A new connection of mRNP biogenesis and export with transcription-coupled repair

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ABSTRACT

Although DNA repair is faster in the transcribed strand of active genes, little is known about the possible contribution of mRNP biogenesis and export in transcription-coupled repair (TCR). Interestingly, mutants of THO, a transcription complex involved in maintenance of genome integrity, mRNP biogenesis and export, were recently found to be deficient in nucleotide excision repair. In this study we show by molecular DNA repair analysis, that Sub2-Yra1 and Thp1-Sac3, two main mRNA export complexes, are required for efficient TCR in yeast. Careful analysis revealed that THO mutants are also specifically affected in TCR. Ribozyme-mediated mRNA self-cleavage between two hot spots for UV damage showed that efficient TCR does not depend on the nascent mRNA, neither in wild-type nor in mutant cells. Along with severe UV damage-dependent loss in processivity, RNAPII was found binding to chromatin upon UV irradiation in THO mutants, suggesting that RNAPII remains stalled at DNA lesions. Furthermore, Def1, a factor responsible for the degradation of stalled RNAPII, appears essential for the viability of THO mutants subjected to DNA damage. Our results indicate that RNAPII is not proficient for TCR in mRNP biogenesis and export mutants, opening new perspectives on our knowledge of TCR in eukaryotic cells.

INTRODUCTION

Nucleotide excision repair (NER) is an evolutionarily conserved DNA repair pathway that deals with severely distorting DNA lesions including intrastrand crosslinks such as UV-induced pyrimidine dimers [reviewed in (1,2)]. Within NER two damage-sensing pathways are recognized: one for the entire genome, global genome repair (GGR), and one for the transcribed strand of active genes, transcription-coupled repair (TCR). In yeast, GGR requires Rad7, a protein carrying leucine-rich repeats, and Rad16, a member of the SWI2/SNF2 subfamily of putative helicases (3). These proteins presumably act in a complex that might be required in chromatin remodeling to facilitate damage recognition by Rad4/Rad23 [reviewed in (1,4)]. As ongoing transcription is required for TCR, damage recognition is likely done by the elongating RNA polymerase (RNAP) itself. RNAP arrests at injuries in the template strand triggering, likely via additional specific factors, the recruitment of the DNA repair machinery [reviewed in (5–7)]. Interestingly, TCR appears to be functional once a low and basal rate of transcription is achieved, beyond which there is no simple correlation between transcription and repair rates (8).

In Escherichia coli, the stalled RNAP leads to the recruitment of the transcription-repair coupling factor (TCRF) Mfd, allowing for the release of RNAP and further recruitment of the repair factors (9,10). In eukaryotes the precise mechanism of TCR remains poorly understood. Mutations in proteins required for NER lead to severe disorders known as Xeroderma pigmentosum and Cockayne’s syndrome [for review see (11)]. One of these proteins, Cockayne syndrome B protein (CSB), and its yeast ortholog Rad26, share conserved functions (12,13) and represent putative eukaryotic TCRF candidates. CSB and Rad26 belong to the SWI2/SNF2 helicase superfamily. Although CSB has been shown to have DNA-dependent ATPase activity, an ATPase-deficient mutant partially restores CSB activity in vivo (14). The putative function of CSB as a TCRF has been substantiated by in vitro reconstitution of the TCR initiation steps, in which an elongating RNAPII arrested at a DNA lesion was shown to mediate an ATP-dependent incision of the damaged DNA only in the presence of CSB (15). XPG, one of the structure-specific DNA endonuclease responsible for the removal of an oligonucleotide containing the DNA lesion in NER, is another protein involved in TCR. Recent results imply a coordinated recognition of stalled RNAPII by XPG and CSB in TCR initiation in mammalian cells and suggest that TFIIH-dependent remodeling of stalled RNAPII without

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release may be sufficient to allow repair (16). In yeast, the Rpb9 subunit of RNAPII has also been shown to contribute to TCR (17,18). Alternatively, and analogous to the mRNA-dependent loading of termination factors in E. coli (19,20), it is also conceivable that the nascent mRNA, or proteins bound to it, may be required to load repair enzymes at stalled polymerases.

On the other hand, RNAPII is subject to ubiquitination and proteosome-mediated degradation in response to UV-generated DNA damage (21–24). It has been proposed that degradation of damage-stalled RNAPII complexes might be an alternative to TCR (25). Indeed, recent studies have shown that arrested RNAPII elongation complexes are the preferred substrate for ubiquitlation, which is dependent on the C-terminal repeat domain (CTD) of RNAPII and on the Def1 protein in yeast (26,27).

In eukaryotic cells, export of nuclear mRNA to the cytoplasm requires correct RNA-processing and the association of a number of RNA-binding proteins to form export-competent ribonucleoprotein particles (mRNP) [for review see (28–30)]. Although there is growing evidence for transcription-coupled mRNA export, the physical nature of this coupling is not known. A connection between mRNP biogenesis and transcription is provided by THO, a conserved four-protein complex composed of stoichiometric amounts of Tho2, Hpr1, Mft1 and Thp2 (31). Strikingly, mutants of the Mex67-Mtr2 and Sub2-Yra1, Mex67-Mtr2 and Thp1-Sac3 are required for efficient TCR in yeast, thus linking mRNP biogenesis and export to TCR. Using a construct in which a self-cleaving Hammerhead ribozyme was cloned between two hot spots for UV damage, we demonstrate that TCR does not depend on the nascent mRNA, neither in wild-type nor in THO and Thp1-Sac3 deficient strains. Chromatin immunoprecipitation (ChIP) analyses revealed that, beside a severe UV damage-dependent loss in processivity, RNAPII is found to be bound to chromatin upon UV irradiation in THO mutants. Interestingly, Def1, a factor responsible for the removal of stalled RNAPII from a DNA template, is essential for the viability of THO mutants subjected to DNA damage. Our results support a model in which mRNP biogenesis and export is required for efficient TCR by preventing the occurrence of defective RNAPII complexes, which may remain stalled at a DNA lesion.

**MATERIALS AND METHODS**

**Strains and plasmids**

We used strains W303-1A, rad1Δ::LEU2 (W839-5D, R. Rothstein), rad7Δ::URA3 (MGSC97, 3), rad26Δ::HIS3 (MGSC102, 13), hpr1Δ::HIS3 (U674-1C), tho2Δ::KAN (RK2-6D, 45), hpr1-101 (WH101-1A, 37), sub2Δ::HIS3 pCM185-sub2-206 (TRP1, DLY33), sub2Δ::HIS3 (Ura- segregant from DLY23, 46), mex67-5 (WMC1-1A, 36), thp1Δ::KAN (WFBE046, F. Fabre), which have been reported previously, and isogenic derivatives obtained by genetic crosses. The def1Δ::HYGR strains were obtained by replacement of the DEF1 gene in rad7Δ::URA3 hpr1Δ::HIS3 diploids and subsequent tetrad dissection.

For ChIP analyses, the GAL1 promoter fused to the 5′-most 300 bp of the YLR454w open-reading frame was integrated at the YLR454w locus (32). Plasmids pHG001-Rib7 and pHG001-ribm were obtained by insertion of a fragment containing 39 bp yeast T-tracts (DED1 promoter), 52 bp Hammerhead ribozyme sequence (47), the Eco47III-BssHII LacZ fragment, and 39 bp yeast T-tracts (DED1 promoter) into the Eco47III-BssHII sites of plasmid pRWE005 (48).

**UV survival curves**

Yeast cells were grown in YPD-rich medium to an OD600 of 0.6. Plating, UV irradiation, and quantification were performed as described (42). All survival curves shown represent the average of at least three independent experiments.

**UV irradiation and repair**

Irradiation and repair was carried out as described (49) with minor modifications. Yeast cells were grown in 400 ml YPD-rich medium, or SG supplemented with the appropriate amino acids in the case of cells harboring the plasmid pHG001, to an OD600 of 0.8, harvested, and resuspended in SD or SG to an OD600 of 1.2. A 200 ml aliquot was irradiated with 230 J/m2 UV light using germicidal lamps (Philips T UV 15 W). The medium was supplemented to YPD-rich or with the appropriate amino acids and the cells incubated at 30°C in the dark for...
Gene- and strand-specific DNA repair assays

CPDs were mapped by indirect end-labeling and quantified as described (49). DNA was cut with appropriate restriction enzymes and aliquots were cut at CPDs with T4-endonuclease V (T4endoV, Epicentre) or mock treated. The DNA was electrophoresed in 1.3% alkaline agarose gels, blotted to Nylon membranes and hybridized with radioactively labeled strand-specific DNA probes. Strand-specific probes were generated by primer extension with T4-endonuclease V (T4endoV, Epicentre) or mock treated. The DNA was electrophoresed in 1.3% alkaline agarose gels, blotted to Nylon membranes and hybridized with radioactively labeled strand-specific DNA probes. Strand-specific probes were generated by primer extension with radioactively labeled strand-specific DNA probes. Strand-specific probes were generated by primer extension and probes that hybridize to the TS- and NTS-strand, respectively, were: Rpb2-A: 5′-TCCTGGAGATAATAACTTCGCGGC-3′; Rpb2-B: 5′-GGTTGAGTACAGATACATGCC-3′; pHG001-A: 5′-ATTITGAGACACAGACACTG-3′; pHG001-B: 5′-TCTGCCATTGTCAGACATGTAT-3′.

Membranes were analyzed and quantified with a PhosphorImager (Fuji FLA3000). The CPD content was calculated using the Poisson distribution, -ln(RF_a/RF_b), where RF_a and RF_b represent the signal intensities of the intact restriction fragment of the T4endoV- and mock-treated DNA, respectively. Region-specific damage was calculated as the average of the signal of that region in the T4endoV-treated DNA divided by the signal of the whole lane. The corresponding signal of the mock-treated DNA was subtracted as background. The average of the initial damage generated with 230 J/m² was 0.3 CPD/kb. To allow direct comparison between different strains, repair curves were calculated as the fraction of CPDs removed versus repair time. The initial damage was set to 0% repair.

Northern analyses

RNA was extracted and northern analyses performed according to standard procedures. For RNA synthesis recovery analyses, filters were hybridized with a 324-bp long RPB2 fragment obtained by PCR using primers RPB2-A, RPB2 B. Northern blots were quantified using a Fuji FLA 3000 and normalized to the rRNA levels of each samples. For pHG001 ribozyme cleavage analyses, filters were hybridized with a 314-bp long fragment obtained by PCR using primers HG001-A and HG001-B.

ChIP analyses

Cells were grown and irradiated as described above. Forty milliliter aliquots were taken at the indicated repair times and cross-linked with a 1% formaldehyde solution for 15 min at RT. Glycine was added to a final concentration of 125 mM, and the cell pellets frozen in liquid nitrogen and kept at −80°C. ChIP assays were performed as described (50). Monoclonal 8WG16 antibody (COVANCE) and protein A-Sepharose were used to immunoprecipitate RNAPII. The GFX purification system (GE Healthcare) was used for the last purification step. All samples were treated with 200 ng photolyase (TREVIGEN) for 30 min under photoreactivating light (Sylvania F15T8 BLB) prior to real-time quantitative PCR analysis. We used 20–30 bp oligonucleotides for the PCR amplification of two fragments of YLR454c (3–43 and 7621–7674) and the 9716–9863 intergenic region of chromosome V, which was used as non-transcribed control. Real-time quantitative PCR was performed using SYBR green dye in the 7500 Real Time PCR system (Applied Biosystems). Standard curves for all three pairs of primers were performed for each PCR analysis, all PCR reactions being performed in triplicate. The enrichment of each PCR amplification of interest was calculated as the ratio between the region-specific signal and the intergenic signal of the precipitated fractions normalized with respect to the corresponding ratios of the input fractions. At least three independent experiments were performed for each condition. Primer sequences are available upon request.

RESULTS

mRNA export-deficient cells are sensitive to UV in the absence of global genome repair

To analyze whether defects in mRNP biogenesis and export result in impaired TCR, we studied mutants defective in both GGR and mRNA export. Isogenic sub2-206, mex67-5, rad7Δ, sub2-206 rad7Δ, mex67-5 rad7Δ mutants were generated and survival after UV irradiation was determined (Figure 1, upper panel). Isogenic repair-proficient W303-1A, repair-deficient rad1Δ and TCR-deficient rad26Δ rad7Δ strains were used as controls. The sub2-206 and mex65-7 single mutants show no increased UV sensitivity as compared with wild-type cells. However, upon UV irradiation viability of the sub2-206 rad7Δ and mex67-5 rad7Δ double mutants dropped below the levels of the rad7Δ single mutant. Survival of the sub2-206 rad7Δ strain was similar to survival of the rad26Δ rad7Δ strain, whereas mex67-5 rad7Δ was less affected. Next, we analyzed mutants of the Thp1-Sac3 complex, which acts downstream of Mex67-Mtr2 on the mRNP biogenesis and export route. Isogenic thp1Δ, sac3Δ, thp1Δ rad7Δ, and sac3Δ rad7Δ mutants were generated and UV survival was determined (Figure 1, middle panel). The thp1Δ and sac3Δ single mutants show no increased UV sensitivity as compared with wild-type cells. However, viability of the thp1Δ rad7Δ and sac3Δ rad7Δ double mutants was below the level of the rad7Δ single mutant upon UV irradiation.

Since THO mutants have been shown to be sensitive to UV irradiation in the absence of GGR (42), we performed UV survival curves of hpr1Δ rad7Δ and tho2Δ rad7Δ strains for comparison of phenotype strength (Figure 1, lower panel). As expected, hpr1Δ rad7Δ and tho2Δ rad7Δ survival were reduced below the levels of the rad7Δ single mutant upon UV irradiation. UV sensitivity of sub2-206 rad7Δ cells was stronger than hpr1Δ rad7Δ cells, and weaker than tho2Δ rad7Δ cells, whereas thp1Δ rad7Δ and hpr1Δ rad7Δ showed similar UV sensitivity, consistent with the individual phenotype of the single mutant in other assays (36,40,45,51). We have recently described the
hpr1-101 mutant allele, which exhibits severe transcription defects but weak hyper-recombination (37). We tested the hpr1-101 allele for UV sensitivity in the absence of GGR to check whether the UV sensitivity of THO mutants was rather linked to their transcription deficiencies or to the formation of recombinogenic structures. Survival of hpr1-101 rad7Δ and hpr1Δ rad7Δ cells were similar (Figure 1, lower panel), indicating that the observed UV sensitivity was linked to the transcription defects of THO mutants.

Thus, because UV sensitivity in the absence of GGR is a phenotype mostly associated with TCR deficiencies, we decided to test whether Sub2-Yra1 and Thp1-Sac3 complexes are required for proficient TCR.

**TCR is impaired in cells defective in Sub2-Yra1, Thp1-Sac3 and THO**

Next, we analyzed removal of UV photoproducts in sub2Δ and thp1Δ cells at the molecular level. Isogenic repair-proficient wild-type, TCR-deficient rad26Δ and GGR-deficient rad7Δ were used as controls. Repair after UV irradiation was determined in a 4.4-kb restriction fragment containing the constitutively expressed RPB2 gene by alkaline electrophoresis and indirect end-labeling (Figure 2). As previously reported (3), rad7Δ cells showed wild-type repair levels in the transcribed strand (TS) while repair of the non-transcribed strand (NTS) was strongly reduced. In rad26Δ cells, repair of the TS was significantly reduced while repair of the NTS almost reached wild-type levels (13). In sub2Δ and thp1Δ cells, repair of the TS was severely impaired while repair of the NTS did not exhibit significant repair defects (Figure 2B). Thus, our results indicate that sub2Δ and thp1Δ show defects in TCR, but not in GGR.

In a previous report, molecular analysis of DNA repair in hpr1Δ and tho2Δ cells indicated general defects in NER (42). In these studies, the UV doses used had produced extensive DNA damage, in contrast to the conditions used in this study, in which one repair event is sufficient to restore the intact DNA (about 1 CPD per restriction fragment). The observations that mutants of the Sub2-Yra1 and Thp1-Sac3 complexes, which act downstream of THO in mRNP export, are specifically affected in TS repair lead us to examine CPD removal in tho2Δ and hpr1Δ cells, using our UV irradiation conditions (Figure 2A and C). Repair of the TS was significantly reduced in both strains. As observed by UV sensitivity assays in the absence of GGR (Figure 1), tho2Δ cells were more strongly affected in TS repair than hpr1Δ cells, reaching levels similar to rad26Δ cells. Likewise, repair of hpr1Δ and hpr1-101 cells were equally affected in DNA repair (data not shown). In the NTS, the repair levels of hpr1Δ, tho2Δ and rad26Δ cells were similar, in contrast to previous results obtained with extensive DNA damage and TCR- and GGR-deficient and rad26Δ rad7Δ served as controls (empty symbols). Double mutants carrying the hpr1Δ and tho2Δ mutations in combination with the rad7Δ mutations were used as marker of phenotype strength. Data for the rad26Δ control strain were taken from (42) (dash line). Average values from three independent experiments are plotted for each genotype.
### Table A

| UV | NER time | T4endoV | **RPB2 (TS)** | **RPB2 (NTS)** |
|----|----------|---------|---------------|---------------|
|    | -        | -       | -             | -             |
|    | +        | 0'      | -             | -             |
|    | +        | 30'     | +             | +             |
|    | +        | 60'     | +             | +             |
|    | +        | 90'     | +             | +             |
|    | +        | 120'    | +             | +             |

- **wt**: Wild type
- **rad26**: rad26 mutant
- **sub2**: sub2 mutant
- **thp1**: thp1 mutant
- **hpr1**: hpr1 mutant
- **tho2**: tho2 mutant
- **rad7**: rad7 mutant

### Graphs B

**RPB2 (TS)**
- **wt**: Black square
- **rad7**: Black triangle
- **thp1**: Black diamond
- **sub2**: Black circle
- **rad26**: Black star

**RPB2 (NTS)**
- **wt/thp1**: Open square
- **sub2**: Open circle
- **rado26**: Open star
- **rad7**: Open diamond

### Graphs C

**RPB2 (TS)**
- **wt**: Black square
- **hpr1**: Black triangle
- **tho2**: Black diamond
- **rad26**: Black circle

**RPB2 (NTS)**
- **wt**/**hpr1**: Open square
- **rado26**: Open circle
- **tho2**: Open diamond
- **rad7**: Open triangle
(42), indicating that GGR is not significantly affected in *hpr1Δ* and *tho2Δ* cells in our conditions.

In yeast, RNA synthesis is inhibited shortly after UV irradiation, probably due to the presence of CPDs in the TS of active genes. The ability of wild-type and mutant yeast cells to recover RNAPII synthesis in individual genes has been shown to mirror their strand-specific repair capacity (52). To gain additional information on the connection between the recovery of mRNA levels and TCR, kinetics of the *RPB2* transcript levels were determined by northern analysis in *rad26Δ, tho2Δ, sub2Δ, thp1Δ*, and wild-type cells (Figure 3). A direct correlation between the *RPB2* expression levels prior to UV irradiation and repair rates was not apparent, in agreement with previous work showing the absence of simple correlation between transcription and repair rates (8).

However, upon UV irradiation, *RPB2* transcript recovery appeared to be most efficient in *rad26Δ* cells, while transcript recovery was clearly affected in all other mutant strains. This result points to THO, Sub2-Yra1 and Thp1-Sac3 behaving differently from Rad26 in response to UV damage, since they appear to undergo severe transcription impairment upon UV irradiation, in addition to their TCR deficiencies.

Taken together, our results place THO, Sub2-Yra1, Mex67-Mtr2 and Thp1-Sac3 as new factors needed for efficient TCR. As these factors play a role at the interface between transcription and RNA export, it was important to determine whether the observed TCR defect was mediated by the RNAPII or by the nascent mRNA.

**TCR depends on RNAPII rather than on the nascent mRNA.**

The molecular basis underlying the requirement of functional mRNP biogenesis and export factors for TCR might rely on the proper packaging of the nascent transcript or on their effect on transcription. In repair-proficient cells, the nascent mRNA could mediate the TCR reaction in response to the transcriptional stalling occurring at DNA lesions. To test this possibility, we designed a construct containing two 39-bp long T-tract sequences inserted at different sites within the *GAL1* promoter-driven *LacZ* ORF. Between the two T-tract sequences, 52 bp encoding either an active self-cleaving Hammerhead ribozyme or an inactive mutated form (47) were inserted (Figure 4A). Northern analysis confirmed a complete disappearance of the full length mRNA in the construct carrying the active ribozyme (Figure 4B), indicating that the nascent mRNA was efficiently cleaved between the T-tracts.

We first assessed whether TCR efficiency depends on the integrity of the nascent mRNA in yeast wild-type cells by comparing repair rates in the T-tracts situated upstream (T1) and downstream (T2) of the ribozyme (Figure 4C and D). The initial damage was higher in

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Figure 2. Transcription coupled repair is impaired in mRNA export deficient cells. Southern blot analysis of representative experiments showing repair of a 4.4 kb (Novel) *RPB2* fragment in W303 isogenic *sub2Δ, thp1Δ, hpr1Δ, tho2Δ, rad26Δ, rad7Δ*, and wt cells after UV irradiation (230 J/m²). The initial damage averaged 0.28 ± 0.05 CPD/Kb in the transcribed strand (TS, left) and 0.26 ± 0.04 CPD/Kb in the non-transcribed strand (NTS, right). The remaining intact restriction fragment after treatment of damaged DNA with T4endoV (+UV, +T4endoV) corresponds to the fraction of undamaged DNA. Non-irradiated DNA (-UV) and DNA not treated with T4endoV (-T4endoV) were used as controls. The 90 min time point of *tho2* cells was taken from a different gel derived from the same experiment (*). As *rad7* was merely used as a negative control, the analysis was restrained to the 60 and 120 min time points. (B and C) Graphical representation of the repair analysis as shown in A. Repair of TS (full symbols and lines) and NTS (empty symbols and dashed lines) are shown for each strain. The percentage of repair was determined from the signal intensities as described in ‘Materials and Methods’. Average values derived from at least two independent experiments are plotted.

Figure 3. Delayed RNA synthesis recovery after UV irradiation in mRNA export deficient cells. (A) Northern blot analysis of *RPB2* expression prior and after UV irradiation (230 J/m²) in the indicated strains. Transcript levels prior to UV irradiation (pre UV) expressed in percent of the wild-type level are shown below the gels. (B) Graphical representation of *RPB2* synthesis kinetics after UV irradiation as shown in A. Northern blots were quantified using a Fuji FLA 3000 and normalized to the rRNA levels of each samples. The transcripts levels during DNA damage recovery were normalized to the pre UV values for each strain. Average values derived from at least two independent experiments are plotted.
T2 than in T1, likely reflecting local differences in chromatin structure. Importantly, our results show no significant difference between repair of T1 and T2, neither in the active (Rib\(^{+}\)) nor in the mutated (rib\(^{m}\)) ribozyme constructs, indicating that an intact and 5'-capped nascent mRNA is not required for efficient TCR in wild-type cells.

Nevertheless, in contrast to wild-type cells, the occurrence of sub-optimal mRNP might impede the process of TCR in mutants of THO/TREX and Thp1-Sac3. To test
this possibility, we used the ribozyme system to assess whether wild-type TCR can be restored in T2 after ribozyme self-cleavage of the nascent mRNA in tho2/C1 and thp1/C1 cells (Figure 5). Repair in the full length LacZ fragment was clearly below repair levels achieved in wild-type cells, confirming the TCR deficiency of tho2/C1 cells.

Comparison of repair efficiencies in T1 and T2 did not show any significant difference, neither in the active (Rib+)) nor in the mutated (ribm) ribozyme constructs. Similar results were obtained in thp1/C1 cells (data not shown).

Thus, our results indicate that cleavage of the nascent mRNA does not restore TCR in THO and Thp1 mutants. Consequently, we assume that the key player in the organization of TCR is the RNAPII itself, or some associated factors, rather than the nascent mRNA.

Def1 is required for removal of stalled RNAPII complexes in THO mutants

Since we can rule out an active role of the nascent mRNA in TCR, it is conceivable that an intact RNAPII complex is sufficient to mediate proficient TCR. Recently, a protein called Def1 was shown to trigger ubiquitylation and degradation of RNAPII in response to UV damage as an alternative pathway to DNA repair (26,27,53). To study the possible requirement for Def1 in the removal of trapped RNAPII presumably present in THO mutants, hpr1Δ def1Δ double mutants were generated and analyzed. hpr1Δ def1Δ double mutants were viable, but very slow growing. Since all THO/TREX mutants grow poorly at 37°C, we first investigated whether deletion of DEF1 might increase the temperature sensitivity (ts) phenotype of hpr1Δ cells (Figure 6A). An additive effect that made cells inviable at 37°C was observed in the double mutant as compared to the single mutant. Next, we performed UV survival curves in isogenic def1/C1, hpr1/C1 and def1/C1 hpr1/C1 mutants (Figure 6B). Viability of the def1Δ hpr1Δ double mutants was reduced below the levels of the def1Δ and hpr1Δ single mutants upon UV irradiation, indicating a synergistic effect of the two mutations on UV sensitivity. In the absence of GGR, def1Δ has been shown to be highly sensitive to UV irradiation (53). Nevertheless, the UV sensitivity of def1Δ hpr1Δ rad7Δ cells was increased as compared to def1Δ.
These genetic interactions between DEF1 and HPR1 indicate that Def1 is important for the viability of THO mutants subjected to stress and DNA damage.
the gene in THO mutants, in contrast to def1Δ and hpr1Δ def1Δ cells, in which RNAPII accumulated at the 5'-end after UV irradiation as a result of the lack of RNAPII degradation (Figure 7). These results imply that TCR-deficient RNAPII complexes remain stalled at DNA lesions in THO mutant and have to be removed from the template in order to provide access for DNA repair machineries, a process that depends on Def1.

**DISCUSSION**

**TCR and mRNP biogenesis are coupled**

The nuclear envelope marks a fundamental difference between eukaryotic and prokaryotic cells. The obligatory passage of eukaryotic mRNA through the nuclear envelope to reach protein translation machineries is tightly controlled by mRNA export systems. TCR has to cope
with mRNA export in eukaryotes, possibly explaining why it cannot take advantage of one transcription-repair coupling factor as found in prokaryotic cells. This study unveils that the repair of active genes indeed depends on the correct interplay between transcription and mRNA export. We found that removal of proteins all along the mRNP export route, including the transcription-associated THO and Sub2-Yra1 complexes, the Mex67-Mtr2 export receptor as well as the Thp1-Sac3 nuclear pore-associated complex, confer TCR-deficient phenotypes. Thus, our results establish a general dependency of TCR on proficient mRNP biogenesis and export.

The inability of human Cockayne’s syndrome cells to recover RNA synthesis after DNA damage was recently found not to be due solely to the failure of these mutants to remove lesions in the transcribed strand of active genes, but to defects in the re-initiation of the transcriptional program after UV irradiation (55). Although TCR of the constitutively expressed RPB2 gene is strongly affected in cells depleted of Rad26—the yeast CSB homologue—we found that, in rad26 mutants, mRNA recovery is much faster as compared to THO, Sub2-Yra1 and Thp1-Sac3 mutants (Figure 3). The mRNA export-dependent TCR-deficient phenotype could reflect a substantial lag in transcription initiation. This is not the case, as ChIP analysis indicated that, in contrast to CSB, RNAPII loading after UV irradiation was not affected in THO mutants (Figure 7). However, the amount of polymerases reaching the 3’-end of the YLR454w gene was substantially lower in hprl. Thus, the RNAPII processivity defects of hprl appear to be strengthened upon UV-irradiation, presumably leading to severe delays in mRNA recovery. Even though it is a priori conceivable that poor transcription rate and/or low RNAPII processivity alone might lead to defective TCR, several lines of evidence argue against this possibility. First, no simple correlation could be found between repair and transcription rates neither in different mutants (Figure 3) nor at different loci (8). Second, transcription-elongation mutants like spt4, in which transcription elongation is impaired and RNAPII processivity significantly reduced (54,56), do not show defects in TCR (57). On the contrary, spt4 suppresses the TCR defects of rad26, indicating that reduced RNAPII processivity can even act positively on TCR.

There is growing evidence for transcription-coupled mRNA export [for review see (28–30)], though the physical nature of this coupling is not known. The possible functional and physical connection between a subset of transcribed genes and the NPC (58–60) suggest that deficient mRNA export could negatively affect RNAPII transcription. Our results, providing evidence for a functional link between TCR and mRNA export, support the existence of such a feedback mechanism, which would alter transcription so that it is no longer proficient for TCR. A major player in the coupling of transcription and mRNA processing is the CTD of the largest subunit of RNAPII, which acts as a loading platform for pre-mRNA processing factors [reviewed in (28,61,62)]. Several lines of evidence indicate that CTD phosphorylation occurs upon UV irradiation and might be involved in the signaling of stalled RNAPII for TCR (24,63,64). These features place the CTD tail as a potential target for feedback modifications of RNAPII in response to mRNP export defects.

**TCR depends on the RNAPII machinery rather than on the nascent mRNA**

Given the association between mRNA export and efficient TCR, the nascent mRNA could act as a mediator for TCR. However, we discard the possibility that the nascent mRNA actively supports the TCR process by monitoring the repair of UV-lesions encountered by a RNAPII-complex containing either the full-length nascent mRNA or about 70 bp left over after ribozyme-mediated cleavage (Figure 4). It is worth noticing that the available nascent mRNA might be even shorter than 70 bp considering that 15 to 20 bp are covered by the RNAPII holoenzyme (65,66). Furthermore, our results indicate that RNA 5’-capping is dispensable for TCR. Thus, our results support the idea of the RNAPII machinery being the main mediator for an active DNA damage response.

Different evidence led to the proposal that the hyper-recombination phenotype of THO mutants is mediated by the formation of R-loops (RNA:DNA hybrids) behind the elongating RNAPII, as well as impaired replication fork progression (48,67). Here, we show that ribozyme-mediated cleavage of the nascent mRNA does not suppress the TCR deficiency of tho2 (Figure 5), suggesting that the hyper-recombination and TCR phenotypes might be mediated by different intermediates. This conclusion is further supported by the observation that the non-hyper-recombinant hprl-101 allele shares the transcription, mRNA export and TCR phenotypes of hprl cells (Figure 1) (37).

**Def1 is required for removal of defective RNAPII complexes in THO mutants**

Recently, RNAPII processivity has been shown to be significantly reduced in THO mutants (54). Our ChIP analysis of UV-irradiated cells revealed that RNAPII processivity was further impaired while RNAPII loading remained unaffected in hprl cells (Figure 7). A conclusion of these findings is that the TCR defect of hprl cells is a consequence of damage-trapped RNAPII. Since transcription is a one-track copying process, a trapped RNAPII can only be resolved by repairing the damage so that transcription can resume or by physically removing the stalled RNAPII [reviewed in (25)]. Thus, it is understandable that RNAPII degradation activities, such as Def1, might be important in backgrounds in which aberrant stalled RNAPII are formed. Indeed, we observed that the hprl def1 mutant exhibited profound growth defects and was highly temperature- as well as UV-sensitive (Figure 6). In contrast to wild-type and hprl cells, RNAPII accumulated at the 5’-end of YLR454w upon UV-irradiation in def1 mutant strains (Figure 7). Hence, Def1-mediated RNAPII degradation appears to be important for the removal of stalled RNAPII after UV damage, in agreement with previous works showing that UV-dependent RNAPII...
ubiquitylation depends on Def1 (26,27,53). Worthy of note, we find that the amount of RNAPII tends to decrease 90 min after UV irradiation in backgrounds in which TCR or RNAPII degradation is compromised. This decrease might reflect the physiological turnover of stalled RNAPII, could mediate the TCR phenotype of mRNA export mutants. Alteration of these factors might result in an incomplete or modified transcription apparatus, which in turn would be incompetent to promote TCR, possibly as a consequence of its impaired transcription processivity (Figure 8). A future new challenge will be to unravel the configuration of the RNAPII machinery in mRNP biogenesis and export mutants.

CONCLUDING REMARKS

Our results present a model by which TCR-deficient RNAPII complexes remain stalled at DNA lesions in THO, Sub2-Yra1, Mex67-Mtr2 and Thp1-Sac3 mutants, thereby preventing repair (Figure 8). On the mRNA export route, THO works in immediate proximity to the ternary complex formed by the polymerase holoenzyme, template DNA and nascent RNA, whereas Sub2-Yra1, Mex67-Mtr2 and Thp1-Sac3 work downstream in the mRNP biogenesis and export route. Here, we show that cleavage of the nascent mRNA, which is the only known physical link between all these complexes, is not sufficient to rescue TCR. Thus, yet to be discovered bridging factors, whether or not functionally connected to the RNAPII, could mediate the TCR phenotype of mRNA export mutants. Alteration of these factors might result in an incomplete or modified transcription apparatus, which in turn would be incompetent to promote TCR, possibly as a consequence of its impaired transcription processivity (Figure 8). A future new challenge will be to unravel the configuration of the RNAPII machinery in mRNP biogenesis and export mutants.

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