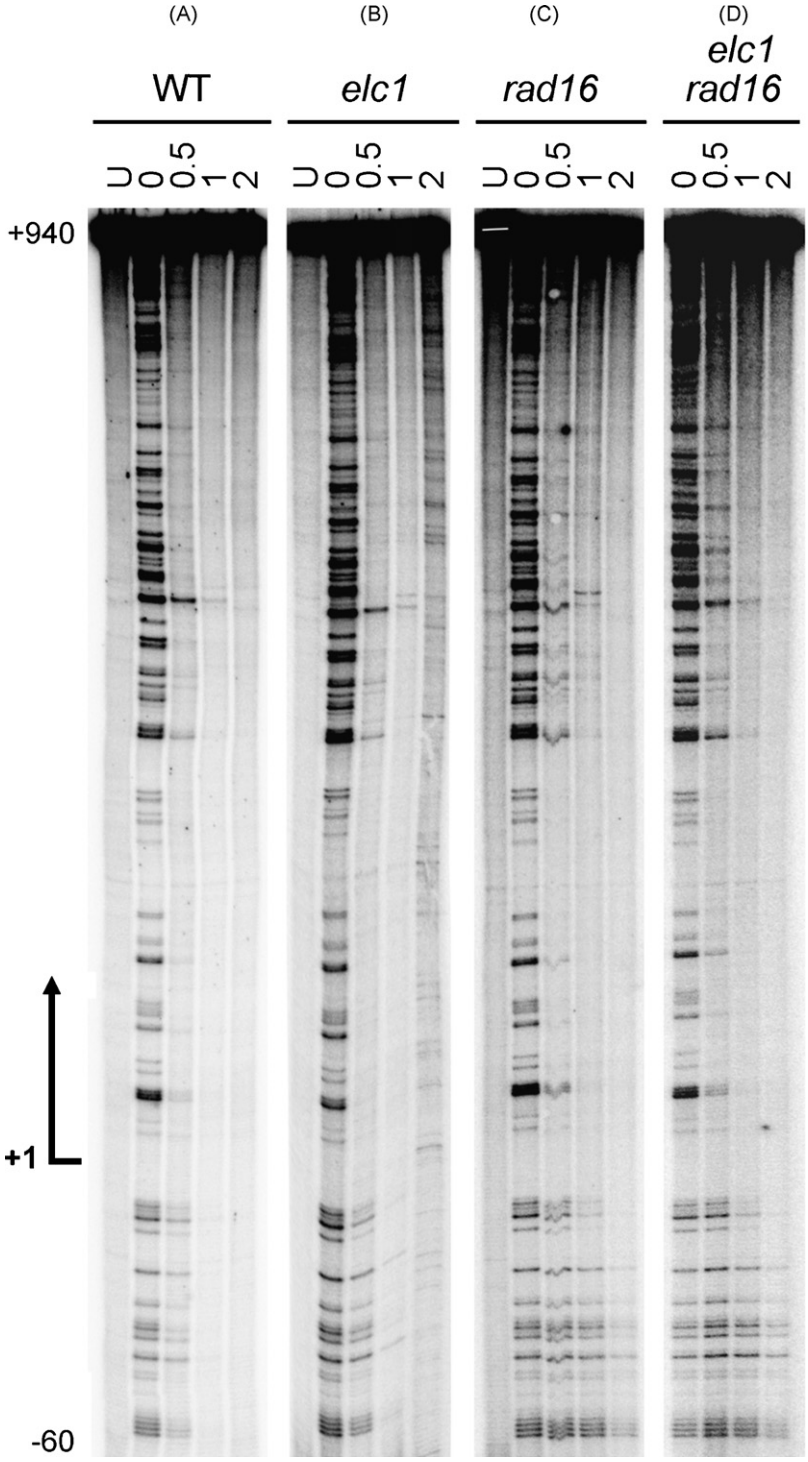

Fig. 2 – The *elc1 deletion* has no effect on TCR. Gels show NER in the TS of the *RPB2* gene. The lanes are DNA samples from unirradiated (U) and UV irradiated cells following different times (hours) of repair incubation as indicated at the top of the gels. The arrow on the left of the gels marks the transcription start site.

rad26 rpb9 cells, which are completely deficient in TCR [14,15] (Fig. 1A). Taken together, these observations support the idea that Elc1 plays a role in GGR but does not significantly affect TCR. However, the role of Elc1 in GGR may not be as significant as Rad7 and Rad16, as *elc1* single deletion mutants are not UV sensitive (Fig. 1A), whereas *rad7* and *rad16* cells are (Fig. 1B) [19,40,41].

3.2. *The role of Elc1 in GGR and TCR*

To examine to what extent Elc1 may contribute to GGR and to definitively determine if Elc1 plays a minor role in TCR that is not reflected by the UV sensitivity test, we directly analyzed the effect of *elc1* deletion on different NER pathways. Repair of CPDs in the constitutively transcribed *RPB2* gene was measured with a high resolution method, which uses streptavidin magnetic beads and biotinylated oligonucleotides to facilitate end-labeling of DNA fragments of interest [36,37]. Yeast cells were cultured to late log phase ($A_{600} \approx 1.0$), UV irradiated, and incubated in a repair medium for varying lengths of time. Total DNA was isolated, digested with a restriction enzyme to excise the fragment of interest, and incised at the UV-induced CPDs with an excess amount of T4 endonuclease V [42]. The incised fragments were strand-specifically end-labeled, resolved on a DNA sequencing gel, and exposed to a Phosphoimager screen [36,37]. The band intensities in the gel lane denoted "0" time

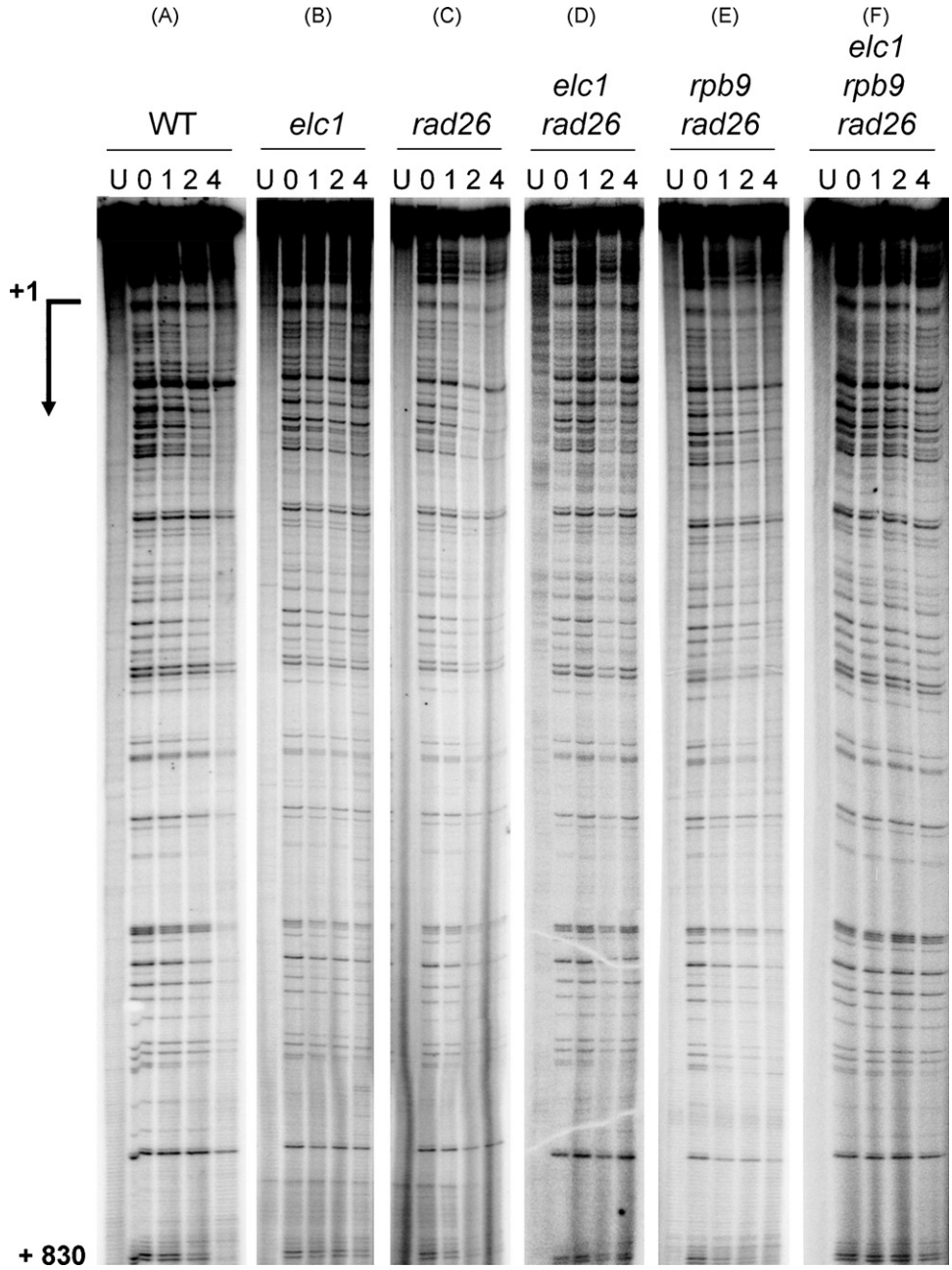

Fig. 3 – The *elc1* deletion results in undetectable GGR. Gels show NER in the NTS of the *RPB2* gene. The lanes are DNA samples from unirradiated (U) and UV irradiated cells following different times (hours) of repair incubation as indicated at the top of the gels. The arrow on the left of the gels marks the transcription start site.

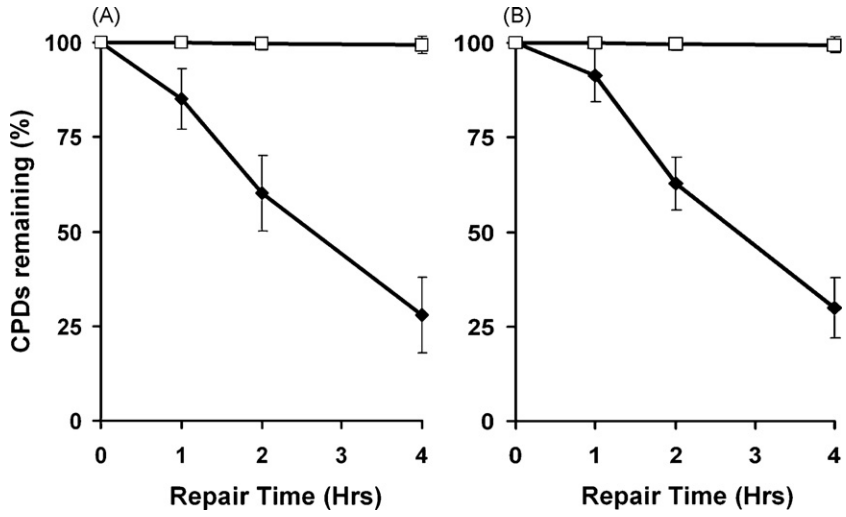

Fig. 4 – Plots showing repair of CPDs in the NTS of the *RPB2* gene. The data were obtained by quantification of the gels showing GGR in the different strains (as shown in Fig. 3). Plot in (A) shows the average (mean ± S.D.) of percent CPDs remaining at individual sites in the non-transcribed strand of the *RPB2* gene at different repair times in the cells of wild type (solid diamonds, quantified from gels as shown in Fig. 3A) and *elc1* (empty squares, quantified from gels as shown in Fig. 3B). Plot in (B) shows the average (mean ± S.D.) of the percent CPDs remaining at individual sites in the non-transcribed strand of the *RPB2* gene at different repair times in *rad26* cells (solid diamonds, quantified from gels as shown in Fig. 3C) and *rad26 elc1* cells (empty squares, quantified from gels as shown in Fig. 3D). It should be noted that some minor repair may not be able to be detected due to the semi-quantitative nature of the gels.

repair indicate the yields of CPDs at different sites. A decrease in band intensities at respective sites indicates CPD removal and DNA repair at these sites.

The NER rates in the TS of the *RPB2* gene were similar between wild type and *elc1* and between *rad16* and *rad16 elc1* cells (Fig. 2), indicating that Elc1 is entirely dispensable for TCR. It has been established that the NER rates in the NTS of an active gene reflect GGR [2]. Indeed, NER rates in the NTS of the *RPB2* gene of *rad26* and *rad26 rpb9* cells were similar to that of wild type cells (Fig. 3, compare panels A, C and E), indicating that Rad26 and Rpb9, two factors involved in TCR [14,15], do not play a role in the repair. In the period of 4 h incubation, no

Table 1 – Frequencies of canavanine resistant (Can^R) mutations in different isogenic strains

UV dose (J/m^2)		0	5	10	20	40	80
Wild type	Can^R frequency[a]	1.68 (0.83)	16 (1.01)	43 (19)	93 (6.2)	126 (10)	1014 (109)
elc1	Can^R frequency	0.77 (0.14)	6.6 (0.85)	41 (0.24)	122 (4.8)	285 (11)	668 (22)
	Fold increase[b]	0.46	0.42	0.96	1.31	2.27	0.66
rad16	Can^R frequency	27 (0.95)	1081 (113)	2856 (416)			
	Fold increase	16	69	67			
rad16 elc1	Can^R frequency	28 (0.97)	1048 (104)	3262 (673)			
	Fold increase	17	67	76			
rad26	Can^R frequency	9.2 (2.08)	24 (1.86)	37 (7.8)	77 (2.7)		
	Fold increase	5.45	1.55	0.86	0.83		
rad26 elc1	Can^R frequency	4.24 (0.54)	49 (9.47)	65 (20)	313 (129)		
	Fold increase	2.52	3.13	1.52	3.36		
rad26 rpb9	Can^R frequency	1.42 (0.23)	12 (2.16)	7.52 (0.64)	10 (2.18)		
	Fold increase	0.84	0.74	0.18	0.11		
rad26 rpb9 elc1	Can^R frequency	15 (1.44)	923 (19)	4909 (195)	6858 (921)		
	Fold increase	9.1	59	115	74		

[a] The frequencies (per 10^6 viable cells) of Can^R mutants are shown as the mean of at least three experiments. Numbers in parentheses are standard deviation.
[b] Relative to the frequency of Can^R mutants of wild type cells.

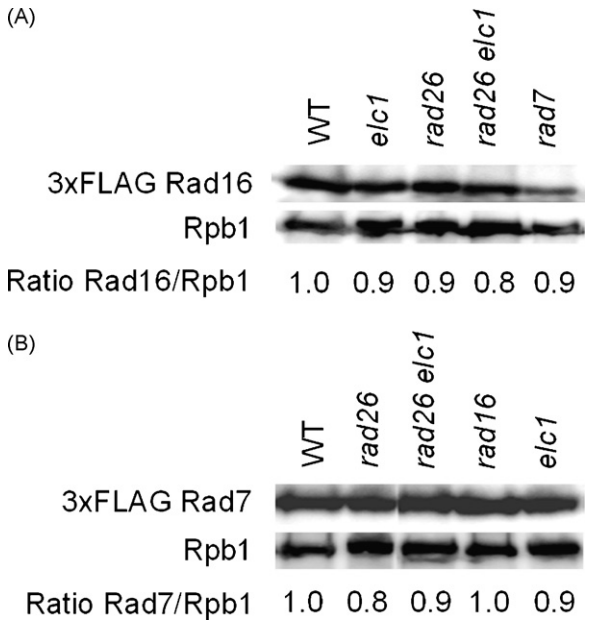

Fig. 5 – Rad7 and Rad16 protein levels are not decreased in *elc1* cells. Western blots show cellular levels of Rad7 (A) and Rad16 (B) levels in wild type and different mutant cells. Rpb1 serves as an internal loading control.

obvious repair can be detected in the NTS of the *RPB2* gene in all types of cells analyzed that lack Elc1 (Fig. 3B, D and F; Fig. 4), indicating that Elc1 plays an important role in GGR. This is surprising in view of the fact that *elc1* single deletion mutants are not UV sensitive, whereas the well-known GGR-deficient *rad7* and *rad16* cells are (see above). Repair in the time period of over 4 h was not performed because significant cell growth occurs if UV irradiated cells are incubated in repair media for over 4 h, and this obscures the fraction of repaired DNA in the samples (because of the increased fraction of newly replicated, undamaged DNA).

3.3. *The effect of elc1 deletion on UV-induced mutagenesis*

We also examined the effect of *elc1* deletion on UV-induced mutagenesis. All mutations resistant to canavanine, the toxic analog of arginine, occur at the single *CAN1* gene, which encodes the arginine permease [43]. Can^R mutants can be selected by plating cells on media containing canavanine instead of arginine [43]. The frequencies of UV-induced Can^R mutations in *rad16* cells was over 60 times of those in wild type cells (Table 1), indicating that Rad16 plays a significant role in preventing UV-induced mutagenesis. In contrast, the mutation frequencies were not dramatically higher in *rad26* (partially deficient in TCR) and *rad26 rpb9* (completely deficient in TCR) [14] cells than in wild type cells (Table 1). In fact, for an unknown reason, the mutation frequency appeared to be somewhat lower in *rad26 rpb9* cells than in wild type cells (Table 1). These results indicate that Rad26 mediated TCR or the entire TCR may contribute little to the prevention of UV-induced mutations.

The frequencies of UV-induced Can^R mutations were not dramatically different between *elc1* and wild type cells and between *rad16 elc1* and *rad16* cells (Table 1), indicating that Elc1 does not dramatically affect UV-induced mutagenesis in these TCR-proficient cells. However, the UV-induced mutation frequencies in *rad26 elc1* cells were ~2–4 times of those in *rad26* cells (Table 1), indicating that Elc1 may play a minor role in preventing UV-induced mutagenesis when TCR is partially defective. Intriguingly, the UV-induced mutation frequencies were dramatically higher (~70–680 times) in *rad26 rpb9 elc1* cells than in *rad26 rpb9* cells (Table 1), indicating that Elc1 plays a critical role in preventing UV-induced mutagenesis when TCR is completely abolished.

3.4. *Cellular Rad7 and Rad16 levels are not affected in the absence of Elc1*

It has been shown that, in *elc1* cells, the level of Rad7 is dramatically decreased, although the level of Rad16 is normal [31]. Furthermore, it has previously been demonstrated that the addition of the purified Rad7/Rad16 complex to a reconstituted NER reaction *in vitro* enhances incision of UV-damaged DNA [20], indicating that Rad7/Rad16 facilitates GGR in the absence of Elc1. These observations prompted us to examine if the function of Elc1 for GGR is achieved by keeping Rad7 from degradation and therefore maintaining the level of the Rad7/Rad16 complex. To this end, we genomically tagged the Rad7 and Rad16 proteins with three consecutive FLAG sequences (3 × FLAG) in wild type and different *elc1* deletion mutants. The 3 × FLAG tag did not cause any noticeable phenotypic changes to the cells (not shown). Expression levels of the tagged proteins were analyzed by probing with an anti-FLAG

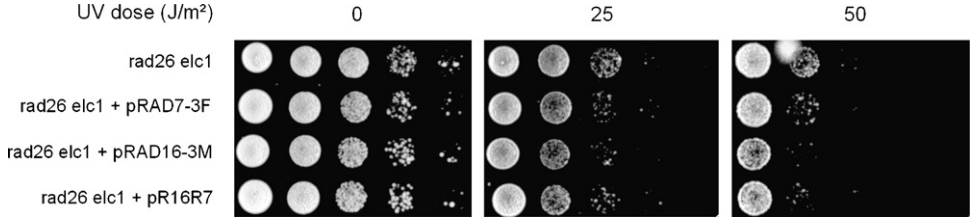

Fig. 6 – Overexpression of Rad7 and/or Rad16 does not restore UV resistance of *rad26 elc1* cells. Yeast cells containing plasmids overexpressing either 3 × FLAG tagged Rad7 or 3 × myc tagged Rad16, or both 3 × FLAG tagged Rad7 and 3 × myc tagged Rad16 and their parent strains (without plasmids) were sequentially 10-fold diluted, spotted onto agar plates containing galactose (to induce overexpression of the tagged proteins), and irradiated with different doses of 254-nm UV light.

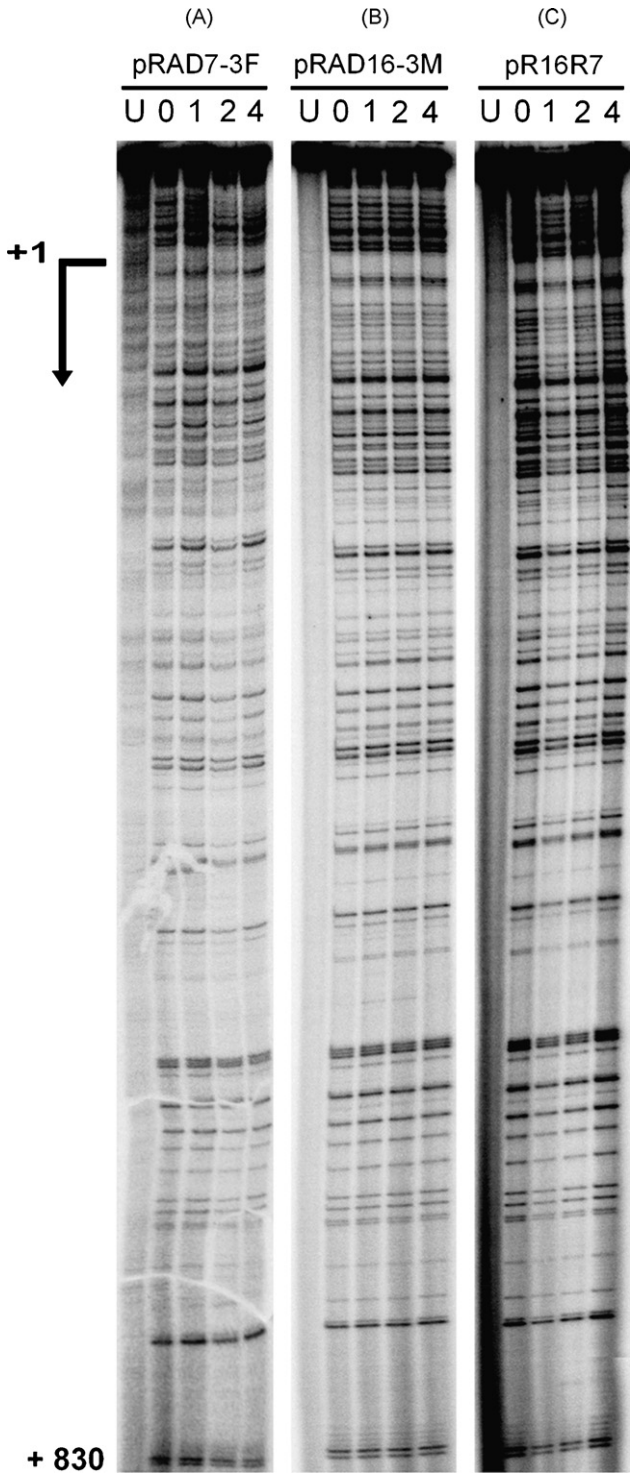

Fig. 7 – Overexpression of Rad7 and/or Rad16 does not restore GGR in *rad26 elc1* cells. Gels show NER in the NTS of the *RPB2* gene in *rad26 elc1* cells transformed with plasmids overexpressing 3 × FLAG tagged Rad7 (pRAD7-3F) or 3 × myc tagged Rad16 (pRAD16-3 M), or both (pR16R7). Lanes labeled U are unirradiated samples. Other lanes are samples with different times (hours) of repair incubation. The arrow on the left of the gels marks the transcription start site.

antibody on a Western blot. In agreement with the previous report [31], Rad16 levels were not significantly decreased in *elc1* and *rad7* cells (Fig. 5A), and Rad7 levels were not significantly decreased in *rad16* cells (Fig. 5B). Different from the previous report, however, Rad7 levels were not dramatically decreased in all *elc1* cells we analyzed (Fig. 5B). Our results indicate that the role of Elc1 in GGR may not be achieved by maintaining the levels Rad7 and Rad16 in the cells.

3.5. *The role of Elc1 in GGR can not be substituted for by overexpressing of Rad7 and Rad16 individually or simultaneously*

Next, we examined if overexpression of Rad7 and Rad16 individually or simultaneously could restore GGR in *elc1* cells. We surmised that if Elc1 merely served a subsidiary role, e.g., by assisting the Rad7/Rad16 complex, overexpression of the absolutely required Rad7 and/or Rad16 should be able to recover some, if not all, of the cell's capacity for GGR. To address this, we constructed multicopy plasmids overexpressing 3 × myc tagged Rad16 (pRAD16-3 M) and 3 × FLAG tagged Rad7 (pRAD7-3F) under the control of the highly inducible *GAL1* and *GAL10* promoters, respectively. The divergent *GAL1* and *GAL10* promoters share the same upstream activating sequence (UAS) and are induced identically by galactose [44]. We also created a plasmid (pR16R7) that simultaneously overexpresses 3 × FLAG tagged Rad7 and 3 × myc tagged Rad16. The plasmids overexpressing the different proteins were able to complement the UV sensitivity of the cells lacking the respective protein (not shown), indicating that the proteins encoded by them are functional for GGR.

We examined the effect of the overexpressions of Rad7 and/or Rad16 on the UV sensitivity of *rad26 elc1* cells cultured in a galactose medium (to induce the tagged proteins). The reason for using *rad26 elc1*, rather than *elc1* cells, for the examination is that *elc1* deletion enhances UV sensitivity in *rad26* cells (Fig. 1A) so that the restoration of UV resistance (and presumably GGR) can be easily observed. As shown in Fig. 6, the overexpressions did not significantly change the UV sensitivity of *rad26 elc1* cells.

We also directly examined the effects of Rad7 and/or Rad16 overexpressions on NER in the NTS of the *RPB2* gene in *rad26 elc1* cells. As can be seen in Fig. 7, the overexpressions did not restore the repair. Taken together, our results indicate that the role of Elc1 in GGR cannot be substituted for by Rad7 and Rad16, even when they are overexpressed.

4. Discussion

In this paper, we present the first direct evidence that Elc1 has no function in TCR but plays an important role in GGR. How Elc1 functions in GGR remains to be elucidated. One possibility is that Elc1, Rad7 and Rad16 need to be in one complex to be functional for GGR. It was shown that Elc1, along with Cul3, Rad7 and Rad16, is present in a ubiquitin E3 ligase complex, which regulates the efficiency of NER by ubiquitylating Rad4 [32]. However, it is unlikely that the role of Elc1 in GGR is achieved primarily by ubiquitylating Rad4. First, abol-

ishment of Rad4 ubiquitylation by mutating the Rad7 SOCS domain results in only a slight decrease in overall NER [32], which contrasts to the undetectable GGR in *elc1* cells. Second, Rad4 is an essential NER protein, required for both TCR and GGR [22], whereas Elc1 is only involved in GGR but not TCR.

It was shown that Elc1 also interacts strongly with a class of proteins that can be loosely defined as stress-responsive proteins [45]. Some of these interacting proteins were shown to be stabilized by Elc1 [46]. We did not observe significant changes in the levels of either Rad7 or Rad16 in *elc1* cells, indicating that Rad7 and Rad16 are not stabilized by Elc1. Furthermore, we observed that overexpression of Rad7 and Rad16 individually or simultaneously did not significantly enhance GGR in *elc1* cells, suggesting that the role of Elc1 in GGR is not achieved by stabilizing Rad7/Rad16. Therefore Rad7/Rad16 and Elc1 may mainly exist in different complexes and participate in GGR independently with each other.

The ubiquitin E3 ligase complex that is involved in ubiquitylation of Pol II was proposed to contain Elc1, Ela1, Cul3, and Roc1, but not Rad7 and Rad16 [34]. It was suggested that, in the absence of Rad26 or in the regions of the TS where Rad26-dependent TCR may be unable to act (e.g., in the promoter regions), Pol II removal upon the E3 ligase-mediated ubiquitylation may be a necessary precondition for repair to take place [34]. This proposition perfectly explained the observed epistasis of *rad7* over *elc1*, *ela1* and *cul3*, and the synergism between *rad7* and each of the E3 ligase components. However, in *elc1* cells, the abolition of NER in the NTS of a gene, where Pol II is not stalled by a lesion [47], cannot be explained by this proposition. Therefore, the role of Elc1 in GGR may not be achieved primarily by ubiquitylating Pol II.

In some cases, UV sensitivity of a mutant does not faithfully reflect its NER capacity, presumably due to the fact that UV sensitivity is caused by a combination of different DNA damage responses, such as NER, activation or suppression of certain genes, and checkpoint activation and recovery. The *elc1* single mutant, which is not UV sensitive but is deficient in GGR, is one of these cases. One possibility is that, deletion of *elc1* may activate a DNA damage response mechanism that somehow increases the cell viability following UV irradiation, although GGR is abolished or greatly compromised. If this is the case, the activation of the DNA damage response mechanism may require either TCR or the transcription elongation functions of Rad26 and Rpb9, as *elc1* deletion only increases the UV sensitivities of *rad26* and *rad26 rpb9* cells. Our observations that *elc1* deletion does not affect UV-induced mutagenesis in TCR-proficient cells but dramatically increases the mutagenesis frequency in *rad26 rpb9* cells support this idea. It is also possible that GGR in *elc1* cells is too slow to be detected. This slow repair, alone or combined with an activation of a DNA damage response mechanism, may be able to ensure cell viability following UV irradiation. A similar slow repair mechanism has been proposed for human TTD-A (trichothiodystrophy type A) cells, which lack a functional Tfb5, the 10th subunit of TFIIH, and are mildly UV sensitive but have very little NER activity [48–50]. Similar to human TTD-A cells, yeast cells lacking Tfb5 are also mildly UV sensitive [51] but have very little NER activity [52].

Conflict of interest statement

The authors state that there is no conflict of interest.

Acknowledgement

This study was supported by NIH grant ES012718 from the National Institute of Environmental Health Sciences, and by NSF grant MCB-0745229.

REFERENCES

[1] E.C. Friedberg, G.C. Walker, W. Siede, R.D. Wood, R.A. Schultz, T. Ellenberger, DNA Repair and Mutagenesis, ASM Press, Washington, DC, 2006.
[2] P.C. Hanawalt, Subpathways of nucleotide excision repair and their regulation, Oncogene 21 (2002) 8949–8956.
[3] C.P. Selby, A. Sancar, Molecular mechanism of transcription-repair coupling, Science 260 (1993) 53–58.
[4] M. Fousteri, L.H. Mullenders, Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects, Cell Res. 18 (2008) 73–84.
[5] J.P. Laine, J.M. Egly, When transcription and repair meet: a complex system, Trends Genet. 22 (2006) 430–436.
[6] A. Sarasin, A. Stary, New insights for understanding the transcription-coupled repair pathway, DNA Repair (Amst.) 6 (2007) 265–269.
[7] L. Lommel, P.C. Hanawalt, The genetic defect in the Chinese hamster ovary cell mutant UV61 permits moderate selective repair of cyclobutane pyrimidine dimers in an expressed gene, Mutat. Res. 255 (1991) 183–191.
[8] C. Troelstra, A. van Gool, J. de Wit, W. Vermeulen, D. Bootsma, J.H. Hoeijmakers, ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes, Cell 71 (1992) 939–953.
[9] A. van Hoffen, A.T. Natarajan, L.V. Mayne, A.A. van Zeeland, L.H. Mullenders, J. Venema, Deficient repair of the transcribed strand of active genes in Cockayne's syndrome cells, Nucleic Acids Res. 21 (1993) 5890–5895.
[10] J. Venema, L.H. Mullenders, A.T. Natarajan, A.A. van Zeeland, L.V. Mayne, The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 4707–4711.
[11] A.J. van Gool, R. Verhage, S.M. Swagemakers, P. van de Putte, J. Brouwer, C. Troelstra, D. Bootsma, J.H. Hoeijmakers, RAD26, the functional *S. cerevisiae* homolog of the Cockayne syndrome B gene ERCC6, EMBO J. 13 (1994) 5361–5369.
[12] S. Li, X. Chen, C. Ruggiero, B. Ding, M.J. Smerdon, Modulation of Rad26- and Rpb9-mediated DNA repair by different promoter elements, J. Biol. Chem. 281 (2006) 36643–36651.
[13] S. Li, B. Ding, R. Chen, C. Ruggiero, X. Chen, Evidence that the transcription elongation function of Rpb9 is involved in transcription-coupled DNA repair in *Saccharomyces cerevisiae*, Mol. Cell Biol. 26 (2006) 9430–9441.
[14] S. Li, M.J. Smerdon, Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in *Saccharomyces cerevisiae*, EMBO J. 21 (2002) 5921–5929.
[15] S. Li, M.J. Smerdon, Dissecting transcription-coupled and global genomic repair in the chromatin of yeast GAL1-10 genes, J. Biol. Chem. 279 (2004) 14418–14426.

[16] J. Venema, A. van Hoffen, V. Karcagi, A.T. Natarajan, A.A. van Zeeland, L.H. Mullenders, Xeroderma pigmentosum complementation group C cells remove pyrimidine dimers selectively from the transcribed strand of active genes, Mol. Cell Biol. 11 (1991) 4128–4134.
[17] J. Venema, A. van Hoffen, A.T. Natarajan, A.A. van Zeeland, L.H. Mullenders, The residual repair capacity of xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA, Nucleic Acids Res. 18 (1990) 443–448.
[18] J.Y. Tang, B.J. Hwang, J.M. Ford, P.C. Hanawalt, G. Chu, Xeroderma pigmentosum p48 gene enhances global genomic repair and suppresses UV-induced mutagenesis, Mol. Cell 5 (2000) 737–744.
[19] R. Verhage, A.M. Zeeman, N. de Groot, F. Gleig, D.D. Bang, P. van de Putte, J. Brouwer, 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 (1994) 6135–6142.
[20] S.N. Guzder, P. Sung, L. Prakash, S. Prakash, Yeast Rad7-Rad16 complex, specific for the nucleotide excision repair of the nontranscribed DNA strand, is an ATP-dependent DNA damage sensor, J. Biol. Chem. 272 (1997) 21665–21668.
[21] S.N. Guzder, P. Sung, L. Prakash, S. Prakash, The DNA-dependent ATPase activity of yeast nucleotide excision repair factor 4 and its role in DNA damage recognition, J. Biol. Chem. 273 (1998) 6292–6296.
[22] S. Prakash, L. Prakash, Nucleotide excision repair in yeast, Mutat. Res. 451 (2000) 13–24.
[23] F. Thoma, Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair, EMBO J. 18 (1999) 6585–6598.
[24] S.H. Reed, Z. You, E.C. Friedberg, The yeast RAD7 and RAD16 genes are required for postincision events during nucleotide excision repair. In vitro and in vivo studies with rad7 and rad16 mutants and purification of a Rad7/Rad16-containing protein complex, J. Biol. Chem. 273 (1998) 29481–29488.
[25] T. Aso, M.N. Conrad, Molecular cloning of DNAs encoding the regulatory subunits of elongin from *Saccharomyces cerevisiae* and *Drosophila melanogaster*, Biochem. Biophys. Res. Commun. 241 (1997) 334–340.
[26] T. Aso, W.S. Lane, J.W. Conaway, R.C. Conaway, Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II, Science 269 (1995) 1439–1443.
[27] T. Aso, N. Mokady, D. Haque, R.C. Conaway, J.W. Conaway, Assignment of a human gene encoding the 110-kDa subunit of general transcription factor elongin (SIII) to chromosome 1p36.1, Genomics 30 (1995) 393–394.
[28] J.N. Bradsher, K.W. Jackson, R.C. Conaway, J.W. Conaway, RNA polymerase II transcription factor SIII. I. Identification, purification, and properties, J. Biol. Chem. 268 (1993) 25587–25593.
[29] J.N. Bradsher, S. Tan, H.J. McLaury, J.W. Conaway, R.C. Conaway, RNA polymerase II transcription factor SIII. II. Functional properties and role in RNA chain elongation, J. Biol. Chem. 268 (1993) 25594–25603.
[30] C.M. Koth, M.V. Botuyan, R.J. Moreland, D.B. Jansma, J.W. Conaway, R.C. Conaway, W.J. Chazin, J.D. Friesen, C.H. Arrowsmith, A.M. Edwards, Elongin from *Saccharomyces cerevisiae*, J. Biol. Chem. 275 (2000) 11174–11180.
[31] K.L. Ramsey, J.J. Smith, A. Dasgupta, N. Maqani, P. Grant, D.T. Auble, The NEF4 complex regulates Rad4 levels and utilizes Snf2/Swi2-related ATPase activity for nucleotide excision repair, Mol. Cell Biol. 24 (2004) 6362–6378.
[32] T.G. Gillette, S. Yu, Z. Zhou, R. Waters, S.A. Johnston, S.H. Reed, Distinct functions of the ubiquitin-proteasome pathway influence nucleotide excision repair, EMBO J. 25 (2006) 2529–2538.
[33] B. Ribar, L. Prakash, S. Prakash, Requirement of ELC1 for RNA polymerase II polyubiquitylation and degradation in response to DNA damage in *Saccharomyces cerevisiae*, Mol. Cell Biol. 26 (2006) 3999–4005.
[34] B. Ribar, L. Prakash, S. Prakash, ELA1 and CUL3 are required along with ELC1 for RNA polymerase II polyubiquitylation and degradation in DNA-damaged yeast cells, Mol. Cell Biol. 27 (2007) 3211–3216.
[35] M.E. Gelbart, T. Rechsteiner, T.J. Richmond, T. Tsukiyama, Interactions of Isw2 chromatin remodeling complex with nucleosomal arrays: analyses using recombinant yeast histones and immobilized templates, Mol. Cell Biol. 21 (2001) 2098–2106.
[36] S. Li, R. Waters, Nucleotide level detection of cyclobutane pyrimidine dimers using oligonucleotides and magnetic beads to facilitate labelling of DNA fragments incised at the dimers and chemical sequencing reference ladders, Carcinogenesis 17 (1996) 1549–1552.
[37] S. Li, R. Waters, M.J. Smerdon, Low- and high-resolution mapping of DNA damage at specific sites, Methods 22 (2000) 170–179.
[38] J. Sambrook, D.W. Russell, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
[39] N.E. Thompson, T.H. Steinberg, D.B. Aronson, R.R. Burgess, Inhibition of in vivo and in vitro transcription by monoclonal antibodies prepared against wheat germ RNA polymerase II that react with the heptapeptide repeat of eukaryotic RNA polymerase II, J. Biol. Chem. 264 (1989) 11511–11520.
[40] D.D. Bang, R. Verhage, N. Goosen, J. Brouwer, P. van de Putte, Molecular cloning of RAD16, a gene involved in differential repair in *Saccharomyces cerevisiae*, Nucleic Acids Res. 20 (1992) 3925–3931.
[41] G. Perozzi, S. Prakash, RAD7 gene of *Saccharomyces cerevisiae*: transcripts, nucleotide sequence analysis, and functional relationship between the RAD7 and RAD23 gene products, Mol. Cell Biol. 6 (1986) 1497–1507.
[42] R.S. Lloyd, Investigations of pyrimidine dimer glycosylases – a paradigm for DNA base excision repair enzymology, Mutat. Res. 577 (2005) 77–91.
[43] W.L. Whelan, E. Gocke, T.R. Manney, The CAN1 locus of *Saccharomyces cerevisiae*: fine-structure analysis and forward mutation rates, Genetics 91 (1979) 35–51.
[44] D. Lohr, P. Venkov, J. Zlatanova, Transcriptional regulation in the yeast GAL gene family: a complex genetic network, FASEB J. 9 (1995) 777–787.
[45] T. Jackson, E. Kwon, A.M. Chachulska, L.E. Hyman, Novel roles for elongin C in yeast, Biochim. Biophys. Acta 1491 (2000) 161–176.
[46] L.E. Hyman, E. Kwon, S. Ghosh, J. McGee, A.M. Chachulska, T. Jackson, W.H. Baricos, Binding to Elongin C inhibits degradation of interacting proteins in yeast, J. Biol. Chem. 277 (2002) 15586–15591.
[47] S. Tornaletti, B.A. Donahue, D. Reines, P.C. Hanawalt, Nucleotide sequence context effect of a cyclobutane pyrimidine dimer upon RNA polymerase II transcription, J. Biol. Chem. 272 (1997) 31719–31724.
[48] G. Giglia-Mari, C. Miquel, A.F. Theil, P.O. Mari, D. Hoogstraten, J.M. Ng, C. Dinant, J.H. Hoeijmakers, W. Vermeulen, Dynamic interaction of TTDA with TFIIH is stabilized by nucleotide excision repair in living cells, PLoS Biol. 4 (2006) e156.
[49] M. Stefanini, W. Vermeulen, G. Weeda, S. Giliani, T. Nardo, M. Mezzina, A. Sarasin, J.I. Harper, C.F. Arlett, J.H. Hoeijmakers,

et al., A new nucleotide-excision-repair gene associated with the disorder trichothiodystrophy, Am. J. Hum. Genet. 53 (1993) 817–821.
[50] W. Vermeulen, E. Bergmann, J. Auriol, S. Rademakers, P. Frit, E. Appeldoorn, J.H. Hoeijmakers, J.M. Egly, Sublimiting concentration of TFIIH transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder, Nat. Genet. 26 (2000) 307–313.
[51] Y. Zhou, H. Kou, Z. Wang, Tfb5 interacts with Tfb2 and facilitates nucleotide excision repair in yeast, Nucleic Acids Res. 35 (2007) 861–871.
[52] B. Ding, C. Ruggiero, X. Chen, S. Li, Tfb5 is partially dispensable for Rad26 mediated transcription coupled nucleotide excision repair in yeast, DNA Repair (Amst.) 6 (2007) 1661–1669.