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-