Rad26, the Yeast Homolog of the Cockayne Syndrome B Gene Product, Counteracts Inhibition of DNA Repair Due to RNA Polymerase II Transcription

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Transcription-coupled DNA repair (TCR) is responsible for the preferential removal of DNA lesions from the transcribed strands of RNA polymerase II transcribed genes. Saccharomyces cerevisiae rad26 mutants and cells from patients suffering from the hereditary disease Cockayne syndrome display a TCR defective phenotype. Whether this lack of preferential repair has to be explained by a defect in repair or in general transcription is unclear at present. To discriminate between both possibilities, we analyzed repair of UV-induced cyclobutane pyrimidine dimers at single base resolution in yeast cells lacking RAD26, the homolog of the Cockayne syndrome B gene. Disrupting RAD26 affects nucleotide excision repair of transcribed DNA irrespective of the chromatin context, resulting in similar rates of removal for individual cyclobutane pyrimidine dimers throughout the transcribed strand. Notably, repair of transcribed sequences in between core nucleosomal regions is less efficient compared with nontranscribed DNA at these positions, pointing to a nucleotide excision repair impediment caused by blocked RNA polymerase. Our in vivo data demonstrate that the TCR defect in rad26 mutant cells is not due to a general transcription deficiency but results from the inability to release the transcription complex trapped at sites of base damage.

Ever since the first observation of transcription-coupled DNA repair (TCR), it has been suggested that RNA polymerase II (RNAPII) stalled at the site of base damage acts as a molecular beacon to signal repair proteins toward the damaged strand (1, 2). Studies in Escherichia coli have provided a frame of reference for such a mechanism by the identification of a molecular matchmaker that physically links RNAP transcription and nucleotide excision repair (NER). It was shown that the mfd encoded transcription-repair coupling factor (TRCF) is able to release blocked RNAP and via interaction with UvrA stimulates efficient targeting of the E. coli NER machinery to the damaged template (3). Consequently, cells lacking TRCF fail to remove cyclobutane pyrimidine dimers (CPDs) preferentially from transcribed strands (4). In human cells, a TCR-deficient phenotype has been associated with Cockayne syndrome (CS), whereas in yeast Rad26, the Cockayne syndrome B (CSB) homolog has been implicated in TCR (5, 6). Primarily based on the resemblance in the molecular defect and the limited sequence similarity between CSB/RAD26 and mfd, it has been proposed that the encoded proteins operate as the eukaryotic analogs of the TRCF either to disrupt or to modify the RNAPII-DNA interaction at the site of base damage and to recruit NER proteins providing a molecular basis for the enhanced repair rate of the transcribed strand.

An alternative hypothesis that implicates CSB/Rad26 as an intrinsic component in the transcription machinery has been suggested to explain the phenotypic complexity of patients suffering from CS (7). Hypothetically, any protein required for efficient transcription by RNAPII, e.g. transcription-initiation or elongation factors, could instigate a TCR deficiency when mutated. In such a scenario, a failure to efficiently transcribe through a template molecule will automatically reduce or abrogate the damage-signaling function of RNAP simply because the complex does not encounter the damage within a certain time frame. Although not easily reconciled with the notion that both yeast and human can cope with the complete absence of Rad26/CSB, this hypothesis has recently gained impetus by the observations that CSB cells display a reduced level of transcription (8) and that purified CSB enhances elongation by RNA polymerase in vitro (9).

Based on the present biochemical and genetic data the question of whether Rad26/CSB is a bona fide repair factor or a general transcription efficiency factor is unanswered, especially because both scenarios predict a reduction in the rate of lesion removal from transcribed DNA when the encoding genes are mutated. However, we reasoned that the two scenarios can be distinguished by the characteristics of repair when analyzed at nucleotide resolution. If a TCR defect in rad26 cells results from impaired transcription, in other words from the absence of RNAPII-mediated damage detection, then lesions in the transcribed strand are repaired by the same mechanism that operates on lesions in the nontranscribed strand i.e. global genome repair (10). Because this mode of repair in the URA3 nontranscribed strand is highly influenced by the chromatin environment of the damage (11, 12), this should also be observed for repair of lesions in the transcribed strand in rad26 mutant cells. However, if a TCR defect results from a deficiency to act upon stalled RNAPII molecules at the site of base damage, the stalled polymerase will still be the substrate to act upon in subsequent steps of NER. Because uniform repair rates for individual damages in the transcribed strand in wild type cells indicates that RNA polymerase II-mediated damage recognition is not profoundly influenced by the sequence or chromatin context (12, 13), this model predicts that individual lesions will be equally affected by a Rad26 deficiency, and similar repair rates for differently positioned dinucleotides will be observed.

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1 The abbreviations used are: TCR, transcription-coupled DNA repair; RNAP, RNA polymerase; RNAPII, RNA polymerase II; NER, nucleotide excision repair; TRCF, transcription-repair coupling factor; CPD, cyclobutane pyrimidine dimer; CS, Cockayne syndrome; CSB, CS group B homolog.

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FIG. 1. Repair of UV-induced CPDs at single nucleotide resolution along the URA3 locus. A, CPD removal from the nontranscribed strand (nucleotides 60 to 275 relative to the start codon ATG designated +1) in rad26 mutant cells irradiated with 70 J/m² and assayed at 0, 40, 80, and 120 min after irradiation. Samples mock treated or treated with the CPD-specific enzyme T4endoV are denoted − and +, respectively. A black box indicates the internal protected regions of nucleosomes U2 (15). B, CPD removal from the transcribed strand (nucleotides 387 to 601) in repair proficient cells (RAD⁺) compared with repair in rad26 mutant cells. The black box indicates the internal protected region of nucleosome U4 (15). C, graphic representation of quantified CPD repair rates along part of the nontranscribed (upper panel) and transcribed strand (lower panel) of the URA3 locus in rad26 mutant cells. Repair half-time values, defined as the time at which 50% of the initial CPD signal was removed, were calculated for each individual CPD position with a sufficient signal to noise ratio and are plotted above their corresponding dipyrimidine position.
Experimental Procedures

Strains—The Saccharomyces cerevisiae NER proficient (rad+) strain used for this study is W303-1B, genotype: MATα his1-100 ade2-1 trp1-1 leu2-3,112 his3-11,15 ura3-1, which was rendered URA3 by transformation of a linear polymerase chain reaction fragment containing the complete locus. Uracll prototrophs were checked by Sanger sequencing for proper recombination at its chromosomal position. Subsequently the rad26 disruption was introduced into this background by one-step gene replacement. Strains were kept on selective YNB (0.67% yeast nitrogen base, 2% glucose, 2% bacto agar) supplemented with the appropriate markers. Cells were grown in complete YEPD medium (1% yeast extract, 2% bacto peptone, 2% glucose) at 28 °C under vigorous shaking.

CPD Analysis—Cells diluted in chilled phosphate-buffered saline were irradiated with 254 nm UV light (Philips T U V 30W) at 70 J/m², collected by centrifugation, resuspended in complete medium, and incubated for various times in the dark at 28 °C prior to DNA isolation. DNA samples (25 μg) were digested with appropriate endonucleases and, precipitated, and, and (ii) the chromatin architecture of the nontranscribed and the transcribed strands in the yeast URA3 locus, as measured by the influence of positioned nucleosomes on NER, is not changed in the absence of Rad26.

we then monitored repair of CPDs in the URA3 transcribed strand. Fig. 1B shows, as expected, that the rate at which CPDs are removed from the transcribed strand is severely affected when Rad26 is disrupted. Whereas CPDs are removed with a t₁/₂ of approximately 8 min in rad+ cells, the t₁/₂ for rad26 cells increases to 52 min, when individual dimer sites are averaged. However, in contrast to repair of the nontranscribed strand where lesions only at core nucleosomal regions persist after 120 min, here, all dimer sites are still visible after 120 min of repair. To allow a visual comparison between the repair patterns of the nontranscribed and the transcribed strands in rad26 cells, we determined the repair kinetics along both strand over an approximately 550-base pair DNA region, occupied by nucleosomes U2–U4 (15), and plotted the repair half-times above the corresponding dinucleotide sequence (Fig. 1C).

In contrast to the high level of repair heterogeneity on the nontranscribed strand, repair of individual dimer sites in the transcribed strand is markedly more homogeneous and not influenced by the chromatin environment of the damaged DNA and in this respect resembles repair in NER proficient cells. This indicates that the transcribing polymerase, also in the absence of Rad26, is capable of suppressing the inhibitory effect of chromatin at the core regions of positioned nucleosomes and thus favors an explanation in which RNP II transcription proceeds normally and is blocked when it encounters a CPD in the transcribed strand, irrespective of the exact position of the lesion. As this blocked transcription complex constitutes an equal obstacle for each individual dimer site in subsequent steps of NER, similar repair kinetics for individual lesions will result. Importantly, Fig. 1C also demonstrates that at certain positions in the transcribed strand, for instance nucleotides 151–152, 164–165, and 165–166, repair halftimes were determined from multiple independent repair experiments.

Results and Discussion

We first analyzed whether the absence of Rad26 had any effect on the removal of DNA damage from the nontranscribed strand of the yeast URA3 locus. Fig. 1A shows an example of repair analysis of UV-induced CPDs performed on the URA3 nontranscribed strand in rad26 mutant cells. Slow repair coincides with the internal protected region of positioned nucleosomes, whereas lesions in linker DNA or at the boundaries of the nucleosome are removed more efficiently. For example, lesions induced at sequences occupied by the core of nucleosomes U2 (exemplified by the arrows in Fig. 1A) persist even after 2 h of repair, whereas most flanking sequences are repaired after 80 min. When the kinetics of individual dimer sites were determined from multiple independent repair experiments, no significant difference was detected between repair of the nontranscribed strand in rad+ versus rad26 cells (Table I). This indicates that (i) nontranscribed DNA is repaired independent of Rad26, in line with previously published data (6, 13) and (ii) the chromatin architecture of the URA3 locus, as measured by the influence of positioned nucleosomes on NER, is not changed in the absence of Rad26.

We then monitored repair of CPDs in the URA3 transcribed strand. Fig. 1B shows, as expected, that the rate at which CPDs are removed from the transcribed strand is severely affected when Rad26 is disrupted. Whereas CPDs are removed with a t₁/₂ of approximately 8 min in rad+ cells, the t₁/₂ for rad26 cells increases to 52 min, when individual dimer sites are averaged. However, in contrast to repair of the nontranscribed strand where lesions only at core nucleosomal regions persist after 120 min, here, all dimer sites are still visible after 120 min of repair. To allow a visual comparison between the repair patterns of the nontranscribed and the transcribed strands in rad26 cells, we determined the repair kinetics along both strand over an approximately 550-base pair DNA region, occupied by nucleosomes U2–U4 (15), and plotted the repair half-times above the corresponding dinucleotide sequence (Fig. 1C).

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sequences is obstructed in the transcribed strand.

Our *in vivo* data strongly suggest that Rad26 is required for the efficient processing of NER blocking transcription complexes. Whether this feature resides in the Rad26 protein itself or in additional factors that are required/recruited remains elusive. Purified CSB, the human homolog of Rad26, on its own was not able to dissociate stalled RNAPII in a defined *in vitro* system (19). However, Rad26 and CSB contain DNA-dependent ATPase activity (16, 17) and belong to the Swi2/Snf2 family of DNA-dependent ATPases. The notion that some members of this gene family are implicated in remodelling specific DNA-protein interactions (reviewed in Ref. 18) supports a function of Rad26/CSB in modifying DNA/RNAPII contacts at the site of transcriptional arrest. In such a scenario, alternative routes to destabilize (or displace) RNA pol II might compensate for the loss of CSB/Rad26 in TCR.

Previously, we have reported that efficient removal of CPDs from a small but distinct region immediately downstream of transcription initiation does not depend on Rad26, because these lesions are repaired strand-specifically in *rad26* mutants (13). A similar observation has been made for human CSB and more recently CSA cells (20, 21), demonstrating that Rad26/CSB is required for efficient TCR only during the elongating stages of RNAPII transcription. In the light of the results presented here, these data could indicate that Rad26/CSB is not essential for the alleviation of NER inhibition due to stalled RNAPII complexes prior to the point where the transition from transcription-initiation to competent transcription-elongation occurs. The question of whether this is a direct result of the presence of TFIH in the transcription machinery during the first steps of nascent mRNA synthesis (as discussed in Ref. 13) or alternatively results from a less stable RNAPII-damaged DNA complex when the polymerase is still in its initiation mode is unanswered at present time and probably awaits the development of an *in vitro* TCR system that has not been established yet with eukaryotic factors.

Finally, one can envisage that the processing of stalled RNAPII complexes is not confined to NER substrates but can also extend to base excision repair substrates and even to naturally occurring pause sites. The observation that some forms of oxidative damage (which are generally not repaired by NER but by base excision repair) are not repaired strand specifically in CS cells (22) indicates that the function of CS extends to a broader substrate range that might ultimately be just a stalled RNA polymerase, irrespective the cause of stalling. Consequently, a subtle transcription-elongation defect could result when Rad26 in yeast or CSB in humans is mutated. Maybe more important than the inefficient removal of lesions from transcribed DNA, a failure to recover trapped RNA polymerase II complexes as suggested by our *in vivo* repair analysis might contribute to the complex clinical phenotypes observed in Cockayne syndrome (7, 23).

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